AN EXAMINATION OF SPASTIC AND TYPICALLY DEVELOPING MUSCLE FIBRE LENGTH RESPONSES TO HIGH VELOCITY RESISTANCE TRAINING

A thesis submitted for the

Degree of Doctor of Philosophy

By

JESSICA F DAVIS

Centre for Human Performance, Exercise and Rehabilitation

Brunel University London

January 2024

Publications Arising from Research

Journal Articles

A review of literature was published in *Medical Hypotheses* in 2020, and can be found in the Appendix.

Davis, J. F., Khir, A. W., Barber, L., Reeves, N. D., Khan, T., DeLuca, M., & Mohagheghi, A. A. (2020). The mechanisms of adaptation for muscle fascicle length changes with exercise: Implications for spastic muscle. *Medical hypotheses*, *144*, 110199. https://doi.org/10.1016/j.mehy.2020.110199

Chapters 4 & 5 were combined and published in *MDPI's Bioengineering* in 2024.

Davis, J. F., Khan, T., Thornton, M., Reeves, N. D., DeLuca, M., & Mohagheghi, A. A. (2024). High Velocity Passive Stretching Mimics Eccentric Exercise in Cerebral Palsy and May Be Used to Increase Spastic Muscle Fascicle Length. *Bioengineering (Basel, Switzerland)*, *11*(6), 608. https://doi.org/10.3390/bioengineering11060608

Acknowledgements

Firstly, I would like to thank my supervisory team, Dr Amir Mohagheghi and Dr Carla Harkness-Armstrong for providing their expertise to guide and support me throughout the project. Amir, thank you for your unwavering support, especially during challenging times where I was uncertain of the direction – you were always able to provide clarity, day or night. I greatly appreciate all the hours you spent guiding me through the process, always taking the time to ensure I was making progress and achieving the goals set, especially when deadlines seemed unachievable, you always provided hope. And to Carla, thank you for always providing support, whether it was technical or emotional, I appreciate all the help you provided. Needless to say, this thesis would not have been possible without you both providing help and support throughout, so thank you.

Secondly, I would like to thank Dr Lee Barber and Dr Neil Reeves, for providing expert objective feedback on project ideas and written work. You both provided different perspectives from within the field that were invaluable throughout the process.

I would also like to thank my fellow doctoral researchers, many of whom I have shared an office with for the past few years. Mara, thank you for all your help in the office and in the lab, both technical and emotional. Tamara, Desmond and Hannah, for all the times we collectively felt like giving up, thank you for always providing a laugh (and sometimes a cry), which always reassured me that my problems are fixable, and we are all going through them together. There are so many memories created in that office that I will cherish, especially when times were difficult, you always gave me a reason to smile and laugh.

To all my friends and family outside of academia, thank you for always providing emotional support, and putting up with me when I am stressed and panicking. I cannot count the times I called you Dad, and Jasmine, in a complete melt down and you both always provided a shoulder to cry on, as well as a positive attitude and encouragement to keep going. To you both, and Lady, thank you for always supporting me unconditionally; I would not be here today without you all. And to Marian, thank you for always providing a distraction when I needed one, for always being at the end of the phone to make me laugh or listen to me complain, I am eternally grateful.

Finally, I would like to thank The Royal National Orthopaedic Hospital Charity for their collaboration and financial support, without which, this research would not have possible.

Statement about COVID-19

The COVID-19 pandemic has undeniably had an impact on academia and research, inflicting profound challenges on methodologies reliant on human participants. The unprecedented global health crisis has disrupted established norms, imposing barriers on the conduct of research and the collection of data from human participants. The rigorous measures necessitated by the pandemic, including lockdowns, social distancing protocols, and heightened health concerns, have significantly affected traditional approaches to participant interaction. The research within this thesis began before the pandemic in 2018 and mainly concentrated on the systematic review of relevant literature, developing the rationale for experimental studies and obtaining ethics approval from relevant research ethics committees, but was significantly impacted by the inability to conduct in-person research throughout 2020 due to university closure. Moreover, the research was focussed on individuals within the clinical population, and recruitment was done through NHS sites, making recruitment and data collection impossible for months, as non-essential research projects were put on hold within the NHS. Once recruitment and undertaking research was once again possible, the impact of the pandemic on individuals became increasingly apparent; while all measures were taken to ensure participants were protected from COVID-19 during participation, health concerns surrounding participation inevitably became a barrier to recruitment as a result of the pandemic. Despite this, the data collection continued, not without difficulty and at much slower pace with fewer than expected participants (mainly affecting the second and third experimental studies presented in Chapters 5 and 6), and the research was completed in a timely manner. It would be prejudicial to disregard the impact the pandemic had on research, especially projects necessitating in-person human participation, therefore I ask that you take this into account when reading the work presented in this thesis.

Thesis Abstract

Muscle fascicle length (FL) is the most important architectural parameter affecting function; there have been inconsistencies in literature as to how to successfully increase FL. Using eccentric (ECC) training has reportedly increased FL, and 3 commonalities occur: muscle fibers undergo strain, are microscopically damaged, and a drop in joint moment during elongation occurs. We propose these three parameters need to be met to successfully increase FL. Individuals with Cerebral Palsy (CP) possess spastic muscles which are smaller than typically developing (TD) peers, which limits function; therefore, they would benefit from interventions which increase FL and improve functional abilities. Thus, an exploratory study was undertaken to establish if passive stretching of spastic muscles can mimic eccentric training due to the presence of a reflex contraction during stretching, and whether the criteria stated above can be induced in individuals with CP. The reliability of the measures used in the exploratory study were tested concurrently to this, to establish user reliability at measuring muscle FL using ultrasonography, and the minimal detectable change of muscle CK as an indicator of muscle microdamage in healthy adults, to inform whether microdamage occurred as a result of stretching in individuals with CP. A training program was then designed, informed by the exploratory study to induce possible increase in the length of fascicles in typically developing muscles over a period of 10 weeks. This was then undergone by an individual with CP as a case study using high velocity passive stretching (HVPS), to determine whether spastic muscles respond similarly to HVPS as do TD muscles to ECC training. Both TD and CP groups experienced an increase in isometric and ECC torque as a result of training, and CP participant improved balance and walking abilities. Future interventions could use HVPS to improve function in a larger sample size.

Table of Contents

List of abbreviations

CP – Cerebral Palsy

TD – Typically developing

SSN – Serial sarcomere number

FL – Fascicle length

MTU – Muscle-tendon unit

HVPS – High velocity passive stretching

ECC – Eccentric

US – Ultrasonography

CK – Creatine kinease

EMG – Electromyography

ICC – Intraclass correlation coefficient

GMFCS – Gross motor functioning classification system

ROM – Range of motion

PA – Pennation angle

pCSA – Physiological cross-sectional area

GM – Gastrocnemius

VL – Vastus lateralis

BF – Biceps femoris

VM – Vastus medialis

SENIAM – Surface electromyography non-invasive assessment of muscles

List of Tables

List of Figures

CHAPTER 1: General Introduction

Background

Cerebral Palsy (CP) is a lifelong, non-progressive neurological disorder that occurs during early development (pre-partum, intra-partum, or post-partum), with a prevalence of 1.5 - 4 per 1000 live births, making it the most common disability amongst children (CDC, 2022). The cause of the disorder is currently unknown; however, it occurs as a result of lesions or abnormalities in the brain. The most common type of CP is spastic, which is a result of damage to the motor cortex, and accounts for approximately 70-80% of all CP cases; Dyskinetic CP is characterized by involuntary movements, and occurs as a result of damage to the basal ganglia, resulting in hypertonia causing stiff movements. Ataxic CP is caused by damage to the cerebellum, and results in hypotonia (loss of muscle tone) and loss of muscle coordination (Sewell et al., 2014).

Spastic CP causes a number of symptoms, both primary and secondary, that can affect the upper/lower limbs (diplegia), one side of the body (hemiplegia), or all four limbs collectively (quadriplegia). Primary impairments are apparent at the point of the initial diagnosis, and include differences in muscle tone (hyper/hypotonia), postural instability and difficulties with motor coordination. Secondary impairments occur over time as a result of primary impairments, such as reduced range of motion, muscular strength and size.

Although CP is the most common disability in children, there are recognized barriers for early diagnosis amongst clinicians; infants can receive diagnoses ranging from 12 months to five years of age. This is attributed to a lack of definitive biomarkers, the possibility of false positives, as well as the grief and stigma associated with childhood disability (te Velde et al., 2019). Early diagnosis is considered important amongst clinicians to ensure essential treatment can be accessed in the early stages of life. This is especially important as neuroplasticity is greatest from birth to two years, when the brain forms double the synaptic connections of an adult brain; post two years of age these cells undergo apoptosis if the synapses are not activated frequently enough (Mundkur, 2005). Therefore, the neurological symptoms that are dominant initially, result in the extinction of many spinal reflex responses due to inhibition of the central nervous system (Theis et al., 2013). Intervention at an early age is important to ensure that synapses are activated frequently

enough to prevent cell apoptosis, with the aim of limiting the extinction of the spinal cord reflexes.

Various treatment approaches in CP aim to address primary or secondary contributors to disability. Clinicians focus on either influencing neural control mechanisms or modifying muscle-tendon unit properties to enhance functional movements. Neural control impairments, including the lack of selective muscle control, are considered one of the primary causes of functional impairment in individuals with CP. These impairments significantly impact motor function and the ability to execute coordinated movements, leading to various functional limitations. These neural impairments, are often characterized by increased muscle tone (resistance to stretch) and altered motor control, which interact with the characteristics of the affected muscles, such as shorter fascicle lengths and increased muscle stiffness to affect function (Barber et al., 2011a). Increased muscle tone and resistance to stretch which has neural origin (i.e., neural hypertonicity/spasticity) affects the length-tension relationship by keeping muscles in a partially contracted state, altering their resting length and eventually leading to biomechanical hypertonicity where resistance to stretch is partly due to the altered biomechanical (e.g., stiffness) and material (e.g., Young's modulus) properties of the muscle-tendon unit. This overall vicious cycle of events associated with altered length-tension relationship affects the muscle's ability to generate force efficiently across various muscle lengths. Accordingly, techniques in the latter approach to combat secondary adaptations of the muscle-tendon unit after CP involve muscle-tendon unit stretching, resistance training, and functional movement exercises.

The interaction between neural signals and muscle-tendon unit characteristics impacts the coordination, precision, and efficiency of movement execution. This interplay influences the ability to modulate force output and perform activities that require controlled and coordinated muscle actions, such as walking and stair climbing. Altered neural control in spastic CP results in alterations in the force-velocity relationship by affecting muscle activation patterns and coordination. Spasticity often results in modulating muscle activation levels during movement, limiting force generating capacity and speed of contraction.

As stated above, in response to neural impairments CP, adaptations occur within the muscles and tendons, creating secondary causes of functional impairment. These adaptations include the shortening of muscle fascicles, increased stiffness, and alterations in the properties of intramuscular connective tissue. The shortened fascicles and increased stiffness impact the length-tension and force-velocity characteristics of the muscle unit, compromising its ability to efficiently generate force across different lengths and affecting the speed of contraction. Additionally, these adaptations contribute significantly to the development of contractures, limiting the range of motion (ROM) in affected muscles. As a consequence, these adaptations not only influence the mechanical properties of the muscles but also contribute to the restriction of movement, leading to functional limitations in individuals with CP.

It is common observation that individuals who present with spasticity and contracture present shorter muscle fascicle lengths (FL) when compared with typically developed individuals (Mohagheghi et al., 2007, Mohagheghi et al., 2008, Theis et al., 2013, Theis et al., 2015) or normal fascicle lengths with fewer number of sarcomeres which are overly stretched (Lieber and Friden, 1993). As muscle fascicle length has been suggested as the most important architectural parameter affecting muscle function, this observed difference in FL may contribute to the impaired movement and functional abilities often reported within the population (Lieber and Friden, 1993). Muscle fascicle length can affect the joint angle at which the optimal muscular force is produced and the velocity of contraction, therefore increasing muscle fascicle length could be beneficial to individuals with CP, allowing more efficient force generation of a muscle at higher velocities, making functional activities easier to execute (Rose and McGill, 2005).

Alterations in muscle characteristics, such as fascicle length and stiffness, have a bidirectional relationship with neural components. Changes in muscle properties can impact neural responses, including the threshold for tonic stretch reflexes. By targeting muscle characteristics, especially fascicle length, it is therefore possible to influence and potentially modulate neural responses. This interplay between neural and muscular elements offers an opportunity to leverage rehabilitation strategies aimed at improving impaired function in CP by targeting muscle adaptations that might, in turn, affect neural control. Increasing muscle

fascicle length plays a crucial role in preventing and improving contractures in individuals with CP. Contractures, a decrease in the range of motion (ROM), involve shortening and tightening of muscles and connective tissues. By focusing on increasing fascicle length, interventions aim to counteract the development of contractures. Addressing contractures not only helps alleviate the burden of care for caregivers but also reduces the risk of deformities associated with prolonged muscle shortening. Increasing muscle length contributes to maintaining or enhancing the functional abilities and overall quality of life for individuals with CP. Increase in the length of muscle and muscle-tendon unit, in healthy animals have been achieved by chronically maintaining muscle-tendon unit at long lengths (Goldspink et al., 1974). Interventions through casting and or surgery in humans however has been less successful in CP and alternative approaches are required (Lee et al., 2011, Lieber and Theologis, 2021, Iwase et al., 2023).

It is well established that eccentric training (lengthening contractions) allow more force to be exerted by the muscle, meaning the load experience by the muscle can be greater than concentric contractions (Reeves et al., 2009a). This makes it more effective at altering the muscle size/volume, which is a common goal of intervention in individuals with CP. The body of research on the outcomes of eccentric resistance training in CP is continually expanding, however it is currently inconsistent – some interventions involving eccentric contractions successfully increase muscle size, and some find there are no alterations in size as a result of eccentric training (Davis et al., 2020, Franchi et al., 2014, Franchi et al., 2017, Reeves et al., 2009a). Although increasing muscle strength and volume are desired outcomes in CP, they will not address contracture and altered optimal angles for force development during movement which are the direct consequence of shorter than normal muscle/fascicle and muscle-tendon unit lengths. Training conditions suitable for increasing spastic fascicle lengths are unknown.

One study, involving healthy young adults, specifically tested different modes of eccentric training, and found that only high velocity eccentric training can induce increases in muscle fascicle length, suggesting this was a direct result of increasing the serial sarcomere number (SSN)(Sharifnezhad et al., 2014b). However, it is not feasible for individuals with CP to undergo eccentric training due to lack of selective muscle recruitment; therefore, new

treatment interventions with the aim of increasing muscle fiber length need to be established. Based off literature in which healthy muscle fascicle length was successfully increased using eccentric training in humans and animal models, it was proposed (Chapter 5, Appendix A (Davis et al., 2020)) that three criteria were required to be met for any training intervention to lead to increase in FL: that muscle should undergo appropriately high velocity training which induces positive fascicle strain associated with momentary deactivation of the muscle characterized by a drop in torque during lengthening. Such situation may lead to muscle microdamage and trigger sarcomerogenesis to add serial sarcomeres. It was then theorized that training conditions to induce these criteria need to be created in order to successfully increase muscle fascicle length in spastic muscles (Chapter 5). As eccentric training may not be possible in individuals with CP due to their limitations in selectively recruiting muscles and engaging with training, it was necessary to establish if the three criteria, presumed necessary to increase muscle fascicle length, could be met using passive high velocity stretching of spastic muscles. Whether a reflex contraction could be triggered in these circumstances in spastic muscles as a result of the high velocity passive stretching, and the muscle would be elongated while contracting, would mean high velocity passive stretching could mimic eccentric contractions in healthy muscles induce beneficial effects of eccentric training. If so, this could be extremely beneficial to the population, and could influence future intervention strategies that aim to increase muscle fascicle length with training within this population.

The overall purpose of this doctoral research was to identify exercise criteria to increase healthy muscle fascicle lengths, establish whether such conditions could be induced in individuals with spastic CP, and if so, whether spastic muscles fascicle length would be increased in response to a training program under such conditions to improve function. Results of the studies presented can be used to inform future clinical interventions that aim to improve muscle function and functional abilities within CP.

Next chapters of the current thesis are formatted in the following order: First, a review of literature (Chapter 2) provides the history of the methodologies used in interventions with the aim of increasing muscle fascicle length, and how these interventions have been applied to individuals with spastic muscles. The review of literature led to the proposition that

satisfaction of three criteria (high velocity of training associated with positive fascicle strain, and muscle deactivation which can lead to microscopic damage to the muscle) in any training intervention were required to increase fascicle length in healthy individuals. This point of view has been published in Medical Hypothesis journal (Davis et al., 2020). Chapter 3 shows an overview of the experimental methodology used throughout the course of this series of studies. Chapter 4 reports the reliability of the techniques used to measure the outcome parameters (FL and fascicle strain via ultrasonography, microdamage by blood creatine kinase measures), and Chapter 5 examines whether the proposed criteria for increasing FL could be met in those with spastic CP and. A further experimental study (Chapter 6) was designed to establish whether the assumed optimal training condition/criteria for increasing muscle FL were indeed reproducible in healthy muscles with eccentric training and led to increase in FL. Whether similar training condition could lead to the increase in FL in a participant with CP (Chapter 7) were also examined. Experimental study in Chapter 7 was informed based on the outcomes of the experimental study in Chapter 5 where it was established assumed optimal training condition for increasing FL in healthy muscles could be recreated in spastic CP muscles.

Aims of this research

Chapter 4

A non-confirmatory (exploratory) study, investigating muscle fascicle responses to stretching at high velocity in healthy individuals, is presented. Specifically, the study determines the examiner's reliability of measuring muscle fascicle length using ultrasonography, and establishes the minimal detectable change in blood CK in response to high velocity stretching in typically developed adults, not attributed to biological variation, to inform the future research in in the absence of relevant literature.

Chapter 5

A non-confirmatory (exploratory) study is presented which aims to establish whether the three presumed criteria necessary to increase muscle fascicle length, can be met in individuals with spastic muscles using a single-bout of high velocity passive stretching.

Chapter 6

To determine whether results reported by Sharifnezhad et al (2014)'s following eccentric exercise (Protocol 4) for increasing quadriceps muscle fascicle length in healthy adults can be replicated, and whether their theory that a drop in knee joint moment during flexion of the knee joint is a result of a momentary drop in the muscle activation during lengthening.

Chapter 7

To establish whether high velocity passive stretching can mimic eccentric training in an individual with spastic CP, where selective muscle recruitment is compromised, and therefore be used as a means of increasing fascicle length with training in this population.

CHAPTER 2: Critical Review of Literature

Evolution of the term “CP”

The definition of the term ‘CP’ has sparked debate for over 150 years, and these debates, which are focussed on how different manifestations of the condition can be classified, continues to the present. The correlation of brain lesions with their physical manifestations began in French publications debating the relationship between hemiplegia of the body with hemiatrophy of the brain post mortem. This was then furthered by British orthopaedic surgeon William Little, who focussed lectures on the ‘Deformities of the Human Frame’ in 1843. His research was centred on joint contractures resulting from spasticity and paralysis, and indicated that damage to the brain preterm and during infancy was the underlying cause of spasticity and paralysis(Dunn, 1995).

As the debate about the classification of CP continued, Sigmund Freud encouraged the classification of CP using only clinical findings; he recognised that the pathological findings of post mortem examinations were the result of a combination of the brain lesion and the process of repair, and so were only partly related to the clinical manifestation of CP. Freud then went on to create a classification system that concatenated the terms for all bilateral disorders to ‘diplegia’, to describe all disorders that manifested general rigidity of a cerebral origin, that is distinct from hemiplegia(Accardo, 1982). This then led to Freud’s identification of three causal factors: maternal and idiopathic congenital, perinatal, and postnatal. It was unclear to Freud whether the observed spasticity and rigidity was the result of trauma during birth, as previously described by Little, or whether there were predisposing factors that caused the trauma during the births.

Winthrop Phelps then developed the modern treatment approaches to the population, advocating physical therapy, nerve blockers and orthoses. He then identified four treatment goals still used to this day: locomotion, self-help, physical appearance, and speech(Morris, 2009). As Phelps’ approach to surgery was conservative, he recognised the need for a neurological classification system for diagnostic purposes. He then continued to use his own classification system to treat the population, which incorporated both mental and physical ability, as well as social assessment. Phelps then went on to found the American Academy

for CP in 1947, which continues to inspire research into the understanding of these conditions and in improving the care and rehabilitation of affected persons.

It was then proposed by Myer Perlstein that the classification of CP should be done by the anatomical site of the lesion within the brain, as well as the severity of the involvement, degree of muscle tone and clinical symptoms. He defined CP as any symptom complex derived from lesions within the brain. A separate measurement for the functional capacity of individuals was also incorporated in Perlstein's classification; it included four levels which were determined by level of activity, such as mild to moderate. This classification was criticised for the use of undefined terms describing the level of activity, making classification subjective from individual to individual(Perlstein, 1952).

The classification was then addressed from an epidemiological perspective by (Evans et al., 1987), which then lead to the creation of the Evans form. This recorded information on central motor disabilities in terms of neurological type, including: hypertonia (spasticity, stiffness and rigidity), hypotonia, dyskinesia, and ataxia. This form also allowed the recording of functional mobility and manual dexterity, as well as other functional difficulties reported in the population such as intellectual and sensory impairments, communication difficulties, seizures, and other genetic disorders.

A publication by Mutch et al., 1992 (Mutch et al., 1992) furthered the proposed definition of the term CP by focussing on the heterogeneity of the condition, classifying it as a term that encompasses a group of non-progressive, but evolving, motor impairment syndromes as a result of lesions or anomalies of the brain occurring during early development.

A functional classification of CP is the Gross Motor Function Classification System (GMFCS), which was developed by Palisano et al., 1997 (Palisano et al., 1997), as they recognised the need to have a standardised classification system for assessing the severity of movement disability amongst the population. It assesses the ability to perform motor skills such as standing and walking independently or with the use of aids(Beckers and Bastiaenen, 2015). The levels range from I-V: I meaning children can ambulate independently, V meaning children use a wheelchair at all times. There are common descriptors for each level, such as

the ability to use stairs without a handrail (level I), with a handrail (level II), with a handrail and supervision/assistance (level III). Descriptors for levels IV and V include using methods of mobility that require physical assistance or mobility aids (level IV), and limited ability to maintain antigravity head and trunk posture (level V)(Palisano et al., 1997).

It is evident that the definition and classification of CP is still a complex and ever evolving term, however throughout this thesis, a working definition for the classification of CP will be used. As the most widely accepted definition, the definition suggested by Hutton and Pharoah will be used throughout: CP is associated with non-progressive motor and sensory impairments due to primary neural damages, which are the result of lesions or abnormalities of the brain(Hutton and Pharoah, 2002). These impairments often include reduced sight and hearing, and functional impairments of balance, ambulation and manual dexterity. Importantly, secondary adaptations in the architecture and mechanical properties of the muscle, such as shortened muscle and fascicle length, and increased intramuscular connective tissue contribute to the development of contracture, and limit muscle-tendon unit functional capacity that can further contribute to the observed movement difficulty in this population.

Classification of CP

The symptoms associated with CP vary greatly and are leading to the classification system used for the condition. There are three main types that exist: ataxic, dyskinetic and spastic, each characterised by the area of the brain that is damaged. The specific mechanical properties of muscles and tendons affected by neural damage are dependent upon the area of damage. The most common is spastic CP, affecting approximately 85 % of patients(Cans, 2000), exhibited as increased muscle stiffness and hypertonia, which may make functional movements difficult to execute smoothly. This is then subcategorised by localisation: hemiplegia affecting one side of the body, diplegia affecting the upper or lower limbs, and quadriplegia affecting all limbs. These subcategories are all caused by the location and severity of the damage to the brain. Ataxia results in impaired coordination and often, hypermetric movement (Minear, 1956). Dyskinetic CP is subcategorised into athetoid and dystonic; the former is characterised by involuntary movement, and the latter is

characterised by strong contractions of the agonist and antagonist muscles simultaneously. Both ataxic and dyskinetic CP cause whole body movement, whereas spastic CP only causes partial body movement, depending on the limbs affected. Rigidity is also a mechanical property associated with CP, which is defined as resistance to passive movement, regardless of the speed or direction of movement (Morris, 2007).

CP is the result of a non-progressive injury to the brain, causing permanent disturbances to the development of movement and posture; these disturbances are characterised as either primary or secondary. Primary impairments are often apparent at the time of diagnosis, and are neural in nature, and are associated with motor impairments affecting postural stability and movement coordination. These primary neural impairments directly result in impaired motor unit recruitment (Rose and McGill, 1998). For example, impulses can be sent to simultaneously activate both agonist and antagonistic muscles (co-contraction), or that there is inadequate recruitment of motor units (not enough motor units are activated for the desired action). This co-contraction, as a result of impaired motor unit recruitment, results in a reduction in the net force generating capacity of the desired muscle and reduced net torque about a joint (Poon and Hui-Chan, 2009). This reduction of force produced over time could be a contributing factor to the smaller muscle volumes observed in children and adolescents with CP (Damiano et al., 2000).

Secondary impairments are musculoskeletal in nature, and are considered secondary as they occur over time in response to the state of neural control and maintenance and therefore are secondary to the primary impairments. These mainly refer to the muscle and tendon structural (architectural, material and biomechanical) impairments, which can present themselves by a decrease in functional range of motion about a joint (contracture), and the increased muscle-tendon unit (MTU) resistance to stretch (i.e., increased stiffness). Secondary impairments are deemed preventable and/or treatable, as most interventions within the population are centred on improving the functional capabilities of the population by decreasing the effects of secondary impairments(Jeffries et al., 2016). Although decreased force production is in fact a primary impairment of CP, it is often treated as a secondary impairment due to its known changes over time which may be due to the

reduced level of activity and development of contractures leading to the reduced level of physical activity (Rimmer, 2001).

Jeffries et al., (2016) investigated the distribution of primary and secondary impairments across a functional spectrum of children with CP. They used three different scales to assess primary impairments (Modified Ashworth Scale, Early Clinical Assessment of Balance, Gross Motor Performance Measure), and three scales to assess secondary impairments (Spinal Alignment and Range of Motion Measure, Functional Strength Assessment, Early Activity Scale for Endurance). Scores from all six measures were compared then to the Gross Motor Function Classification System (GMFCS) scores using a one-way analysis of variance. This study found differences in primary and secondary impairments among GMFCS levels, with increasing severity of impairment as GMFCS levels increased, validating that children with greater functional limitations exhibit greater primary and secondary impairments. However, differences between GMFCS levels and primary and secondary impairment scores varied greatly; specifically, there was no difference in secondary impairment scored between children in GMFCS levels II-III for any of the three scales(Jeffries et al., 2016). The group attributed this to the required differentiations between GMFCS levels II and III in young children; as the differences in GMFCS are clear compared to the subtle differences that may not have been captured by the measures of strength, stability and range of motion used in this study. The team also attributed a lack of differences between GMFCS levels and scores of primary impairment assessments to the sensitivity of the tools used. The significant finding of this paper was the demonstration of children as young as 18 months exhibiting secondary impairments, even at GMFCS level I, and that there were significant differences in impairments amongst GMFCS levels. Therefore, it could be necessary to begin treatment of secondary impairments as early as 18 months old, and different approaches to treatment could be required depending on the GMFCS level of the individual. More aggressive treatment techniques and beginning treatment earlier in life could be more beneficial for those with GMFCS levels IV and V. Researchers and healthcare professionals should be aware that a singular approach to treatment may not be beneficial to everyone within the population, as the severity of secondary impairments vary.

Muscle Force and Ability to Produce a Net Torque

The structure of spastic muscle is also altered within the population, which has a direct effect on the movement of the joint segment. The ability to produce a net torque requires the production of a muscular force to rotate the body segment around the joint. In CP, the ability of the individuals to be able to produce a net torque is limited, due to primary neural impairments, and secondary changes in the muscle and tendon architecture. In the next section, the literature on the primary and secondary impairments will be discussed, and their influence on CP individuals' ability to produce a net torque.

Neural Manifestations of CP: Motor Unit Recruitment

As stated before, there are many suggested causes of neural origin for the decreased muscle force and torque production ability within CP population, such as presence of spasticity, and lack of selective muscle recruitment. A suggested result of dysfunctional movement within the population is spasticity, which is defined as the velocity dependent increase in tonic stretch reflexes(Lance, 1980a). Spasticity (neural hypertonicity) is manifested by a decrease in the threshold of tonic stretch reflex of the muscle (where the muscle is reflexively activated at relatively shorter lengths), and an exaggerated response to the stretch (higher gain of the reflex where the muscle produces larger force in response to the induced elongation). This is different from biomechanical hypertonicity, which is an increased resistance to stretch due to alterations in the mechanical properties of the muscle-tendon unit, such as the shortening of the muscle fibres(Sanger et al., 2003b). However whether spasticity is a negative contributor to disability has been debated amongst researchers; many are of the opinion that spasticity is an important contributor to disability in CP (Bonow et al., 2018), and that it only needs to be treated when the resultant muscle tightness causes pain or impaired function (Roy and McLaughlin, 2009). Others believe that spasticity is allows for increased trunk control, standing and ambulation, and therefore should be maintained or even facilitated (S. et al., 2020). As spasticity results in changes in soft tissue and skeletal muscle, it is natural to conclude that interventions aimed at reversing these muscular changes would in turn, reduce spasticity in the targeted muscles.

Trompetto et al., (2014) characterised spasticity in the population as a disorder of the stretch reflex that is manifested as increased muscle tone, which becomes more apparent when the muscle is stretched (Trompetto et al., 2014). This study described the clinical manifestation of spasticity as a result of an upper motor neuron disease. The researchers explored the phenomenon in relation to its dependence on the velocity of the stretch, as well as the length of the muscle at the time of stretch. The quadriceps for example, display greater spasticity when the muscle is stretched at a longer length compared to a shortened length, and as a result of this, the resistance to stretch is greater. The clasp knife phenomenon is an example of a display of resistance to stretch and stretch reflex occurring; as the muscle is stretched initially from a short length, the muscle resists to a certain point, at which it then suddenly gives way and allows the stretch to lengthen the muscle until it reaches peak extension. This could also be due to the excitation of higher threshold muscle receptors within the quadriceps. In direct opposition to upper limb flexor muscles, the triceps surae, a lower limb flexor muscle, experiences greater stretch reflex at shorter muscle length. Trompetto and colleagues assessed the correlation between the muscle activity, measured by surface electromyography, and the velocity of the stretch; they found that when the velocity of the passive stretch was low, the stretch reflex response was also low, and this stretch reflex increases as the velocity of the stretch is increased. There is also observed increase in muscle tone at higher velocities. This study presents the idea that spasticity in upper motor neuron diseases is due to an exaggerated stretch reflex.

Co-contraction of the antagonist muscles (inability to recruit appropriate motor units) is also a factor commonly reported that impairs muscular function; co-contraction causes reductions in the torque produced by a muscle. This will result in motor skills, such as walking, not being performed optimally.

Ostensjo et al., (2003) assessed spasticity, ROM, and motor control in children with CP, and found an inverse relationship between the level of spasticity in the lower limb muscles, and selective control of the ankle joint. Children with less spasticity had a greater ability to selectively activate the tibialis anterior for dorsiflexion, compared to those with more spasticity, who relied predominantly on the toe extensors. Although the researchers emphasised that this could reflect a correlation between spasticity and the underlying cause

of motor deficit (Ostensjo et al., 2003), reduction in spasticity has been a treatment goal for the management of CP.

Another way inappropriate motor recruitment limits muscular power can be attributed to a low frequency (rate) of muscle recruitment (Hong et al., 2014). According to Henneman's size principle, the force of a contraction is regulated by the number and type of motor units recruited. Therefore progressively increasing recruitment enables muscular force to increase in a stepwise fashion (Jones et al., 2004). However, recruitment is not the only factor limiting motor unit function; both rate and frequency of firing, and synchronisation on firing impact the efficiency of motor unit function (Rose and McGill, 2005). Rose and McGill (2005) studied the neuromuscular activation and motor unit firing in CP and reported that with the same level of neuromuscular activation, there was no difference in the motor unit recruitment or rate of firing. There was however, a decrease in short term synchronisation in participants with CP compared to TD participants, reporting this as the key limiting factor with regard to muscular weakness due to motor unit function.

As stated before, neural hypertonia (spasticity) is a manifestation of CP, in which the soft tissue adaptation is mediated by the velocity-dependent exaggerated response to stretch reflex (spasticity). However, increased passive stiffness of the muscle/MTU, commonly referred to as intrinsic (biomechanical) hypertonia, can also be observed in CP. Contrastingly to spastic hypertonia, intrinsic hypertonia is not related to the velocity of the stretch, and it can often be difficult to distinguish between the two subcategories in a clinical setting. Hypertonia, as a general term, is defined by Sanger et al., (2003)(Sanger et al., 2003a) as an atypically increased resistance to a passive movement that is externally applied to a joint, but it does not differentiate between the two sources of the increased resistance and does not indicate whether there is an involuntary (reflex) contraction at rest. Sanger et al., (2003) also indicated that although an increase in muscle tone affords increase functional abilities in children with neuromuscular disorders, severe hypertonia is often associated with contractures, which severely disables the joint. The inability to voluntarily and appropriately contract a muscle about a joint not only decreases the force production capabilities by altering the length-tension relationship of the muscle and tendon, but also reduces the

functional abilities of the individual, as ROM about a joint is often essential to perform tasks in everyday life.

Musculotendinous Manifestations of CP: Changes in Muscle and Tendon Architecture & Mechanical Properties

The morphology and mechanical properties of a muscle-tendon unit is of great importance when generating muscular force, and changes in the structure and properties of the muscle and tendon are suggested to contribute to the reduction in functional force producing capability of the spastic muscles. The primary representatives of force-producing capacity are the force-length and force-velocity relationships.

The magnitude of muscular force is correlated to the active motor unit size and physiological cross-sectional area of a muscle. As it is widely reported that the muscle volumes are greater in typically developed (TD) individuals than in those with CP (Noble et al., 2017), it is accepted that those with CP produce less force during contraction (Barber et al., 2011c). However, in both TD and CP subjects, passive structures such as tendons produce the majority of the positive mechanical work. The differentiation between TD and CP tendon function is that in TD individuals the tendon produces more work to reduce the metabolic cost of walking (Lieber and Fridén, 2000). Due to muscular stiffness and co-contraction in CP, the majority of the work produced during walking is also produced by the tendon to compensate for the inability to produce work by the muscle, as CP individuals have increased tendon compliance compared to their TD peers. Fukunaga et al., (2001) studied the in vivo muscle tendon behaviour during walking in TD and CP subjects, and compared the differences in tendon length during the stance phase. The results showed that during the single support phase of stance, the tendon length increased by up to 2 % in those with CP; however in TD participants the tendon length was maintained until the end of the single support phase, at which point it decreased slightly (Fukunaga et al., 2001). This study also compared heel-toe walking and toe walking in TD subjects, and found that the percentage change in muscle belly length was lower in toe walking than in heel-toe walking. As toe walking is common for those with CP, it is concluded that because muscle-tendon

interaction is altered due to stiffness and co-contraction, the muscle belly of the GM muscle can be exposed to eccentric lengthening during locomotion.

Differences between the muscles of individuals with CP and TD individuals can lead to the development of joint contractures. Contracture is the presence of limited range of motion about a joint due to increased stiffness of surrounding muscles. Changes which increase the passive stiffness of the muscle can contribute to the development of contracture over time (Smith et al., 2011a). Contractures are commonly reported complications as a result of lesions within the central motor pathways present in CP, stroke, and spinal cord injury. It is suggested that contractures occur as a result of unusually high muscle activity due to spasticity (Gracies, 2005). However, the pathology underlying contracture is still debated, as it is not yet clear whether increased stiffness is due to elastic elements within muscle fibres, extracellular matrixes or both. As contractures can cause joints to gradually become fixated in awkward positions, the extent to which structural changes to the muscle fibres, such as reduction in the length and number of sarcomeres, and how these changes contribute to the gross muscle anatomy and joint positions, is still unclear. This could be due to the current difficulty in measuring tissue stiffness and muscle fibre lengths in vivo (Mathewson and Lieber, 2015)

Pingel et al., (2016) suggested that contractures were an adaptation in tissue homeostasis in the neuromuscular-tendon-connective tissue complex induced by the lesion. As individuals with CP possess smaller muscles, in both muscle belly length and cross sectional area, it appears that this is related to a decrease in muscle growth caused by reduced use of the muscle and neural activity (Pingel et al., 2016), and a decrease in the number of satellite cells in CP individuals (Dayanidhi et al., 2015). The hypertrophy and atrophy of a muscle is mainly dependent upon the diameter of the muscle fibres, specifically the number of sarcomeres in parallel. However due to the pennate nature of some muscles, such as the soleus, the diameter of muscle fibres also contributes considerably to the length of the muscle. Because of this, muscle atrophy may cause muscles to be too short in relation to the adjoined bone length, resulting in increased stress on the muscle fibres, eliciting the development of contractures (Gough and Shortland, 2012).

Strength is a significant contributor to motor control, and children with CP have decreased strength (Damiano and Wiley, 1998). However, shifts in the muscle-tendon unit (MTU) length-tension relationship could also be a contributing factor to what appears to be a deficit in force production (Bartlett and Palisano, 2000) during movement. Contractures may interfere with the length-tension relationship, and therefore the force producing capacity of a muscle; the involuntary shortening of the muscle may place the MTU at length that is suboptimal for producing forces necessary for functional activities. It has been reported that CP individuals have longer sarcomeres than their TD peers (Mathewson and Lieber, 2015), and when combining this increase in sarcomere length with an increase in muscle stiffness, the outcome results in an increase in the stiffness of muscle contractures (Smith et al., 2011b). This tells us that contracture is the result of multiple factors, all of which lead to higher passive strain on the muscle. Over time, this constant strain has resulted in injured muscle, which directly reduces the contractile force, and may reduce the tension producing capabilities of the muscle. Due to the variation within the population, the combination of contractures and spasticity, which lead to the increased strain and decreased ability to produce tension, it is unsurprising that functional abilities are reduced across the population. It is clear, however, that interventions to reduce the symptoms of these issues are needed.

Taking into consideration the intertwined nature of the neural and musculoskeletal factors, which can cause or are correlated with the clinical manifestations of the CP, the following section attempts to provide a theoretical underpinning of stretching as the most commonly employed intervention for the rehabilitation of individuals with CP.

Stretching Interventions in CP

There are four behavioural properties of muscle tissue: tension, elasticity, extensibility and excitability, all of which are altered in individuals with CP. Excitability refers to the muscles ability to respond to a stimulus such as a hormone or motor neuron, and tension is the ability of the muscle to forcibly shorten (Whitehead et al., 2001). Contrastingly, extensibility is the ability of the muscle to be stretched or extended, and elasticity is the ability of the muscle to return to its original resting length after being stretched. Other descriptors of

muscle state and determinants of its response to an external load include its passive and active stiffness. Passive muscle and muscle-tendon unit stiffness (to differentiate between the responses of a muscle in isolation or in series with its tendon, to a stretching force) refers to the ratio between the change in stretching force (or load) and the change in muscle or muscle-tendon unit length when the muscle is not active. Active muscle and MTU stiffness represent such change in an active state of the muscle. Active and passive stiffness can influence muscle and MTU extensibility and elasticity, and hence, are important in the control of posture and function (Komi, 2011).

Passive stretching (e.g. applied by passive rotation at a joint) results in stress on the MTU, and such stress leads to deformations characterised by a stress-strain curve of the stretched tissue (Komi, 2011). A passive muscle is significantly more compliant than the connected tendon, and therefore muscle fascicles/fibres will receive the majority of the mechanical straining load generated during a bout of passive stretching. However, the magnitude of stress muscle fibres will undergo is dependent on their orientation relative to the longitudinal axis of the muscle, or the angle of pennation in addition to the fibre's cross-sectional area. When the ankle is stretched passively, stress applied to the Achilles tendon is passed on to the muscle fascicles of the GM, however this depends on how compliant the tendon is; increased tendon compliance which is common in CP, results in increased strain on the tendon, and less strain being transferred to the muscle (Theis et al., 2016). When a muscle-tendon unit undergoes passive stretching, it deforms according to its material properties, however this deformation occurs in a time dependent manner; when a low velocity of stretching occurs over a prolonged period of time the deformation is known as 'creep'. Creep is the result of viscoelastic properties of the muscle and tendon, and when the stretch is no longer being applied, the muscle will return to its original length, in a time dependent manner (Glantz, 1974). A structural protein within the muscle, titin, is suggested to be the major determinant of the elastic properties of the muscle fibres, and contributes to the passive resistance of the muscle (Whitehead et al., 2001).

It is widely accepted that the factors most limiting movement in those with spastic CP include shortness of the MTU (Kruse et al., 2018) and increased muscle, MTU and fascicle

stiffness (Barber et al., 2011a, Fridén and Lieber 2003, Smith et al., 2011a). This impairs function and movement in the affected limbs with varying severity. Because of this, the majority of rehabilitative interventions performed on individuals with CP have the objective of increasing muscle, MTU, and fibre lengths and reducing stiffness with the use of passive stretching.

Although there has been some debate amongst researchers about the shortness of muscle fibre length in CP relative to the typically developing individuals (TD), some studies have found shorter fascicles in CP individuals, such as Mohagheghi et al., (2007, 2008)(Mohagheghi et al., 2007, Mohagheghi et al., 2008). The group found a significant decrease in spastic muscle fascicle length of the gastrocnemius compared to the unaffected lower leg and TD peers respectively. This shortness was suggested to be due the reduction of sarcomeres both in series and in parallel, as atrophy occurs when the muscle is disused at short lengths. This disuse may be due to spasticity, which leads to the presence of contractures, which as stated above, causes reduced ROM and extensibility. Opposing this view, Barber et al., (2011) and Malaiya et al., (2007) both found no reduction in the muscle fascicle length of CP individuals compared to their TD peers (Barber et al., 2011c, Malaiya et al., 2007).

Many animal studies have investigated the disuse of the muscle, and how maintaining shorter muscle lengths over a prolonged period leads to a decrease in the number of sarcomeres in series and in parallel (alongside each other, resulting in an increase in pCSA); Wirtz et al., (1988) investigated the effects of immobilising the hind leg muscles of mice with and without muscular dystrophy (Wirtz et al., 1988). This study found that when immobilising TD mice calf muscles for a week, the muscular atrophy of the calf muscle was approximately 35 % when compared with their non-immobilised limb, and that the muscle fibre number had decreased by 15 % compared to the non-immobilised limb. Interestingly, when the calf muscles were remobilised, they resumed normal development, and were at the expected growth rate 3 months after the immobilisation protocol had occurred. Dystrophic mice showed an increase in atrophy in agonistic and antagonistic muscles; however, this was significantly less than their TD counterparts. This study directly indicates that maintaining a muscle in a fixed position for as little as a week can have detrimental

effects to the growth of a muscle; the atrophy and reduction in muscle fibre length show a decrease in the cross-sectional area of the muscle and muscle thickness due to a reduction in the number of sarcomeres in parallel and in series. If, like in CP individuals, this immobilisation had been maintained for a long period of time, the effects on the muscle could result in the muscle being permanently shortened.

Such effects have also been shown in humans by Grosset et al., (2008), who measured quadriceps, hamstring and triceps surae muscle volumes using magnetic resonance imaging (MRI) before and after a four week immobilisation period (Grosset and Onambele-Pearson, 2008). They found decreases in muscle volumes after the 4-week period immobilisation period of 24.1 %, 6.5 % and 21.9 % respectively. This group also measured the same muscle volumes following a two-month recovery period, and found that the quadriceps and triceps surae muscles were still 5.2 % and 9.5 % smaller than before the immobilisation period, whereas the hamstrings had increased by 2.7 % compared to before the immobilisation. This indicates that not only do the quadriceps and triceps surae lose muscle mass at a quicker rate than other muscles, they also require a longer to increase recover after atrophy occurs. This could have detrimental effects to our target population of CP individuals, as both of these muscle groups are often the target of interventions as they are not only reportedly smaller than their TD peers, but they are necessary for maintaining balance and walking ability.

Due to this shortness of the muscle, many interventions have been undertaken by researchers to specifically reduce the effects of muscle shortness, such as contracture and reduced ROM, by using passive stretching. One of several studies that have performed these interventions, such as a study by Theis et al., (2016) examined if acute passive stretching increases muscle and fibre length in children with CP (Theis et al., 2016). While this study acknowledged that it is not clear if the common MTU length changes experienced as a result of stretching are due to lengthening of the muscle or tendon, it did however, find that muscle and fascicle strain increased immediately after stretching. This caused an increased ROM about the joint. This study is one of many that have successfully shown that stretching interventions increase ROM immediately after stretching, and may acutely decrease MTU stiffness by increasing the muscle and fascicle strain, however this does not account for the

long-term effects of such interventions. Increased ROM directly after a passive stretching protocol can also be explained by the viscoelastic properties of the muscle, and short term adaptation in the extensibility of the muscle (De Deyne, 2001).

Despite the importance of increasing muscle and fibre length on improving muscle function, majority of work supporting the positive effect of stretching on increase muscle fibre length involved animals. These studies have shown that the muscular response to passive and intermittent stretching is the addition of new sarcomeres in series (Holly et al., 1980).

This was also found by Coutinho et al., (2004) who investigated the effects of passive stretching on the soleus muscle. The researchers used 18 rats, which were split into three groups: The first group spent the period with the soleus immobilised, the second underwent immobilisation and a stretching protocol, and the third underwent just the stretching protocol. The duration of this study was three weeks, in which time the second and third groups underwent stretching three times per week for a duration of 40 minutes. At the end of the three-week period, the soleus muscles were dissected from the rats and divided longitudinally; the medial section was used for histological measurements, and the lateral section was used for sarcomere measurements. The number and length of sarcomeres was measured, as well as the muscle fibre cross sectional area. They reported muscle fibre atrophy (decreased weight, length, serial sarcomere number and muscle fibre area) in both groups that underwent immobilisation, but no change in the group that only underwent stretching. Moreover, they found a significant increase in the soleus length of the stretching only group, but they found a decrease in the length of both immobilised groups, although this wasn't significant. Interestingly, in the stretching only group, they found an increase in the number of sarcomeres in series, but no change in sarcomere length; the immobilisation and stretching combined group had no change in the number of sarcomeres in series, but an increase in the length of the sarcomeres. The study concluded that stretching and immobilisation applied three times per week was sufficient to reduce muscle atrophy, but did not prevent muscle shortening, suggesting that stretching regulates serial sarcomere number and cross-sectional area of a muscle differently. They also acknowledged that more research was needed to understand why stretching caused significant protection of the

cross sectional area, but did not prevent the loss of the number of sarcomeres in series (Coutinho et al., 2004b).

Williams (1990) aimed to prevent the loss of serial sarcomeres using intermittent stretching in mice; attributing the loss of serial sarcomeres to a short muscle that has adapted to the change in functional length, increasing the tension, resulting in the length tension curve being shifted to the left. This is similar to participants with CP, who possess short muscles. As Williams found that stretching for just 30 minutes a day could not only prevent most of the atrophy associated with immobilised short muscles, it could also increase the number of sarcomeres in series, many interventions within the population are aimed at this phenomenon, with the overall aim of gaining functional improvements (Williams, 1990).

Replicating findings of the animal studies in humans has been less successful. Investigating the effects of active and passive stretching on muscle length, Riley et al., (2012) found that there was no conclusive evidence demonstrating that passive stretch alone can increase muscle length in humans. They were also aware that passive stretch may induce the addition of sarcomeres if the muscle fibres are lengthened sufficiently. They presented the limitations of stretching in vivo as physiological range of motion, and stretch pain tolerance. As it is clear that children with CP have reduced range of movement, it appears that to meaningfully administer passive stretching, muscle length and pain tolerance need to be increased (Riley and Van Dyke, 2012).

Regardless of the effect of stretching on increasing the length of muscle fibres in humans and in specific in those with CP, increased ROM has also been found immediately after passive stretching, which can be explained by the short-term adaptation of the viscoelastic properties of the muscle, as well as changes in the extensibility. De Deyne (2001) also explained that the increased ROM gained after passive stretching rehabilitation is maintained after stretching is removed, suggesting an adaptive response to stretch (De Deyne, 2001). For chronic longitudinal adaptation to happen in spastic muscle fascicles with stretching or training (maintained or repeated joint rotation), fascicles should experience positive fibre strain during elongation of the MTU. Evidence for positive strain of spastic muscle fascicles during acute stretching of the MTU is available in the literature (Hussain et

al., 2013, Theis et al., 2015, Barber et al., 2012, Burke et al., 2013). The relative stiffness of the muscle and tendon determine how much of the load is applied to the muscle fibres during exercise, both one off sessions and longitudinal training. However, individuals with CP possess stiffer muscle fascicles and more compliant tendons (Barber et al., 2011d, Kalkman et al., 2019), which may result in the load stretching the tendon as opposed to the muscle fascicles as desired (Theis et al., 2015). Therefore, muscle fascicles may not be adequately stretched to allow sarcomerogenesis to be induced, limiting the benefits of such interventions (Bar-On et al., 2018). The general outcome of stretching studies involving spastic MTUs, is that stretching exercises of appropriate duration has generally led to the short-term maintenance and increase of the joint ROM (Pin et al., 2006, Theis et al., 2015, Coutinho et al., 2004a), but magnitude of the associated increase in the length of muscle and fascicle during stretch or following intervention was not quantified in all studies. Hosl et al., (2015, 2018) found that ankle dorsiflexion during gait improved in response to static stretching despite a decrease in FL (Hosl et al., 2015, Hosl et al., 2018). Theis et al., (2015) found a significant increase in ankle ROM post stretching intervention, but no alteration in FL. Inadequate stretching of the fibre during stretching interventions in human studies can be a contributing factor to the failure of these interventions for increasing muscle and fascicle length, and potential improvement in function (Theis et al., 2015).

Strength Training Interventions

In addition to stretching interventions, strength training is suggested to improve function in CP. Strength training is the use of resistive loading on skeletal muscle with the intention of increasing muscular power, strength and size (Kawakami, 2005). However, these effects are widely dependent on the morphology of the muscle: the organisation and placement of the sarcomeres within the muscle fibres, and placement of the muscle fibres within the muscle. Nevertheless, the use of strength training is reported to increase the size of the muscle fibres, and therefore cause an increase in the size of the muscle in healthy adults. This is measured by using ultrasound or magnetic resonance imaging to measure the thickness and the cross-sectional area of the muscle. It has also been reported that the increased volumes resulting from strength training are an indicator of joint functionality and torque production (Fukunaga et al., 2001).

The use of strength training to increase muscle size and volume of the lower limbs is specifically useful to those with CP. It has been observed throughout many studies that the muscle size, particularly the gastrocnemius (GM), of the affected limb is smaller than the unaffected limb in hemiplegic CP. This difference can be as great as 50-75 % reduction in GM muscle volume compared to the unaffected limb (Malaiya et al., 2007). The result of this could be detrimental to the performance of everyday activities such as walking. It has also been observed that people with CP possess an increased volume of intramuscular and extra-muscular connective tissue. These two observations put together equate to a shorter muscle and longer tendon, which impacts not only the contractile force but also the range of motion (ROM).

Some studies investigating the effects of strength training in CP have observed this issue, and have come to the assumption that by increasing the size of the GM in the affected limb, the muscle length may also be increased, and reduce joint stiffness by increasing range of motion as a result (Kubo et al., 2002). The study by Barber et al., (2011) found that the reduction in muscle volume paralleled a reduction in fascicle length, which decreases contractile power (Barber et al., 2011c). In theory, resistance training on the affected muscle(s) could cause physiological and psychological adaptations that could overcome the reduced muscle volume and fascicle length observed.

It is a well-known fact that performing regular strength training will cause adaptations, both physical and neural. System level adaptations are those that occur at a particular system within the body, such as the muscle and connective tissue, or the brain and the central nervous system. Both the neural and muscular systems impact the level of adaptation achievable from strength training. Noble et al., (2014) undertook a study assessing lower limb volumes in TD and CP children using MRI scans of both legs to effectively compare the muscular volumes relative to body mass. This is one of the few studies on relative muscle volume in individuals with CP, which reports that in CP the lower leg muscles show a 27.9 % average muscle volume deficit (Noble et al., 2014). They also found that the deficits were significantly greater in the distal muscles than the proximal. This particular study concluded

that the deficit in lower limb muscle volumes, coupled with commonly reported sarcopenia in adults, may contribute to the early loss of mobility in adults with CP.

It is widely recognised that a common factor causing stiffness in the GM muscle is decreased muscle belly and fascicle length, and increased tendon compliance. One study in particular, researched by Barber et al., (2011) examined the medial GM muscle volume and fascicle length in children with CP. The study found that there was a significant difference in muscle belly volume and cross-sectional area, including decreased fascicle length between participants with spastic CP and those with TD. The researchers also reported a decreased ROM in CP compared to TD subjects, with a maximum of 8° dorsiflexion compared to TD subjects with a maximum of 22 ° (Barber et al., 2011c).

A specific change that could impact the function of the gastrocnemius is changes in the muscle morphology. Many studies that use resistance training as an intervention for individuals with CP report these changes. McNee et al., (2009) undertook a study investigating the increase in muscle belly volume after plantar flexor strength training in CP, which involved four training sessions per week over ten weeks (McNee et al., 2009). Maximum passive plantarflexion and dorsiflexion were both tested, as well as the use of 3D kinematics during barefoot treadmill walking at a self-selected speed. Although no statistically significant increase in function was observed after the twelve-week follow-up, there was a significant increase in medial and lateral GM muscle volumes of 17 % and 14 % at week five; this was maintained at week ten and at follow-up. The mean number of calf raises also increased after five weeks, and was maintained at ten weeks and twelve weeks. While the increase in function was not found to be statistically significant, it is evident that GM strength increase and muscle belly volume increased as a result of strength training, which will cause an increase in contractile force of the GM muscle, decreasing this limitation of muscle capacity. These improvements in muscle force producing capacity as a result of strength training, represented by increased muscle volumes, may eventually alter the length-tension characteristics of the MTU, and therefore increase the functional abilities of the subjects.

In addition to improving strength, resistance training might affect muscle architectural parameters. Fukutani and Kurihara (2015) investigated changes in fascicle length between individuals who underwent resistance training, and untrained individuals. This study's primary finding was that here was no significant difference in muscle fascicle length between the trained and untrained individuals, and that fascicle length alteration was not associated with muscular hypertrophy. This study concluded that resistance training during which muscles were undergoing shortening contractions, did not increase muscle fascicle length (Fukutani and Kurihara, 2015). This was controversial as studies by Lynn et al., (1994) and Butterfield et al., (2005) found a greater addition of sarcomeres in series in rats after downhill, eccentric exercise compared with uphill running, and suggested differential effect of different modes of contraction on muscle architecture in general and fibre/fascicle length in specific (Lynn and Morgan, 1994b, Butterfield et al., 2005a).

This was further supported by observations made in other animal and human studies which also investigated the architectural response of the muscle to resistance training. The number of studies that report no increase in fascicle length in response to resistance training is narrow (Blazevich et al., 2007b, Erskine et al., 2010) in comparison with studies that demonstrated an increase in fascicle length after resistance training, isokinetic training or marathon training (Franchi et al., 2016), and the difference could be attributed to the differences in the mode of contraction and condition of exercise.
However, the definition of resistance training is often considered insufficient; it is generally referred to as the magnitude of the load, number of repetitions, sets, rest periods, and number of intervention/contact hours within a weekly period. Toigo and Boutellier (2006) identified new determinants of the effects of resistance training on skeletal muscle, with the direct aim of identifying why the current definition of resistance training is insufficient. The determinants they establish are comprised of time under tension, muscular failure, ROM and recovery time, amongst others. They outline 13 mechano-biological determinants, including load magnitude, time under tension, ROM, and type of exercise, which are to be followed when prescribing or undergoing future resistance training protocols, to reduce subjective definitions and inconclusive findings (Toigo and Boutellier, 2006).

This is supported by Reeves et al., (2009) who investigated different adaptations to concentric versus eccentric training in healthy adults, and found that architectural changes including an increase in muscle fascicle length, are somewhat contraction specific. The increase in muscle fascicle length was significantly greater in the eccentric exercise group compared with the concentric exercise group, demonstrating that eccentric training increases muscle fascicle length via the addition of sarcomeres in series (Reeves et al., 2009b). Opposing this, they found that concentric training promoted an increase in pennation angle, suggesting an increase in the number of sarcomeres in parallel.

When investigating muscular response to eccentric exercise, many research groups have established that the addition of sarcomeres in series, and therefore increasing muscle fascicle length, appears to be a main method of protecting the muscle against exercise induced muscle damage (EIMD)(Morgan and Talbot, 2002). The increase in the number of sarcomeres in series also leads to secondary adaptations, such as a shift in the optimum length of the muscle to produce tension (Proske and Morgan, 2004). This is useful for individuals with CP, as alterations in the length-tension relationship is a possible contributing factor to their limited force production ability.

It is clear that the increase in the number of sarcomeres in series as a result of strength training is a positive adaptation, which can alter muscle extensibility and the length tension characteristics of the muscle, as well as aid in the prevention of muscular atrophy. However, the best practice (e.g. contraction mode, volume and speed of training) for achieving increase in the muscle fibre length and evidence of the functional improvements as a result of such adaptations is not yet clear. Functional improvements such as walking, as well as investigations into increasing muscle size within the population are common individually, but not combined. It is generally accepted that resistance training is an effective way of improving muscle strength in individuals with CP, but without functional improvements the interventions may not be as effective as once thought. When investigating the effects of a 12 week combined (aerobic and strength) training programme on muscle properties and gait deficiency in young people with CP, Gillet et al., (2015) attributed the ineffectiveness of strength training to improve function to the mismatch between the recommended

prescription of strength training from The National Strength and Conditioning Association, and the exercise protocols used in research (Gillet et al., 2015).

An investigation into the therapeutic effects of strength training on gait and function in individuals with CP was undertaken by Lee et al., (2008). Participants underwent a five-week strength training intervention, with functional measures such as three-dimensional gait analysis, Muscle tone and strength of lower limb, Gross Motor Function Measure, lateral step up, and squat to stand were all measured before and after the intervention as well as at a follow up six weeks later. Hip extensor strength and the number of squat to stand repetitions increased significantly post intervention, as well as a significant increase in stride length and gait speed. This study concluded that strength training interventions could be a useful method of improving functional capabilities including gait in individuals with CP (Lee et al., 2008).

Contrastingly, a study that assessed the effectiveness of strength training in muscle strength and mobility was undertaken by Scholtes et al (2010) and found that although strength training did in fact increase strength, there were no observed functional improvements in mobility (Scholtes et al., 2010). The study randomised fifty-one individuals with CP into exercise and control groups, and compared them before and after a twelve-week training programme. Functional measures were assessed; muscle strength using a hand-held dynamometer, mobility using Gross Motor Functioning Measure, and spasticity using the appearance of catch. The group attributed the lack of functional improvements to the lack of specificity of the training programme; the exercises chosen did not effectively work the desired muscles enough to result in improvements in function.

A similar study that also assessed the effects of mobility in relation to strength training also found that increased strength did not lead to improvements in mobility, but it did increase participant-rated mobility scores (Taylor et al., 2013). Whether this is the result of a placebo effect was not made clear. It is quite clear, however, that there are improvements as a result of strength training in the quality of life of the participants, but there is not yet enough evidence to support the idea that strength training can lead to long term functional improvements; this warrants further investigation. As posed by Gillet et al., (2015) the lack

of functional improvements could be the result of a lack of vigilance when creating a training programme, and adhering to the recommended guidelines. It could also be the case that specific criteria within the training protocol must be met to insure adaptations that will lead to functional improvements such as gait and balance (Gillet et al., 2015). This indicates that training does not only have to be specific in terms of load and intensity, but also action specific, to insure there is a positive transfer of learning from the training action to everyday activities.

Importantly, it is clear from the current literature that not all training results in increased muscle fascicle length; this has been reported by Sharifnezhad et al., (2014) who investigated the effects of eccentric loading on the longitudinal growth of the Vastus Lateralis (Sharifnezhad et al., 2014a). The participants were split into two groups which either underwent eccentric training with different loads (60 % and 100 % of maximum isometric force) or at different angular velocities (90 °/s and 240 °/s). Muscle fascicle length and moment-angle relationship were measured before and after a 10-week training programme, and were compared to an untrained control group. This study found that only one of the four training parameters induced an increase of 14 % in VL muscle fascicle length: the group that underwent training at lengthening velocity of 240 °/s. This is a direct indicator that although eccentric training has the capability to increase muscle fascicle length, not all types of eccentric training can do this. Of the four training conditions, the only one associated with an increase in FL was the fastest lengthening velocity. This condition was also associated with a drop in the knee extension moment toward the end of the eccentric contraction cycle, which could not be explained by the muscle functioning in its descending part of force-length relationship. The researchers defined this drop in the joint moment as a sudden, unexplained drop in EMG activity, resulting in a drop in active muscle force; this was defined as a momentary deactivation of the muscle. In agreement with earlier work in animals by Butterfield and Herzog (2005), Sharifnezhad et al., (2014) argued that the rapid lengthening velocity of the fibres in the descending part of muscle force-length relationship in combination with muscle momentary deactivation at this stage may elicit muscle damage. The muscle damage could lead to sarcomere instability and homeostatic perturbation that would facilitate sarcomerogenesis and elicit longitudinal plastic changes in the length of fascicles.

In neurologically impaired populations, resistance to stretch caused by reflex contraction of the stretched muscle may give way momentarily, which is known as the clasp-knife phenomenon (Turpin et al., 2016, Burke et al., 1972). This could be similar to the momentary deactivation of the healthy muscles during high-velocity eccentric contractions, which was claimed by Sharifnezhad et al. (2014) to be a requirement for muscle damage inducing sarcomerogenesis. Taking part in eccentric training protocols requires active control of the targeted muscle group which may not be possible in clinical populations with spastic muscles, and limited or no selective motor control (e.g., CP, stroke, spinal cord injury). Alternatively, passive stretching of spastic muscles induces velocity-sensitive stretch reflex (Morris, 2007, Bar-On et al., 2014, Lance, 1980b). In these circumstances, moving the joint passively through its ROM at appropriately high velocities could be considered a form eccentric training, provided the stretch reflex occurs and the muscle maintains contraction. There is evidence for the varied electromyography response of the stretched muscles in individuals with CP, where in some individuals, muscle reflex contraction dropped directly after peak EMG activity during stretching (Bar-On et al., 2018). This deactivation occurred in individuals with spasticity alone, and individuals with spasticity and stiffness, but not in individuals with stiffness alone; these individuals may be particularly responsive to an appropriately high velocity (active or passive) eccentric training protocol for increasing SSN and FL.

This furthers the literature presented above about the need for specificity in training, and provides justification for the reason an increase in fascicle length in all studies that use eccentric training may not be observed. Following on from this, the use of eccentric training in CP could be a useful tool for increasing muscle fascicle length provided the training is specific, includes the 13 mechano-biological determinants, and occurs eccentrically at high muscle lengthening velocities, as presented by Sharifnezhad and colleagues.

Exercise Induced Muscle Damage

Strenuous physical exercise is one of the most common causes of muscle damage. Exercise induced muscle damage (EIMD) commonly occurs when untrained individuals undertake

strenuous exercise, particularly eccentric exercise. Symptoms of EIMD include a prolonged decrease in muscle force production, both voluntarily and electrically stimulated, increased muscular soreness, an increase in inflammatory markers, and an increase of proteins in blood plasma (Clarkson and Hubal, 2002). An investigation into the events that are responsible for the presence of EIMD was undertake by Proske et al., (2004); they suggest two contributors, the first being damage to the excitation-contraction coupling mechanism, and the second being disruption at the level of the sarcomeres (Proske and Morgan, 2004). This study presents the idea that EIMD is a defence mechanism the muscle employed post exercise to prevent future damage to the muscle from eccentric exercise. This is supported by the attenuation of the effects of EIMD after a second bout of eccentric exercise, which produces much less damage than the original first bout a week earlier. The researchers indicate that this attenuation in the effects of EIMD is due to an increase in the sarcomere number, which in turn shifts the muscles length-tension characteristics because it alters the muscle fibre. This study concluded that the alteration in sarcomere number after the initial exercise bout, coupled with the increase in passive tension, and the fall in active tension of the muscle post eccentric training, all indicate that sarcomere damage is the initial factor that causes EIMD.

As EIMD with eccentric exercise leads to sarcomere generation, and sarcomere generation is needed to increase muscle fascicle length, it is expected that those exercise interventions aiming to increase muscle fascicle length would design eccentric training to cause EIMD. However, not all eccentric training results in EIMD; as indicated by Sharifnezhad et al., (2014) eccentric training at high velocities of stretching both causes EIMD and an increase in muscle fascicle length (Sharifnezhad et al., 2014b). In fact, all studies that have induced an increase in muscle fascicle length, in both humans and animals, have used eccentric training at high lengthening velocities, and have reported symptoms of EIMD, including alterations in tension post exercise.

In a theoretical paper, Toigo and Boutellier (2006) commented on the cellular and molecular muscular adaptations as a result of RT. According to Toigo and Boutellier (2006), an increase in FL could be inferred from a change in the optimum angle for torque generation, which

occur alongside delayed onset muscle soreness (DOMS)(Toigo and Boutellier, 2006). The authors used “popping sarcomere hypothesis” to explain the findings. According to this theory, muscular damage occurs as a result of stretching sarcomeres in a non-uniform manner when an active muscle is stretched beyond its optimum length.

It should be noted that intensity of exercise a muscle can withstand is limited, and when loading exceeds this limit subcellular damage occurs. This results in the leakage of extracellular ions into the interstitial fluid, which are then returned into the circulation. These ions are referred to as biomarkers; their concentration in blood serum is measured as an indicator of the level of damage a muscle has undergone as a direct result of exercise. These biomarkers include lactate dehydrogenase, aldolase, troponin, and creatine kinase.

What is Blood Creatine Kinase?

Creatine Kinase (CK) is a compact enzyme found in the cytosol and mitochondria of tissues with high energy demands. It consists of two subunits, muscle CK and brain CK, which are further broken down into five isoenzymes, three of which are found in the cytoplasm: skeletal muscle (CK-MM), cardiac muscle (CK-MB), and brain muscle (CK-BB)(Baird et al., 2012a). Two isoenzymes are found in the mitochondria, which are sarcomeric and non-sarcomeric, and are referred to as macro-CK due to their large molecular size(Brancaccio et al., 2007). Macro-CK is used clinically for the prognosis of various conditions, as high levels of macro-CK are associated with cardiovascular and autoimmune diseases, or malignant conditions such as cancer. The type of CK subunits found at the site varies depending on the type of muscle; skeletal muscle contains approximately 98 % CK-MM and 2 % CK-MB, cardiac muscle contains 70-80 % CK-MM and 20-30 % CK-MB, whereas the brain is almost exclusively CK-BB (Cabaniss, 1990).

CK is a catalyst used to convert creatine and adenosine triphosphate (ATP) to phosphocreatine and adenosine diphosphate (ADP). This enzyme reaction occurs continuously throughout tissues that require a constant supply of ATP, a molecule used during muscular contraction (Cabaniss, 1990). Because of this, the presence of CK in plasma augments the contractility of skeletal and cardiac muscle. As CK is plentiful in tissues that

require constant ATP production, such as the heart and kidneys, measuring blood CK levels is commonly used as an indicator of tissue damage such as myocardial infarction, acute kidney failure, and muscular dystrophy. Although CK is no longer the primary method used for diagnosing myocardial infarction, higher levels of serum CK are still associated with muscle cell disruption and cell damage, as the cause of CK leaking from cells into blood serum can often be due to cellular disturbances (J. C. Cook, 1990). CK-MM binds to the M-line structure within sarcomeres of muscle tissues, which accounts for 5-10 % of all CK-MM. The M-line is a myofibrillar structure which connects the thick myosin filaments to each other, providing stability during muscular contractions.

Variation in CK Levels

Many factors are reported to influence CK levels in individuals; baseline levels alone can range from 20 to 16,000 u/L, which can be attributed to a number of factors such as medication, genetic factors, minor injuries, and physical activity levels (Baird et al., 2012a). The concentration of CK isoenzymes changes as age increases, with CK levels being higher in new-borns and decreasing into adulthood. Many report differences in resting CK levels between males and females, with higher values found in males in human and animal models. This gender difference is maintained after exercise, with oestrogen maintaining membrane stability, and therefore limiting the leakage of CK from damaged muscle cells (Amelink et al., 1988, Amelink et al., 1990). Some have also found differences in CK values between races, reporting that black males have higher resting values than white males (Brewster et al., 2012, Black et al., 1986); however other studies report no difference in CK levels between black and white athletes (Eliakim A, 1995).

Training status is a significant factor that influences both resting and post exercise blood CK concentration; with resting CK levels being higher in athletes than in untrained individuals, and greater increases in CK levels post exercise in untrained individuals due to increased muscular damage (Mougios, 2007). A study by Karamizrak et al., (1994) reported that when trained athletes and sedentary individuals perform the same exercise protocol, the post exercise serum CK is lower in the athletes when compared with matched control subjects (Karamizrak et al., 1994). The type, intensity and duration of exercise also influence the

concentration of plasma CK release and clearance; highest post exercise CK levels are reportedly from prolonged competitive exercise, such as marathon and triathlon events (Brancaccio et al., 2007). Resistance training and eccentric exercise are also associated with an increase in serum CK post exercise. Serum CK levels peak approximately eight hours after exercise has ceased and increases to double baseline levels. After eccentric exercise, muscle damage causes an increase in serum CK between two and seven days post exercise and peaks around 96 hours after exercise (Karamizrak et al., 1994). An attenuation of serum CK occurs if a second bout of exercise is undertaken, likely due to an increase rate of enzyme clearance. The isoenzyme found post exercise can also vary depending on the type of exercise undertaken; CK-MM is usually exclusively found in serum, however after strenuous prolonged exercise all three isoenzymes can be found in the blood serum. The decrease in serum CK after exercise can be attributed to the duration of rest post exercise, as physical inactivity, even for a short duration, may reduce both the release of CK from the muscle, and the transport of CK through the lymphatic system. Amino acid supplementation is another method of increasing the rate of CK clearance amongst athletes (Brancaccio et al., 2007).

Discrepancies in CK Measurement

Blood CK levels are a commonly used method of determining muscle damage after an exercise intervention; however other biochemical markers of muscle damage are usually measured alongside CK, such as lactate dehydrogenase, troponin, and aldolase. It has been reported that exclusively measuring serum CK may not accurately reflect structural damage to muscles as a result of exercise. Questions regarding the validity of using create kinase as a direct measure of exercise level and intensity are prominent in literature (Baird et al., 2012a).

As measurements of CK are taken from the blood, it is likely that they reflect complex interactions of proteins within the blood, which are associated with energy status and muscle disturbance, therefore measuring CK will reflect the concentration of CK released, degree of enzyme activity as well as the rate of clearance of CK from the serum [84]. However, measuring serum CK alone may not provide a fully accurate reflection of the

structural damage to the cell post exercise. One particular study by Fielding et al., (2000) reported that CK levels were affected by hydration status prior to exercise, and varied within subject groups of comparable volunteers (Fielding et al., 2000). However, it remains questionable as to whether increased CK levels in response to exercise represent a degree of muscle damage, or a form of disruption to the energy control process, or another molecular reaction. It is suggested increased CK after low to moderate intensity exercise is representative of a disruption to the muscle energy process, as opposed to the type of muscle cell damage that is characteristic of myocardial infarction, stroke or any other physical structural damage (Baird et al., 2012a).

Contrastingly, the validity of creatine kinase as a measure of muscle injury produced by lumbar surgery was measured by Kumbhare et al., (2008) who found that measuring serum CK was in fact a reliable measure of muscle damage as a result of lumbar surgery; albeit the muscular damage due to surgery would differ to that of exercise (Kumbhare et al., 2008). Measuring muscle CK as a minimally invasive and easy to register marker of muscle damage in studies involving eccentric training to stimulate sarcomere genesis could provide some indication for the success and rigour of the intervention employed to induce microscopic muscle damage which may trigger sarcomere genesis.

Based on the literature presented throughout this review, it is clear that our target population, individuals with CP, could benefit from an increase in muscle fascicle length, as they have shorter muscle belly lengths and reduced functional capabilities as a result. The work by Sharifnezhad et al., (2014) in particular, has been hugely influential in the justification of the proposed research (Sharifnezhad et al., 2014b). As stated above, the criteria for inducing an increase in muscle fascicle length is extremely specific, and therefore will be the focus of the present research in individuals with CP. Further, based on the mechanism of sarcomerogenesis, it is assumed that the training protocol will have to be severe enough to induce EIMD, resulting in the increase in sarcomere number post training. To date, there has been no success in increasing the length of the muscle or muscle fibres in CP. The literature presented above have had success in increasing muscle fibre length in animals or healthy individuals, all of which possess a common characteristic: the use of eccentric training. Upon further investigation, it has been presented that not all types of

eccentric training result in an increase in fibre length; with this in mind, the assumed requirements of training to induce an increase in muscle fibre length are at least induced positive strain (to adequate ensure stretch of the muscle) and micro-damage to the muscle (to trigger sarcomerogenesis). To establish the success of the specifically designed training protocol at increasing muscle fascicle length, the required parameters will need to be measured. Muscle fascicle length is commonly measured using ultrasonography (US).

Reliability of Measuring Muscle Fascicle Length

As muscle fascicle length is a commonly measured functional outcome of strength training and stretching protocols, many research groups have evaluated different measurement methods for fascicle/fibre length and their reliability. Kwah et al., (2013) composed a systematic review of the literature that measures the reliability and validity of measuring muscle fascicle length and pennation angle in human skeletal muscle using two-dimensional ultrasonography (US). The study compiled 36 reliability studies, and 6 validity studies that measured the reliability of muscle fascicle length or pennation angle. The results of this systematic review indicate that the use of two-dimensional US to measure muscle fascicle length was reliable across a range of experimental conditions including relaxed and contracted muscle states. While studies on the validity of US to measure muscle fascicle length are limited, the presented evidence suggests that US is in fact a valid method of measuring muscle fascicle length or pennation angle during static condition with a relaxed muscle. Contrastingly, measures of muscle fascicle length are reliable during relaxed or contracted muscle states, when measures are repeated, and when the administrator has had no formal training (Kwah et al., 2013).

Another study that measured the reliability of measuring muscle fascicle length using US compared a standard US tape method with previously validated handheld three dimensional US (Barber et al., 2011b). Resting muscle lengths of the gastrocnemius were measured at three joint angles of nine adults with CP, and fifteen typically developing adults. They hypothesised that there would be a small measurement error when using the US tape method, which would still allow the detection of changes in muscle length. The results of this study report that the US tape method was sufficiently accurate in measuring length

changes of both TD and CP individuals, and therefore would be a useful tool in diagnosis and treatment effectiveness.

Although the use of US as a measure of muscle fascicle length has been established as reliable, the method of analysis of ultrasound images is ever evolving. Gillet et al., (2013) investigated the accuracy and reliability of an auto-tracking algorithm to measure passive and active muscle fascicle length changes. Auto-tracking was undertaken by three experienced assessors, and compared to manual tracking methods that have been previously validated, as well as within and between assessor reliability. The results of this study indicate that muscle fascicle length changes can be accurately and reliably tracked using an auto-tracking algorithm, during both controlled and active conditions, by the same or a different assessor. The auto-tracking method was also found to be significantly quicker at calculating muscle fascicle length, and is currently widely used by researchers (Gillet et al., 2013).

The necessity for an individual study assessing the reliability of measuring VL muscle fascicle length using US is clear from the literature above; although the technique is considered reliable in different conditions, the fact that CP individuals have smaller muscles, and that the leg will be moving during measurement, creates a clear justification for testing assessor reliability at measuring VL muscle fascicle length.

Measures of Functional Improvements in CP

The focus of most interventions that target individuals with CP is not simply to increase muscle size or length, but how these increases can lead to improvements in everyday life. As such, there are many studies that assess the changes in functionality that occur after a training intervention, whether the intervention involves stretching or some form of strength training. As functionality of CP individuals is hugely variable, specific aids have been created with the population in mind, to help with functional tasks such as walking. For example, braces are prescribed to prevent deformity and control joint position and therefore improve stability. Ankle foot orthosis (AFOs) are also commonly used to prevent the equinus position of the foot. AFOs and their effects on walking have been studied using gait analysis

techniques, and no significant differences in cadence, velocity, or stride length were found (Powell et al., 1989). However further investigation by Thomas et al., (1992), using kinematic and EMG information, found that there was a significant improvement in the ankle motion of all the patients, as well as an increase in the ratio of hip to knee movements in 80 % of the participants (Thomas et al., 1992).

Comparison between TD gait and gait in CP has also been studied by Condie et al., (1993); assessment of gait characteristic in CP compared with and without AFOs to TD individuals. This group hypothesised that with the effective use of AFOs, the high impact forces often seen during the early stance phase can be reduced, and vertical ground reaction forces during late stance phase can be increase. They concluded that the use of AFOs improve the ability of CP individuals to not only support their body weight during walking, but also to generate greater forces during push-off (Condie and Meadows, 1993).

Continuing from this, Abel et al., (1998) assessed the gait of children with spastic diplegia using fixed AFOs compared with barefoot walking. The study used kinematics and force plate data to determine joint moments and power. The results of this study show that the percentage of single limb support was increase in those using AFOs compared with barefoot walking; and increase in velocity and stride length in the AFO group was also observed. Kinetic analysis demonstrated a decrease in the abnormal power bursts in the AFO group compared with barefoot walking during early stance, and an increase in ankle moment during late stance, concluding that AFOs enhanced gait function when compared directly to barefoot walking in diplegic participants (Abel et al., 1998). Often gait measurements observe time-distance characteristics, as well as three dimensional joint kinetic and kinematic values and EMG.

Although an increase in functional walking abilities when using AFOs is a positive adaptation, the long-term goal of most researcher and medical professionals would be to improve gait and posture independent of orthosis. A method of progressing toward the desired improvements is assessing and improving dynamic balance during gait, as this not only affects their mobility, but also their safety and likelihood of falling. As balance is the product of complex interactions between the neural control of muscles, strength of

muscles, proprioception and vision, it is not surprising that individuals with CP possess balance deficits. However there is a distinct lack of standardised indexing of the measurement of dynamic balance within the population; there are in fact many methods of assessing balance (Niiler, 2018). Due to the abundance of measurement techniques of postural control, Pavao et al., (2013) compiled a review of currently literature on the topic. It used 25 studies that assessed postural control in CP, showing that postural control is widely studied in children with CP with reliable and robust methods (Pavao et al., 2013). It reported that individuals with spastic diplegia exhibit poorly organised muscular responses with regard to balance and gait; trunk and limb muscles are activated proximally before distal activation occurs, as well as a high degree of antagonistic co-contraction not observed in TD peers. This review also found a link between functionality and postural control, implying that an increase in functionality would also result in an increase in postural control. It is also mentioned that there are very few studies that explore postural control in relation to daily activities, presenting a gap in the literature, and a clear rationale for using strength training to not only increase muscle fascicle length, but with the aim of producing long term adaptations that could improve functional capabilities such as postural control and gait. While many interventions in CP focus on increasing function in muscles surrounding the shank, specifically the gastrocnemius and soleus, there are few interventions within the population which focus on improving the function of the quadriceps and hamstrings, which are important contributors to functional abilities such as walking an balance (Liu et al., 2008), and are high predictors of knee extensor strength in CP (Moreau et al., 2010).

Conclusion

The research presented here details exercise interventions that have successfully lead to muscle damage induced sarcomerogenesis and increase in serial sarcomere number and FL in healthy animal and human models. Here, studies have shown similarities in their methodologies which should be taken into consideration for the design of interventions to increase FL in spastic muscles. All interventions involved: 1) ECC training at appropriately high velocity, and 2) were associated with positive strain of muscle fibres/fascicles. Moreover, such interventions could have been associated with 3) momentary deactivation

in the stretched muscle. Satisfaction of these criteria could lead to microscopic fibre damage and popping of the sarcomeres which act as the sufficient stimuli for sarcomerogenesis.

Several studies reported increase in FL (Zhao et al., 2011) and improved gait (Hosl et al., 2015, Zhao et al., 2013, Herrin and Geil, 2016) as a result of passive and active stretching of the MTU using different approaches (e.g. casting, robot-assisted stretching, and orthoses), however these improvements were small and not investigated over the long term. In contrast, a recent systematic review concluded that there was insufficient evidence to support the effectiveness of stretching (applied manually, using orthosis and casting) for improving ROM or spasticity in CP over the medium to long term (Craig et al., 2016). As individuals with CP possess shorter and stiffer muscles with longer and more compliant tendons, which prevents overstretching of the fibres (Tabary et al., 1972, Theis et al., 2015), commonly employed passive stretching treatment have been reported to be unlikely to produce the required long-term increase in SSN and FL (Bar-On et al., 2018, Kalkman et al., 2019). Therefore, to increase SSN and FL in spastic muscles, new exercise protocols in which the three presumed criteria: 1) ECC at appropriately high velocity, 2) positive strain of muscle fibres/fascicles, and 3) momentary deactivation in the stretched muscle, are satisfied must be developed, and long-term muscle architectural and functional adaptations to such trainings must be examined. The subsequent investigation of the functional adaptations as a result of increased FL should also be undertaken, to determine not only whether eccentric training produces any functional improvements to walking and/or balance, but whether these effects can be maintained over the long term.

CHAPTER 3: General Methods

Overview

This thesis first established a theoretical framework for increasing muscle fascicle length based on a review of the literature. The proposed framework was built on three criteria observed in healthy human and animal muscles during high-velocity eccentric exercise, which possibly lead to an increase in muscle fascicle length. It was argued that if these exercise conditions could be replicated in individuals with CP, similar increases in fascicle length and improvements in functionality might be achieved. However, due to the inability of many individuals with CP to engage in eccentric exercises (employed for healthy muscles), there was a need to introduce and test a training method that could mimic high-velocity eccentric training in healthy participants.

Second, the thesis provided data on the behaviour and response of healthy muscle fascicles to high-velocity passive stretching, which offered an experimental context that could potentially be applied to individuals with spastic CP. Such data was essential to establish a baseline for expected values and to compare with the data from individuals with CP, as no data was available for spastic muscles.

Third, the thesis examined the repeatability of a specific high-velocity eccentric exercise in healthy adults to increase muscle fascicle length, where the theoretical framework's criteria were expected to be met. The results confirmed the anticipated increase in the length of healthy muscle fascicles.

Fourth, the response of spastic muscles in CP to high-velocity passive stretching was examined, confirming that the proposed prerequisite criteria for increasing fascicle length were met, and suggesting the technique's potential for increasing fascicle length through training.

Finally, the feasibility of this training method was tested, and architectural adaptations of spastic muscles, as well as functional improvements in an individual with CP in response to high-velocity training, were evaluated.

Participants

A pragmatic approach to participant recruitment was employed for the experimental studies reported in Chapters 4-6. Healthy adult participants were recruited for the first

experimental study (Chapter 4), in which the primary goal was to determine baseline values for the muscle fascicle response to high-velocity passive stretching in our laboratory (measures of muscle microdamage and biological variation in blood muscle-CK), and to assess the reliability of measurements. For the high velocity eccentric training study in Chapter 6, a sample of convenience of healthy adults who met similar criteria to previous work in the literature was recruited. Given the overlap of the data collection period with the Covid-19 pandemic and the limited access to participants with spastic CP, the use of a pragmatic approach for recruitment of the participants with CP was also justified for the experimental studies reported in Chapters 5 and 7. In these studies, first response of the spastic muscle fascicles to high-velocity passive stretching was monitored (Chapter 5), informing the decision to apply the technique to potentially increase fascicle length (Chapter 6) in a single adult participant with CP. Ethics approval for conduction of the study was obtained from relevant Brunel University London and/or NHS research ethics committees. Ethics approval for conduction of the studies was sought from the College of Health, Medicine and Life Sciences at Brunel University London.

Equipment and Material

An isokinetic dynamometer (Cybex Norm, division of Lumex Inc., Ronkonkoma, New York, USA) was used to passively rotate the knee joint where required. Angular position and velocity of the system input arm were produced at 100 Hz by the dynamometry system, but collected at 2000 Hz rate. For the scanning of muscle fascicle lengths and determination of strain or potential increase in the length of muscle fascicles in response to training, a portable B-mode ultrasound scanner (Echo Blaster 128 Ext-1Z system, Telemed, Vilnius, Lithuania) interfaced with a laptop via Echowave software (Telemed, Vilnius, Lithuania) was used which scanned muscle fascicle/fibre length continuously at 40 Hz. The Reflotron Plus blood analysis machine (Roche Diagnostics GmbH, Mannheim, Germany) was used to analyse blood samples. Samples were taken using spring-loaded single-use 2 mm Unistik 3 lancets, Human GmbH 30 microliter micro pipettes, and Reflotron CK strips (Roche Diagnostics GmbH, Mannheim, Germany). The EMG system used was a Digitimer440 (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK), a 4-channel system. Use of the EMG

system was required to establish suitability of the high velocity passive stretching for inducing eccentric-like effect in the stretching muscle. EMG signals were sampled at a frequency of 2000 Hz. A CED 1401 POWER3A (Cambridge Electronic Design Limited, Cambridge, UK) data acquisition system interfaced with Spike2 was used for data acquisition at 2000 Hz, allowing the synchronization of EMG and dynamometry data which were triggered by the start of US scanning. During the gait analysis of the single subject in the pre-training session (Chapter 6), Trigno Wireless electrode sensors (Delsys Inc., Ltd., Boston, USA) were used for EMG. Gait analysis was conducted using a 10-camera Qualysis system (Qualysis Track Manager 2020.2, Sweden) synchronized with a 60 × 40 cm Kistler piezoelectric force platform (type 9281B11; Kistler Instrumente, Winterthur, Switzerland) to record force and balance data.

Electromyography

Protocol for recording muscle activity (EMG) recommended by SENIAM was followed. Electrode placement for the individuals with CP were adjusted to suit individual muscle geometry which was significantly varied in muscle size and volume. EMG was measured every session to monitor the activation patterns of the VM and BF muscles during induced passive motions at high velocities in participants with CP. EMG data was demeaned (DC removed), rectified, and smoothed (using a moving average of every 0.005s) before being exported as a spreadsheet text file from Spike2 to third party software for offline analysis.

Data Processing and Analysis

Determining Fibre Strain

For the healthy individuals, the US probe was attached midway between the greater trochanter and the lateral epicondyle of the femur, aligned with the line of action of the VL muscle fascicles, and secured using hypoallergenic tape. Location of the probe was adjusted for the individuals with CP due to differences in muscle geometry to allow visualisation of the muscle fascicles. Scanning depth and focus were optimized for each person. VL fascicle

length was defined as a straight line along the orientation of the muscle fascicles between the superficial and deep aponeuroses of the muscle (Figure 3.1).

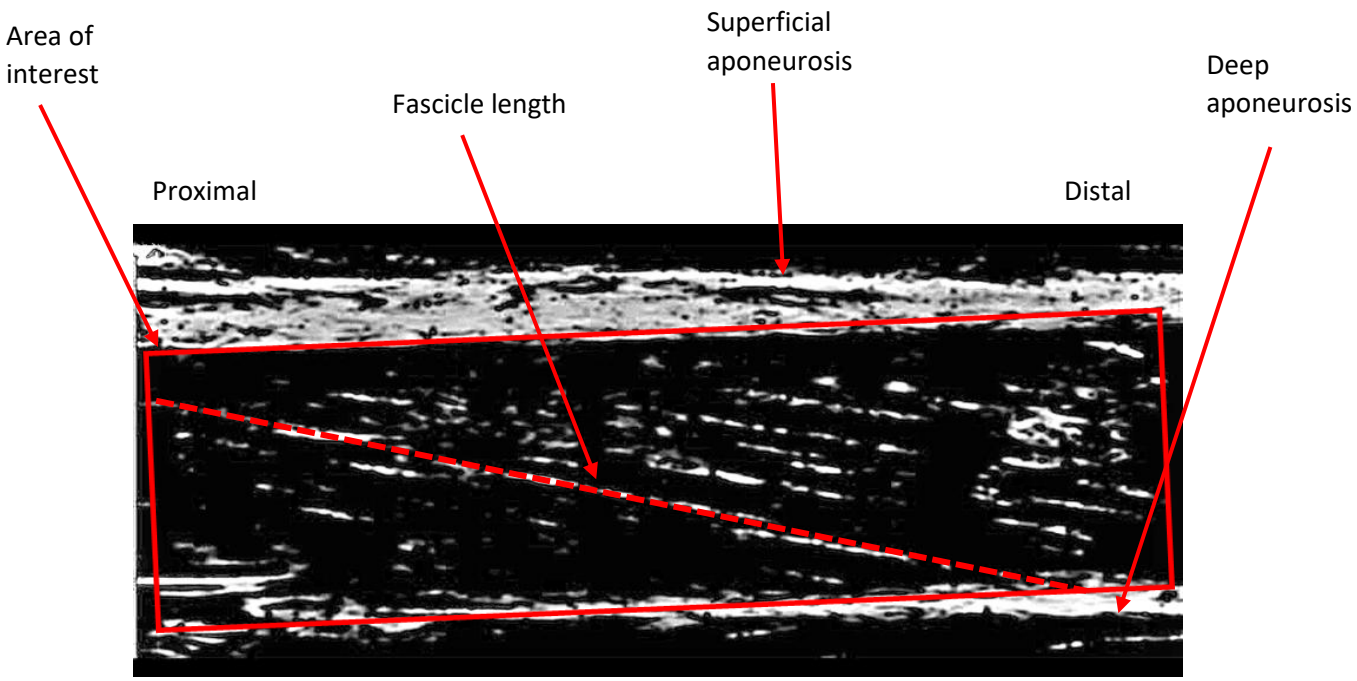
The US scanning was started prior to the beginning of the three experimental rotations of the knee delivered by the dynamometry system. US data was collected in .tvd format, saved, and exported in .avi format, then opened in MATLAB (R2018b, MathWorks, USA). The length changes of a defined muscle fibre were tracked. Positive fibre strain was calculated for the flexion phase of the knee rotation. Flexion of the knee joint during continuous passive motion elongated the quadriceps muscle-tendon unit (MTU). Associated changes in the VL fascicles, measured with respect to their reference length at full extension of the knee joint, were used in the calculation of strain (Equation 1).

Equation 1:

$$Strain = (\text{max FL} - \text{FL origin}) / \text{FL origin}$$

Where FL origin was defined as the length of the muscle fibre, measured as a straight line,at full extension of the knee when no extension torque was recorded, and max FL was the maximum length of the fascicle during flexion of the knee joint.

If the whole fascicle was not present within the view of the probe, the algorithm tracked the change in the position of the defined fascicle start and endpoints, including points outside the range of interest.



(Figure 1 - an Ultrasound image of the VL of a participant)

Ultrasonography was performed on the training leg every 7th session during the 10-week training period; scans were taken during rest at maximum extension angle, maximum flexion angle, and at each angle at which the iMVC was performed.

FL measures were taken at six different angles at the pre training session, follow up session, and every two weeks during training during the iMVC sessions. US data was collected in .tvd format and saved and exported in .avi format, then opened in MATLAB (R2018b, MathWorks, USA) where they were converted to images in .tif format. The participants were relaxed and stationary at a fixed angle during FL measurements. The whole fascicle was not always visible; therefore, the muscle FL was calculated (estimated) manually using ImageJ (1.54g, NIH, USA). To this end, muscle thickness was measured from the superficial to deep aponeurosis, and in combination with the measured pennation angle, were used to calculate the FL (*Equation 3*).

Equation 3:

Fascicle length = muscle thickness (sin θ_p)$^{-1}$ (5)

Muscle-CK

CP participants (Chapter 5) underwent two blood samples that were taken using a standard finger-prick technique, and levels of muscle-CK were measured before (Pre) and immediately after (Post) the stretching protocol to provide some insight into the possibility of microscopic muscle fibre damage due to stretching. On the second (Post-24) and third (Post-48) days, 1 blood sample was taken from these participants while they were seated in a standard office chair which allowed monitoring of the level of the enzymes over 48 h from the imposed stretch. TD individuals in Chapter 5 underwent 6 blood samples on the first day, and levels of muscle-CK were measured before (Pre, 3 samples) and immediately after (Post, 3 samples) the stretching (TD-EG; Chapter 4) or rest period (TD-CG; Chapter 4). On the second (Post-24) and third days (Post-48), an additional 3 blood samples were taken from the participants in both TD groups while they were seated in a standard office chair (below). Such design allowed determination of typical error of measurement, estimation of minimal detectable change (MDC) and smallest clinically worthwhile change (SWC), and biological variation in muscle-CK over 48 h in the group.

The Reflotron Plus blood analysis system was cleaned and checked according to manufacturing guidelines before use; a standardised warm-up period of two hours was undertaken. Blood samples of 30 microlitres were taken from the participants using a standard finger-prick technique; the finger was cleaned using a disposable alcohol wipe, and then pricked using a lancet. The blood sample was then collected using a micropipette and expelled onto a specialised CK strip, which was analysed by the Reflotron Plus blood analysis machine within 15 s of blood contact with the CK strip. This was performed pre and post passive stretching Protocol. Results were expressed in microlitres per litre of blood. Muscular micro-damage was defined as microscopic dam-age to spastic and TD muscle fibres shown by the increase in CK concentration using two approaches: (1) more than 1.5 times the baseline concentration; and (2) more than 1.5 times the typical error above the upper limit of the confidence interval for the CK group mean.

Statistical Analyses

Statistical analyses for all studies were conducted using SPSS (IBM Statistics, version 2022). Each chapter employs a different approach to statistical analysis, and thus, specific methods will be reported in detail on a chapter-by-chapter basis. Briefly, no hypothesis testing was performed for the outcome measures of the non-confirmatory studies reported in Chapters 4 and 7.

To quantify the experimenter's reliability in assessing fascicle length using ultrasonography and to assess variability in muscle-CK values in response to stretching in healthy adults (Chapter 4), an Intraclass Correlation (ICC) analysis and various measures of variability (e.g., SEM, MDC95, and LoA) were used.

In Chapter 5, in addition to descriptive analysis of the outcome measures, a t-test was used to compare baseline (i.e., pre-stretching) muscle-CK values between individuals with CP and healthy individuals from Chapter 4, while an ANOVA examined the differences between the two groups in their muscle-CK responses to stretching.

In Chapter 6, a factorial analysis of the change score was employed to assess changes in fascicle length and normalised peak eccentric torque following an established eccentric exercise protocol. A t-test was also used to examine the change in the angle at which peak normalised eccentric torque occurred.

Methodological Assumptions and Limitations

Measuring muscle FL using 2D ultrasonography is a common practice within the field, however it relies on specific assumptions, which limits interpretation of the results. Firstly, measuring FL using two dimensional US assumes that the full fascicle length is visible inside the scanning/viewing window (reference frame) of the scanner probe, which is not always possible due to limitations of the probe size and scanning depth. This means than the open-source code, previously validated and presented by Farris and Lichtwark (2016)(Farris and Lichtwark, 2016) assumes the position of the end of the fascicle if the full length of the fascicle cannot be seen within the frame. Secondly, the use of 2D US assumes the fascicle as a straight line; it does not account for curvature of the muscle or fascicle, meaning that the actual length measured using the code is the distance between the visible start and end points of the fascicle, and not the true length of it. Moreover, measurement of the fascicular

strain requires determining a reference (original) length. While measurements of the resting FL were taken at a consistent reference point, it is possible that muscle was under some form of compression or tension forces during scanning which may also affect the measured length.

The use of EMG is used to measure muscle activity by placing two electrodes on the surface of the muscle, and one on a local bone as a form of grounding. This measures the electrical signal of the muscle over a specific period on different days; however, as this is measured on the skin surface, it relies on the proper skin preparation and other factors that can affect recorded signal from on session to next. This technique also assumes that the electrodes are placed on the correct location of the muscle belly. There are specific guidelines for the placement of electrodes to get the strongest signal in healthy individuals (SENIAM), but when participants do not have typically formed muscles, this can prove difficult. Individuals with CP have smaller muscle belly length compared to their TD peers (Barber et al., 2011a) making finding the muscle belly more of a challenge. This is a limitation of using the technique within the clinical population, specifically with those possessing irregular muscle structure.

CHAPTER 4: RELIABILITY OF MUSCLE FASCICLE LENGTH AND BLOOD-MUSCLE CREATINE KINEASE MEASURES

Abstract

This study aimed to assess the experimenter's reliability of measuring muscle fascicle length using US and determine the minimal detectible change of blood-muscle creatine kinase (CK) levels in healthy individuals. Muscle fascicle length, a critical determinant of muscle function and performance, was evaluated using B-mode ultrasonography. Concurrently, blood-muscle CK levels, an important marker of muscle damage and recovery, were analyzed through blood sampling. Measurements were taken at rest and following standardized high velocity passive stretching (HVPS) protocols at four time points: baseline, immediately post stretching, 24 hours later and 48 hours later. Intraclass correlation coefficients (ICC) and coefficients of variation (CV) were calculated to determine intra-rater reliability, and minimal detectable change and smallest worthwhile change were calculated to establish any increases in CK not due to biological variation. Results demonstrated high reliability for muscle fascicle length measurements (ICC > 0.866 lower bound, CV < 5%) and a the most conservative MDC_{95} of 273.3 µL /L. These findings suggested that the experimenter measuring muscle fascicle length using US was reliable at assessing muscle architecture, and the use of CK as a measure of exercise-induced muscle damage was highly variable, as biological variation between participants remained high.

Introduction

Muscle fascicle length (FL) is a commonly used outcome measure of strength training interventions, as it is an indicator of the increase in serial sarcomere number (SSN) in response to muscle microscopic damage during training. Evidence from literature supports the notion that long term/chronic training may lead to increases in muscle FL in healthy human and animal models (Franchi et al., 2014, Reeves et al., 2009a, Butterfield et al., 2005a, Franchi et al., 2016, Franchi et al., 2017, Sharifnezhad et al., 2014b, Mohagheghi et al., 2008, Erskine et al., 2010, Williams, 1990); however, this is likely to occur when specific exercise and training conditions are provided, as not all training interventions lead to increase in FL.

Sharifnezhad et al. (2014), used four different eccentric exercise protocols to establish training conditions leading to increase in quadriceps FL. Training was modified for each group, one of which trained at a lower load magnitude than the other three training groups. Training in these three groups was modified by changing the velocity of muscle lengthening during training, and the knee joint angle, and therefore the muscle length at which training was undergone. Muscle FL was examined pre and post training using ultrasonography, which showed a significant increase in FL as a result of training at the highest muscle lengthening velocities only. Interestingly, authors also reported that a drop in the knee joint moment occurred during exercise in the high velocity training group, which they attributed to a drop in muscle activation during the eccentric contraction, however this was not measured directly (e.g., by using electromyography). No significant changes in muscle architecture were found in any of the other four training groups; the researchers concluded that not all modes of eccentric training resulted in FL increases, and lengthening velocity was an important factor for longitudinal muscle growth.

Dissimilarly, Marzilger et al., (Marzilger et al., 2019) investigated the effects of muscle lengthening velocity during eccentric training in the VL muscle on the physiological cross-sectional area (PCSA) and FL, and reported that all angular velocities resulted in similar increases in FL over an 11-week period. The researchers concluded that FL gains as a result of eccentric training are independent of the velocity of the training employed. This directly contradicts the findings of Sharifnezhad et al. (2014) who found that only high velocity eccentric training conditions resulted in increases in quadriceps FL.

Discrepancy within the literature, with regard to optimal training conditions to increase muscle FL in healthy adults, is evident. The optimal exercise conditions which may lead to increase in FL with eccentric training in healthy individuals have been previously proposed (Davis et al., 2020). In 2020, a literature review undertaken by the present researcher suggested that appropriately high velocity eccentric training, as could be manifested by large enough positive fiber/fascicle strain, microscopic muscle damage, and momentary deactivation of the muscle during exercise, may be the three prerequisites for increase in FL in response to exercise. The decision was then made to investigate evidence for such proposal in the present work, as determining the optimal conditions for increasing FL using

eccentric training could be beneficial both within sporting contexts and for rehabilitation purposes where increase in FL may improve functional abilities (e.g., in individuals with CP). However, before conducting any study involving a training regime at high-velocity eccentric exercise to increase FL in healthy or CP population, a number of baseline measures are required. These baseline measurements would provide measures of reliability, smallest worthwhile change (SWC) and minimal detectable changes (MDC) in the outcome parameters of interest due to their day-to-day biological variations. Accordingly, any possible change in the outcome measures could be attributed to either due to normal variability or response to training protocol.

In the present study, possible microdamage in response to a one-off stretching exercise was examined using variations in the levels of Creatine Kinease (CK) assessed via a minimally invasive procedure. Creatine Kinease is an enzyme stored in the heart, brain and skeletal muscle, which is excreted into the blood as a result of muscle damage, and therefore is used to determine whether training programs result in sufficient muscle damage (Brancaccio et al., 2007, Cabaniss, 1990). As stated above, levels of CK is measured within research to establish damage to a muscle in response to training, and therefore determining the smallest worthwhile change (SWC) in CK levels is necessary. Measurements of the level of blood CK have been previously reported as being highly variable, as they are dependent on many factors including gender, age, race, and fitness levels (Baird et al., 2012a, Brewster et al., 2012, Black et al., 1986). Determination of the biological variations in the levels of CK (as could be inferred by estimating MDC and SWC in healthy individuals) under our laboratory practice was therefore required. In the absence of any better alternative, such measurements could also form the basis against which changes in the CK in response to a training program in individuals with CP (population of interest to the present doctoral study) could be assessed.

To this end, the aim of this non-confirmatory (exploratory) study was firstly, to determine the biological variation in the level of blood CK in response to one session of high velocity passive stretching over a three-day period, in a group of healthy adults, to inform future research in CP participants. Secondly, we aimed to determine experimenter's reliability for measuring quadriceps muscle fascicle length using ultrasonography in this group.

Methods

Experimental protocol and equipment details can be found in the General Methods section (Chapter 3).

Participants

Due to the exploratory nature of this study, a convenience sample of 28 healthy adults aged 18-50 (14 female, 14 male, mean (SD) mass (kg) 76.63 +/- 26; mean (SD) height 167.5 +/- 23.5) were recruited to participate in this study. They were recruited from the students and staff at Brunel University London, and were free from lower limb neuromuscular injuries, blood borne viruses, and metabolic conditions. Ethics approval for conducting this study was obtained from Brunel University London College of Health, Medicine and Life Sciences Research Ethics Committee (Appendix B). All participants provided written informed consent to participate in this study. Healthy adults were recruited to determine the immediate effect of stretching, and reliability of measurements of the VL muscle fascicle length and muscle-CK in a group of individuals without CP. All participants attended the Biomechanics Laboratory at Brunel University London on three consecutive days at the same time of day. Participants were randomly allocated into either an exercise group (EG) or control group (CG) using randomly generated numbers in *Excel* according to their order of recruitment. Participants were required to refrain from strenuous exercise for four days prior to attending the laboratory.

Data processing and analysis

Determining changes in muscle-CK

Level of blood muscle-CK was measured to establish if any potential muscle microdamage, which could occur as a result of stretching in individuals in the EG, could be identified by our minimally invasive technique of finger pricking. To this end, as stated above, three measures of blood muscle-CK were obtained from those in the EG. For this group, the mean of the 3 measurements at each time point was used in the analysis.

The standard deviation of changes in the values of blood muscle-CK (change score) between Pre and Post stretching, Pre and Post-24, and Pre and Post-48, was used in the calculation of three standard error of measurements (SEM).

The highest Minimal Detectable Change (MDC_{95}), that could be calculated based on the SEM (Equation 2) across all comparisons made from individuals in the CG, would be the most conservative value to determine changes in the blood muscle-CK due to biological variations. An estimate of the smallest worthwhile change (SWC) was also calculated (Equation 2).

The MDC_{95} and the SWC were used to assess if any increase in the CK levels in the EG was due to stretching, and not the biological variation of the participants or device error. Moreover, in the absence of any better alternative, the MDC_{95} and SWC from individuals in CG, could provide a ballpark figure for assessing alterations in the resting state of the enzyme in individuals with CP (Chapter 4) in response to any interventions that may microscopically damage the spastic muscles in future interventions.

Equation 2:

$$\mathbf{MDC_{95} = SEM \times \sqrt{2} \times 1.96}.$$

$$\mathbf{SEM = SD/\sqrt{2}}$$

$$\mathbf{SWC = SD \times 0.2}$$

where, MDC_{95} is the highest value calculated using SEM obtained across all possible comparisons, SEM is the standard error of measurement, and SD is the standard deviation of the change scores (change in the mean value of the measured muscle-CK levels at different time points).

Measures of reliability

Three measures of reliability, change in mean (change score), typical error, and coefficient of variation, were calculated for EG (N=9) and CG (N=7) at Post, Post-24, and Post-48 compared to baseline (Pre) values. Participant numbers used in this analysis differ to the

total number of participants due to the obsolescence of consumables to measure blood-CK as a result of Covid19. Intraclass Correlation Coefficients (ICC) were calculated following Hopkins, 2015 (WG, 2015) to measure the degree of consistency of the blood muscle-CK values at each time point. ICC was also calculated for the fascicle lengths to assess the experimenter reliability when measuring maximum and minimum fascicle lengths during the three experimental rotations of the knee joint at the same speed for each participant in EG.

Results

Blood-CK

Mean values for muscle-CK at each time-point for individuals in Protocols 1-3 are shown in Table 1. One participant in Protocol 3, had extremely high muscle-CK values (>1000 µL /L) and blood analyser produced an error message (dilute sample). Data of this participant's was removed (not shown in the table).

Table 1 – CK mean values at each time-point for CG and EG

Group	P no	Pre	Post	24hrs	48hrs	Notes
Control	3	42.6	46.4	45.3	42.7	
Control	4	87.9	105.9	86.1	74.5	
Control	6	136	81.2	188.3	M	Missed appointment
Control	9	70.7	122.7	138.7	114.3	
Control	11	128.7	108.3	98.5	104.7	
Control	12	240.3	220	M	M	Felt sick
Control	16	64.8	51.4	125.3	410	
Group Mean		**110.1**	**105.1**	**113.7**	**149.2**	
Exercise	1	59.5	71	59.1	59	
Exercise	2	118.7	147	110.5	90.6	
Exercise	7	53.3	60.7	63.3	47.1	
Exercise	8	63.6	51.8	61.8	67.7	
Exercise	10	156.7	152.3	327.7	286.3	
Exercise	13	190.7	207.7	182	147.3	
Exercise	14	97.1	133.3	79.9	107.8	
Exercise	15	155	137.3	125.3	104.1	
Exercise	17	246.7	166.2	239.3	333.7	
Group Mean		**126.8**	**125.3**	**138.8**	**138.2**	

Note. *Group = control (C) or exercise (E), P no. = participant number, pre = mean CK values pre-stretching, post = mean CK values post-stretching, 24hrs = mean CK values 24 hours post-stretching, 48hrs = mean CK values 48. Muscle-CK values are in μL /L, M = missing datum.*

Changes in the mean values from baseline (Δscore) for the control and exercise groups can be seen in Table 2.

Table 2 - Changes in muscle-CK mean values at each time-point for individuals in CG and EG

Group	P no.	Δscore post-pre	Δscore 24hrs-pre	Δscore 48hrs-pre
Control	3	3.8	2.7	0.1
Control	4	18	-1.8	-13.4
Control	6	-54.8	52.3	M
Control	9	52	68	43.6
Control	11	-20.4	-30.2	-24
Control	12	-20.3	M	M
Control	16	-13.4	**60.5**	**345.2**
Exercise	1	11.5	-0.4	-0.5
Exercise	2	28.3	-8.2	-28.1
Exercise	7	7.4	10	-6.2
Exercise	8	-11.8	-1.8	4.1
Exercise	10	-4.4	171	129.6
Exercise	13	17	-8.7	-43.4
Exercise	14	36.2	-17.2	10.7
Exercise	15	-17.7	-29.7	-50.9
Exercise	17	-80.5	-7.4	87

Note. *Group = control (C) or exercise (E), P no. = participant number, Δscore post-pre = change in the mean of the values from Pre to Post time point, Δscore 24hrs-pre = change in the mean of the values from Pre to 24 hours time point, Δscore 48hrs-pre = change in the mean of the values from Pre to 48 hours time point. Muscle-CK values are* in μL */L, M = missing datum.*

Measures of variability for muscle-CK, estimated based on the values obtained from healthy individuals in the control group, are shown in Table 3. MDC_{95} and SWC values increased at each time-point. The highest value for MDC_{95} (273.3 μL /L), and associated LoA (-234.2-312.4 μL /L) provided the most conservative estimation of biological variation in muscle-CK. These values should be treated with caution as were mainly driven by an extremely high value obtained from participant 16 (highlighted in blue in Table 2). With exclusion of the change scores at the last 2 time points of participant 16, MDC_{95} and LoA would change to 71.2 μL /L; and [-69.9-72.5] at 24 hours, and 50.4 μL /L; [-76.4-24.4] at 48 hours (Table 3.3).

Table 3 - Measures of variability in muscle-CK for typically developing individuals in CG

Measure	**Post-Pre**	**24-Pre**	**48-Pre**
Δ in Mean	-5	3.6	39.1
SD	31.3	36.8	139.4
SEM	22.1	26	98.6
CV	10.0%	10.0%	30.0%
MDC_{95}	61.3	72.1	273.3
SWC	25.07	32.78	136.58
LoA	[-66.3 – 56.3]	[-68.5 – 75.7]	[-234.2 – 312.4]
		[-69.9 – 72.5]	**[-76.4 – 24.4]**

(Post-Pre = change in the mean of the values measured at Post and Pre time points, 24-Pre = change in the mean of the values measured at Post-24 and Pre time points, 48-Pre = change in the mean of the values measured at Post-48 and Pre time points, Δ in Mean = change in mean of repetitions at each time point, SD = standard deviation of Δ in Mean, SEM = Standard Error of Measurement, CV = Coefficient of variation, MDC = Minimal Detectable Change, SWC = Smallest Worthwhile Change, LoA = Level of Agreement. Please see text for highlighted LoA values)

Measures of muscle-CK variability for individuals in CG are in Table 4. As a group, individuals showed an overall small drop immediately post intervention but small increase in muscle-CK

levels at Post-24 and Post-48 time points. Not all individuals in CG followed similar pattern of change to their respective group (*Table 1*)

Table 4 - Measures of muscle-CK variability for typically developing individuals in EG

Measure	Group	Post-Pre	24-Pre	48-Pre
Δ in Mean	Exercise	-1.5	12	11.4
SD	Exercise	32.5	57.2	56.4
SEM	Exercise	23	40.4	39.9
CV	Exercise	30.0%	50.0%	40.0%

(Δ in Mean = change in mean at each time point, SD = standard deviation of Δ in Mean, SEM = Standard Error of Measurement, CV = Coefficient of variation. Post-Pre = mean change of the values measured at Post, 24-Pre = mean change of the values measured at Post-24, 48-Pre = mean change of the values measured at Post-48)

Immediately after stretch, measured muscle-CK increased in 5 participants in the EG. Others showed a drop in the measured muscle CK. After 24 hours, 2 healthy participants had positive muscle-CK values. After 48 hours, 4 healthy participants had positive muscle-CK values. In this group, there was no increase in muscle-CK after stretching above the upper bound of the LoA (312.4 or 24.4 μL /L) established based on Control participants.

Fibre Strain

Table 5 shows maximum strain observed during stretching of the MTU and associated speed at which the maximum strain occurred, for participants in EG. All participants experienced positive fibre strain in response to passive stretching at different velocities, however, increase of 5 % or more in the length of fascicles at any time during stretch (i.e. flexion of the knee joint) with respect to its initial (origin) length (i.e., when the knee joint was at its maximum extension angle) was observed in limited number of participants. As illustrated in table below, positive fibre strain of ≥5 % was observed in four healthy participants (Table 5). Majority of maximum strain recorded at relatively lower velocities (speeds 1-3; i.e., up to 210 °/s in healthy individuals).

An Intraclass correlation coefficient was used to assess the experimenter reliability when measuring maximum and minimum fascicle length of three random trials for each participant in EG. Both maximum and minimum FL measurements show strong correlations of 0.959 and 0.951 respectively (Table 6).

Table 5 – Vastus Lateralis maximum fibre strain and the velocity they occur at

Vastus Lateralis FL Strain in TD		
Participant	Speed	Max Strain
1	4	1.10%
2	1	7.89%
7	1	0.64%
8	3	16.67%
10	1	2.51%
13	3	5.75%
14	3	2.64%
15	2	2.38%
17	5	1.05%
20	2	0.82%
24	1	3.19%
27	2	19.04%
28	1	1.33%

(*Speed = 1 being the slowest and 5 being the fastest velocities used during knee flexion, Max Strain = maximum FL strain observed during knee flexion expressed as a percentage of VL origin length*

Table 6 – Intraclass Correlation coefficient measuring experimenter reliability

Intraclass Correlation Coefficient							
	Intraclass	95% Confidence Interval		F Test with True Value 0			
	Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.887a	0.745	0.960	26.155	12	24	<.001
Average Measures	.959c	0.898	0.986	26.155	12	24	<.001
Single Measures	.866a	0.702	0.953	19.207	12	24	<.001
Average Measures	.951c	0.876	0.984	19.207	12	24	<.001

Discussion

The main purpose of this study was to establish the minimal detectable change and/or smallest worthwhile change of blood CK levels as a result of stretching, not confounded by biological variation or device error, in a group of healthy participants. This was undertaken to determine, via generalization to individuals with CP, whether a passive stretching protocol in spastic muscles would result in sufficient muscle damage. We also aimed to determine the experimenter reliability of measuring typically developing muscle fascicle length using ultrasonography. This is imperative when measuring muscle structure in spastic muscles, as the muscles are smaller in length and volume, making measurements more challenging. Results presented above showed good experimenter reliability at measuring muscle FL using US. We also found that 5 participants showed an increase in CK immediately post stretching, 2 showed an increase after 24 hours, and 4 showed increases after 48 hours, however there was no increase in muscle-CK after stretching above the upper bound of the LoA established in the control group, therefore the protocol employed did not lead to notable muscle CK increases used to indicate muscle damage.

Literature on the matter of biological variability of muscle-CK is inconsistent: demographic factors such as gender, age, ethnicity, as well as activity level all cause baseline CK variation, causing inconsistency in literature regarding acceptable levels of CK as a result of exercise (Baird et al., 2012b). The minimal detectable change and smallest worthwhile change for blood CK have previously been reported as 1.5 times baseline levels, or three times baseline levels in extreme cases, such as myocardial infarction (J. C. Cook, 1990). However, these measures increased at each time-point, meaning as time progresses, a higher rate of increase in CK is necessary to indicate muscle damage occurs due to intervention and not increases in biological variation. Literature reporting CK variation over time as a result of intervention shows increases in CK peak 24-72 hours after intervention occurs (Baird et al., 2012a). Therefore, the rate of extracellular flooding of blood CK as a result of exercise occurs exponentially up to 72 hours post training (Brancaccio et al., 2007). Our results, however, do not reflect this (Tables 4.1-4.4). The most conservative value of increase in muscle-CK calculated in individuals in the control group was used (312.4 μL /L or 24.4 μL /L; Table 3); This was done to inform future protocols in CP populations where determination

of level of muscle damage due to passive stretching is an outcome measure. The pattern of alteration in muscle-CK post stretching in was inconsistent; some participants showed no or minimal increase as a result of stretching, which supports the claim that the high velocity passive stretching protocol employed was not sufficient to induce muscle micro-damage in typically developing muscles as could be measured by the muscle-CK as employed in this study. Furthermore, a few participants showed values of muscle-CK decreased after 24 or 48 hours. Moreover, even when the smaller of the two conservative values for the upper bound of LoA was used, increase in muscle-CK was seen only in 2 participants. Bias due to missing data or selection of participants into the study might have also affected muscle-CK outcomes.

Collectively, the second criterion previously proposed for increasing FL during exercise – i.e., damage to muscle quantified by measuring muscle-CK using finger prick – was not met under current experimental protocol, and due to the number of missing values and bias which may have been introduced due to dropping one participant at the outset from the experimental group, these results were interpreted as being inconclusive.
It is clear from literature that individuals with CP respond differently to stretch than do typically developing muscles (Theis et al., 2013), this is however not an indication of whether the stimuli would be sufficient to induce muscle microdamage as a response to high velocity passive stretching in spastic muscles.

Fiber strain of the muscle-tendon unit (MTU) is an important factor predicting muscle damage, and greater mechanical strain associated with high force muscular contractions likely results in greater damage to contractile proteins (Morgan and Talbot, 2011, Proske and Morgan, 2004). Strain was calculated with respect to the length of fascicle at the most extended position of the knee prior to stretching. Under this experimental condition as the participants were lying supine with the knee joint aligned with the axis of rotation of the dynamometer, the muscles in the quadriceps and/or hamstrings may have been contracting slightly in expectation of the upcoming flexion movement. Therefore, strain values, rather than reflecting maximum strain possible in individual muscles, reflected maximum determined using alteration in the length of fascicles within the viewing probe under the experimental condition of the present study. Our findings suggest that a single bout of

passive stretching, following protocol employed in the current study, can induce positive fascicle strain in typically muscles, however there could be other than methodological factors that dictate the amount of strain that muscles/fascicles experience. The velocities employed were determined based off average angular knee velocities during walking in typically developing adults, therefore these may not have been high enough to induce fiber strain of more than 5 %. While all participants experienced positive fiber strain, only 4 experienced a maximum strain more than 5 %. This is likely due to the velocities employed; the average angular velocity of the knee during fast walking, jogging or running is higher than the velocities used for stretching (Mentiplay et al., 2018), therefore participants were likely accustomed to the movement. This was limited by the use of the isokinetic dynamometer, which has a maximum velocity of 300 °/s for continuous passive motion protocols.

ICC values, to assess experimenter reliability in measuring VL FL, showed strong correlations, supporting out hypothesis that the experimenter was reliable at measuring typically developing muscle FL while stretching. Experimental reliability to assess the effect of intervention is imperative. In contrast to healthy muscles, architecture of muscles in individuals with CP is different as represented by fascicle non-uniformity, and muscle belly volumes and lengths which are having been reported as 37 % smaller, and therefore their location can vary greatly from person to person (Barber et al., 2011b). Reliably measuring FL in CP is hugely important as increasing FL is an important outcome measure of interventions for the population. Due to the uniqueness of muscle shape in every participant with CP, reliability of measurement of FL (and/or other architectural parameters of interest) must be established in a number of baseline measurements prior to starting intervention to alter FL, and not confounded by the errors of measurement.

Conclusion

Whether muscle micro-damage could occur as a result of exercise was inconclusive; our findings suggest that a single bout of high velocity passive stretching following the protocol in the present study is not a sufficient enough stimulus to produce microscopic damage to the muscle, and therefore it would be unlikely to induce sarcomerogenesis within the

typically developing population. This was to be expected, and supports our expectation, as the velocities employed were average velocities exhibited during walking, which when done passively on a relaxed muscle, will be unlikely to cause damage due to the lack of reflex contraction in the population. However, this is not to say that high velocity stretching would have the same (null effect) result in spastic muscles, as it has been determined that individuals who present with spasticity respond differently to stretch due to the clasp knife phenomena, where a stretch induced reflex contraction occurs as a protective mechanism, which then gives way to the stretch. With the lack of research into how spastic muscle respond to stretch, specifically with changes in enzyme activity as a result of muscle damage, determining biological variation in a sample of convenience would be necessary to inform subsequent research studies within this thesis.

As three criteria that are necessary to increase muscle fascicle length were previously proposed, the present study explored whether positive fiber strain and muscle microdamage could be induced in healthy adults, to inform future protocols in spastic muscles. Muscle activation was not measured as it is known that activation patterns present differently in spastic muscles, therefore this would not be suitable. Future interventions in individuals with CP could use passive stretching at high velocities to establish whether these three criteria could be met in spastic muscles, and whether high velocity passive stretching could be used as an effective replacement for eccentric training within the population. The next chapter presents the undertaking of a protocol similar to the present study, in individuals with CP, to determine whether these three proposed criteria can in fact be met using the employed protocol, and to establish whether spastic muscle response to stretching is indicative of the sufficiency of using this as a replacement for eccentric training.

CHAPTER 5: MUSCLE RESPONSE TO PASSIVE STRETCHING IN CP

Abstract

This non-confirmatory pilot study investigated the acute muscle response to a single bout of high-velocity passive stretching in individuals with cerebral palsy (CP), with the aim of determining whether the three specified criteria in the previous chapters which are necessary to increase muscle FL can be induced in this population. These three criteria include: the occurrence of positive fibre strain, microdamage to the muscle, and momentary deactivation of the muscle during the stretch. Seven participants with spastic CP underwent a single session of high-velocity passive stretching targeting the quadriceps muscles. Muscle fascicle length, microdamage as a result of stretching, and any drop in muscle activation were assessed using B-mode US, muscle-CK measures and EMG, respectively. CK measures were taken at three timepoints: before, immediately after, 24 hours and 48 hours post intervention. Results demonstrated that there were no significance differences in the muscle-CK values at the baseline between CP and TD individuals ($t(13) = -0.020$; $p = 0.985$; CI [-74.79 – 73.43]), however muscle-CK after stretching was lower than the upper bound of the LoA (312.4 or 24.4 µL /L) established in the previous chapter. Whether microdamage was induced was inconclusive, as some participants showed greater increases as a result of stretch, while others showed no increase. Positive fibre strain (maximum fibre strain ranging between 0.67 % and 45.27 %) and a drop in muscle activation (of >25 % baseline activation occurred between 180 ms and 360 ms into the flexion period) were shown in all participants to varying degrees. This study provides novel insights into the acute effects of high-velocity passive stretching on spastic muscles in CP. The findings suggest that this approach may allow the three presumed criteria necessary for the increase in FL to be met, warranting further investigation as to whether HVPS done over a longer time period could lead to increases in FL, and whether those increases lead to functional improvements in individuals with CP.

Introduction

Muscle fascicle length has been suggested as the single most important architectural parameter of a muscle affecting its function (Lieber and Friden, 1993). Many motor disabilities with neurological origin such as CP (CP), which present clinically with spasticity

and contracture, are associated with relatively shorter muscle fascicles compared to matched healthy controls (Mohagheghi et al., 2007, Mohagheghi et al., 2008, Theis et al., 2013, Theis et al., 2015) that could contribute to impaired movements often observed within this population. Therefore, increase in muscle fascicle length (FL) maybe be a desirable outcome for individuals with CP, as it could improve functional abilities by altering the length-tension and force-velocity characteristics of the muscle, increasing muscle-tendon unit (MTU) compliance, and threshold of the tonic stretch reflex (Proske and Morgan, 2001, Trompetto et al., 2014). Despite several reports of increases in the length of muscle fascicles in response to various interventions in healthy humans and animals (Sharifnezhad et al., 2014b, Goldspink et al., 1974), necessary and sufficient exercise conditions to consistently increase FL through increases in serial sarcomere number (SSN) in humans are yet to be determined. The situation is less clear for spastic muscles due to CP. Intervention is spastic muscles often involve the use of passive stretching to increase muscle fascicle length, such as Theis et al., (2013) who found a significant increase in FL of 0.6 cm post stretching, as well as an increased ROM, fibre and tendon elongation.

Evidence from literature supports the notion that (long term/chronic) training may result in increase in muscle FL in healthy animals and humans (Butterfield et al., 2005b, Blazevich et al., 2007a, Potier et al., 2009), however, this is likely to occur when specific exercise and training conditions are provided (Lynn and Morgan, 1994a). As previously proposed, three criteria for such optimal training conditions may exist: 1) eccentric exercise at appropriately high velocity to strain fibers, leading to 2) microscopic damage of the stretched fascicles, and 3) momentary deactivation of the elongated/stretched muscle during exercise (Davis et al., 2020). The hypothesis was based on the principle of exercise induced muscle damage triggering sarcomerogenesis: i.e., increase in sarcomere number triggered by muscle fibre damage caused by increased loading, specifically during eccentric contraction (Morgan and Talbot, 2011). Three measurable outcomes to examine viability of our proposition, when exercising or training under these presumed optimal conditions include: positive fascicle strain, increased level of a blood biomarker (e.g., muscle-CK) representing microscopic damage to muscle cells, and drop in muscle electrical activity (EMG) representing a period of reduced activity.

Whether such (theoretical) optimal training conditions could be consistently induced in healthy muscles, and lead to increase in the length of muscle fascicles has not been determined yet and was the subject of another study by the present author (Chapter 4). It was established in the previous chapter that positive fibre strain can be induced in healthy muscles, but whether the single bout of high velocity passive stretching could induce muscle microdamage was inconclusive. Importantly, eccentric training of spastic muscles at high velocity may be limited by the lack of selective motor control in individuals with CP (Komi et al., 1987). In general, behaviour of spastic muscles and fascicles during exercise is not well understood (Reid et al., 2010, Morris, 2007, Bar-On et al., 2014, Kalkman et al., 2019) and it is not clear if the proposed training conditions for healthy muscles can be successfully created in exercise laboratories to train spastic muscles. Answering these questions can lay the foundation for devising training interventions to increase muscle FL in CP.

In addition to problems faced by many individuals with CP to selectively recruit muscles during exercise, relative stiffness of the muscle and tendon can affect behaviour of the spastic muscle fascicles during exercise and training. During stretching of the spastic MTU, reflex contraction may oppose stretch of the muscle and fascicles, as spastic muscles have a hyperactive response to stretching, especially at higher velocities compared to their typically developing peers. In many individuals, such resistance may suddenly give way and disappear with continual attempt to elongate MTU (clasp-knife phenomenon). Continual reflex contraction of the MTU during stretching, therefore is theoretically similar to eccentric exercise.

The present study, as a continuation of the previous chapter, was designed to provide an insight into the acute behaviour of spastic muscle fascicles in response to a specific form of exercise. Accordingly, we examined whether the three presumed criteria for increasing FL can be met in spastic muscles using a single bout of HVPS. It is assumed that passive stretching of the spastic MTU at an appropriately high velocity would induce (stretch) reflex contraction, and hence, resemble eccentric exercise in healthy individuals. In a group of individuals with CP, the behaviour of the spastic muscle fascicles during one session of passive stretching were monitored, and variations in the muscle-CK over three days.

As stated above, based on the reported response of FL to interventions in CP, positive fibre strain of 5 % during exercise was subjectively selected as sufficiently large.
It was expected that sufficient large positive fibre strain would be observed in spastic muscle fibres during exercise that stretch the MTU at relatively high velocities, that stretch reflex contraction would occur as a result of stretching the MTU at relatively high velocities, and that there would be an increase in blood-muscle CK as a result of training due to microdamage to the muscle.

Methodology

The details of the experimental protocol are provided in the General Methods section (Chapter 3).

Participants

7 individuals with CP (CP) (1 female, 6 male, mean (SD) age 21 +/- 9.47) volunteered to participate in this study. CP individuals were either hemiplegic (n=2) or diplegic (n=5), all level I-III on the Gross Motor Functioning Classification System, and were recruited through the Royal National Orthopaedic Hospital (RNOH). Ethical approval for conducting this study was obtained from Brunel University London College of Health, Medicine and Life Sciences Research Ethics Committee (Appendix C), and from the NHS Research Ethics Committee. All participants provided written informed consent to participate in this study. The original sample size outlined for this non-confirmatory pilot study was 15, however due to Covid-19 and time limitations, recruitment of participants ceased in order to continue the research series.

Procedure

Participants with CP were recruited to monitor behaviour of spastic quadriceps muscle fascicles during stretching at different velocities to examine whether the 3 presumed criteria for increase in the length of muscle fascicles with exercise could be observed in an exploratory one-off study. Participants attended the RNOH for gait analysis on an instrumented treadmill. The details of participants' knee joint velocities during gait were

used for individualising experimental protocol, allowing the velocities of stretching to be appropriately high, while remaining safe. Participants then attended the Biomechanics Laboratory at Brunel University London on three consecutive days at the same time of day, and were required to refrain from strenuous exercise for four days prior to attending the laboratory.

Data processing and analysis

Determining fiber strain

The US scanning was started prior to the beginning of the three experimental rotations. Time lag between start of US scanning and synchronous recording of the EMG and dynamometry data was between 70ms and 90ms (scan rate was 40 frames per second). No further action for aligning EMG, dynamometry and US data was required as scans taken prior to the rest of data were at rest.

Determining changes in muscle activation

EMG was recorded to establish whether a) reflex contraction would occur in the stretching quadriceps during knee flexion; and b) whether such reflex contraction would subsequently subside (drop to the baseline muscle activity or below it). EMG data was demeaned to remove DC offset, rectified and smoothed based on an average of three consecutive data points in *Spike2* before being exported as a text (.txt) file, and analysed using a custom-written algorithm in MATLAB (Appendix D).

Baseline muscle activity was defined as the Average Rectified Value (ARV) calculated over the duration of knee extension. The first and last 100 ms of the dynamometry extension period were not included as part of the extension period for the calculation of ARV as it was associated with the changing direction of the dynamometry input arm from flexion to extension, or vice versa, at the end of flexion and extension phases, respectively. Muscle activity during this time could have also been affected by expectation of the movement coming to a halt prior to change in the direction.

Muscle activity (ARV) during knee flexion was calculated and examined using successive (non-overlapping) epochs of 60 ms for the duration of the flexion phase [23]. Reflex contraction was identified when ARV for an epoch of 60 ms was larger than the baseline ARV by 25 % (i.e., >baseline + 25 % baseline), to be conservative, while still indicating noticeable changes in activation. Muscle was considered as "inhibited" if any increased ARV activity dropped back to the baseline in any epoch of 60 ms. Muscle was considered as "deactivated" if the ARV dropped below the baseline by 25% (i.e., <baseline – 25%baseline) in any epoch of 60 ms. It was expected that reflex contraction would be observed during knee flexion in the spastic muscles. No prediction was made with regard to whether the reflex contraction would be maintained, or inhibited/deactivated throughout the flexion phase in this group. However, the former situation, if happened, would mimic eccentric exercise, while the latter situation, would be similar to the clasp-knife phenomenon in CP.

Determining changes in muscle-CK

Level of blood muscle-CK was measured to establish if any potential muscle microdamage, which could occur as a result of stretching in individuals with CP, could be identified by our minimally invasive technique of finger pricking. To this end, one measure of blood muscle-CK was obtained from participants at each time-point. The standard deviation of changes in the values of blood muscle-CK (change score) of participants between pre- and post-stretching, pre-stretching and 24-post, and pre-stretching and 48-post, was used in the calculation of three standard error of measurements (SEM).

Statistical analysis

An independent samples t-test was run on the baseline CK values to determine differences in CP and TD individuals (data from the previous chapter). The change in mean at each timepoint was calculated, along with the coefficient of variation and the standard error of measurement. A multivariate ANOVA (Group (2) x Time effect (4)) was then run on the change in score at each timepoint for TD and CP individuals using between groups as the main effect. The alpha level of significance was selected as 0.05 ($\alpha = 0.05$).

Results

Muscle-CK

Muscle-CK measures for the mean of each time-point in individuals with CP can be seen in Table 7 expressed in microliters per litre of blood (uL/L). Changes in the mean values from baseline (Δscore) can be seen in Table 8.

Table 7 – Mean muscle-CK values for each time-point

P no.	pre	post	24hrs	48hrs
3	191	179	144	78.2
4	39.5	-	71.7	51.6
6	56.3	-	48	58.1
7	173	199	217	192
9	160	121	-	145
12	137	195	114	88.1

(*P no. = participant number, pre = CK values pre-stretching, post = CK values post-stretching, 24hrs = CK values 24 hours post stretching, 48hrs = CK values 48 hours post stretching).*

Table 8 - Changes in muscle-CK mean values at each time-point

P no.	Δscore post-pre	Δscore 24hrs-pre	Δscore 48hrs-pre
3	-12	-47	-112.8
4	M	32.2	12.1
6	M	-8.3	1.8
7	26	44	19
9	-39	-160	-15
12	58	-23	-48.9

(*P no. = participant number, Δscore post-pre = change in the mean of the values from Pre to Post time point, Δscore 24hrs-pre = change in the mean of the values from Pre to 24 hours*

time point, Δscore 48hrs-pre = change in the mean of the values from Pre to 48 hours time point. Muscle-CK values are in μL */L, M = missing datum.*

Two participants showed an increase in muscle-CK levels immediately post intervention, but this is not consistent for all participants. At Post-48 time point, three participants show an increase to pre-stretching, compared with only two at Post-24. Measures of muscle-CK variability are presented in Table 9. As a group, participants show an overall small increase in muscle-CK levels immediately post intervention. At Post-24 and Post-48 time points, however, muscle-CK values were smaller than the baseline values.

Table 9 - Measures of muscle-CK variability

Measure	Post-Pre	24-Pre	48-Pre
Δ in Mean	8.3	-8.7	-39.4
SD	36.9	38.5	48.7
SEM	26.1	27.2	34.4
CV	20.00%	20.00%	30.00%

(Δ in Mean = change in mean at each time point, SD = standard deviation of Δ in Mean, SEM = Standard Error of Measurement, CV = Coefficient of variation. Post-Pre = mean change of the values measured at Post, 24-Pre = mean change of the values measured at Post-24, 48-Pre = mean change of the values measured at Post-48.)

At all time-points, increase in muscle-CK after stretching was lower than the upper bound of the LoA (312.4 or 24.4 μL /L) established based on typically Control participants in Chapter 4, therefore MDC and SWC were not met.

An independent samples t-test on typically developing EG participants (Chapter 4) and CP participants showed no significance differences in the muscle-CK values at the baseline between individuals in the two groups ($t_{(13)}$ = -0.020; p = 0.985; *CI* [-74.79 – 73.43]). Moreover, there was no difference in their muscle-CK response to stretching (Table 10). For this latter analysis, muscle-CK change scores at different time points with respect to

baseline were used in the ANOVA with baseline measures from participants entered into the model as covariates. Group (CP vs TD) was the between-groups factor. Results of the analysis are shown in Table 10.

Table 10 - Results of multivariate ANOVA on change scores (Δscore) between CP and non-CP (EG – Chapter 3) at different time points (post, after 24 and 48 hours)

Statistic	df	Mean Square	F	Sig.	η_p^2
Time Point	2	4510.90	1.98	0.17	0.17
Time Point x Baseline	2	3428.98	1.50	0.25	0.13
Time Point x Group	2	5886.62	2.58	0.10	0.21
Error (Time Point)	20	2281.36			

Fiber Strain

Table 11 shows maximum strain observed during stretching of the MTU and associated speed at which the maximum strain occurred. All participants experienced positive fibre strain in response to passive stretching at different velocities, however, increase of 5 % or more in the length of fascicles at any time during stretch (i.e. flexion of the knee joint) with respect to its initial (origin) length (i.e., when the knee joint was at its maximum extension angle) was observed in limited number of participants. As illustrated in table below, four participants experienced maximum positive fibre strain of 5 % or more during stretching. Maximum fibre strain ranged between 0.67 % and 45.27 % in this group. Majority of maximum strain recorded at relatively lower velocities (speeds 1-3; i.e., 50-75 % above the average knee angular velocity recorded during walking at self-selected speed in CP). There was also large within group variations in these responses to stretching protocol, and the higher number for the CP group was driven by one participant (participant 3; 45 %). For this participant, maximum strain of other trials was 4 %.

Table 11 – Vastus Lateralis maximum fibre strain and the velocity they occur at

Vastus Lateralis FL Strain in CP		
Participant	Speed	Max Strain
3	5	45.27%
4	1	1.54%
6	3	6.25%
7	3	9.14%
9	3	8.15%
12	1	2.68%
13	5	0.67%

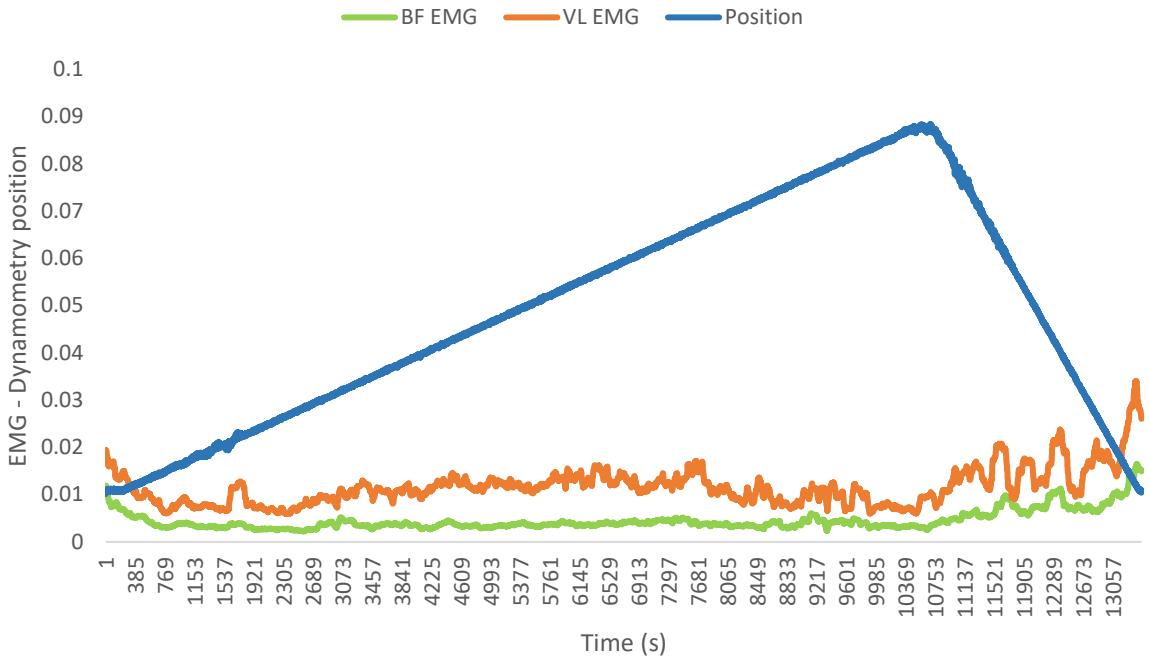
(*Speed = velocity at which the largest fascicle strain occurred is reported. 1 being the slowest and 5 being the fastest velocities used during knee flexion. Max Strain = maximum FL strain observed during knee flexion expressed as a percentage of VL origin length)*

VL Muscle activity during stretching

An example of the EMG output from a single rotation in a participant with CP can be seen in Figure 2. Positive and longer slope of the position trace, represents extension of the knee joint from its most flexed position to full extension which occurred at constant velocity of 15 ◦/s. Negative and shorter slope of the position trace represents flexion of the knee.

VM muscle activity during knee extension was expected to be minimal in CP participants, as VM was shortening during knee extension and assuming that participant could follow the instructions and remain relaxed. It was expected that a reflex contraction would occur, particularly at higher velocity(s) of joint flexion (lengthening of the MTU), which could be interspersed with periods of decreased activity (deactivation or inhibition) due to clasp-knife phenomenon.

Figure 2 – the EMG of the VM and BF plotted superimposed onto the position of the shank represented by dynamometry data



Out of the three experimental rotations, the final two were analysed for the results section. An example of EMG activity during the flexion period for VM and BF every 60 ms can be seen in Table 12. State of VM and BF muscle activity during stretching, i.e. during flexion of the knee joint, at all speeds are illustrated in Table 13. VL reflex activation for at least one epoch of 60 ms did not occur at two speeds (Speed 1 and Speed 3) in one participant (P7), and at the slowest velocity of rotation (Speed 1) in two other participants (P12 and P13). All participants experienced at least one epoch (60 ms) of increased activation of the VM during knee flexion at higher velocities (Speed 4 and/or Speed 5) in at least one of the experimental rotations.

All participants but one (P9), in whom reflex contraction was maintained throughout joint flexion at the highest velocity (Speed 5), experienced at least one epoch of inhibition and/or deactivation. The inhibition/deactivation occurred between 180 ms and 360 ms into the flexion period. EMG was not measured in Protocol 2 as healthy individuals were not expected to experience a reflex contraction during passive stretching.

Table 12 – An example of EMG data collected from a participant during flexion in 60 ms epochs

Vastus Medialis and Biceps Femoris EMG Activity																		
Speed	Rotation	Baseline	AVR	60	120	180	240	300	360	420	480	540	600	660	720	780	840	900
1	R2_EMG2	0.012	0.014	0.014	0.012	0.010	0.013	0.014	0.015	0.015	0.013	0.012	0.012	0.011	0.011	0.014	0.015	0.014
1	R2_EMG3	0.004	0.006	0.004	0.004	0.004	0.005	0.004	0.005	0.005	0.005	0.004	0.005	0.006	0.006	0.006	0.006	0.005
1	R3_EMG2	0.008	0.016	0.009	0.010	0.013	0.014	0.015	0.015	0.013	0.020	0.013	0.013	0.017	0.013	0.014	0.021	0.019
1	R3_EMG3	0.003	0.008	0.004	0.004	0.005	0.005	0.006	0.006	0.005	0.008	0.009	0.007	0.006	0.007	0.008	0.010	0.009
2	R2_EMG2	0.004	0.008	0.003	0.005	0.005	0.004	0.005	0.007	0.006	0.005	0.005	0.006	0.006	0.005	0.007	0.010	0.009
2	R2_EMG3	0.002	0.004	0.001	0.001	0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.002	0.003	0.002	0.003	0.004	0.003
2	R3_EMG2	0.004	0.008	0.006	0.005	0.005	0.010	0.005	0.008	0.007	0.007	0.006	0.006	0.005	0.004	0.008	0.006	0.005
2	R3_EMG3	0.002	0.003	0.002	0.002	0.002	0.002	0.003	0.002	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
3	R2_EMG2	0.011	0.018	0.013	0.009	0.010	0.014	0.014	0.015	0.016	0.017	0.017	0.013	0.018	0.027	0.016	0.015	0.020
3	R2_EMG3	0.004	0.007	0.004	0.003	0.004	0.005	0.004	0.004	0.004	0.007	0.004	0.006	0.005	0.005	0.007	0.006	0.009
3	R3_EMG2	0.009	0.014	0.013	0.011	0.009	0.013	0.011	0.017	0.010	0.010	0.019	0.012	0.009	0.015	0.017	0.010	0.027
3	R3_EMG3	0.003	0.006	0.002	0.003	0.004	0.004	0.004	0.003	0.004	0.004	0.005	0.004	0.004	0.004	0.006	0.005	0.013
4	R2_EMG2	0.015	0.019	0.012	0.016	0.015	0.016	0.017	0.016	0.017	0.013	0.021	0.017	0.011	0.021	0.018	0.020	0.015
4	R2_EMG3	0.005	0.007	0.004	0.004	0.006	0.005	0.003	0.005	0.005	0.005	0.006	0.006	0.008	0.006	0.006	0.006	0.007
4	R3_EMG2	0.015	0.021	0.016	0.016	0.014	0.016	0.020	0.019	0.016	0.026	0.028	0.020	0.015	0.019	0.032	0.023	0.030
4	R3_EMG3	0.004	0.008	0.005	0.005	0.004	0.007	0.005	0.005	0.004	0.006	0.008	0.011	0.008	0.012	0.010	0.010	0.019
5	R2_EMG2	0.003	0.008	0.004	0.002	0.002	0.003	0.003	0.005	0.007	0.005	0.004	0.005	0.006	0.008	0.009	0.011	0.018
5	R2_EMG3	0.002	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.005	0.005	0.007
5	R3_EMG2	0.002	0.007	0.003	0.003	0.002	0.003	0.003	0.004	0.006	0.005	0.004	0.005	0.005	0.007	0.009	0.012	0.015
5	R3_EMG3	0.001	0.004	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.006

(Speed = which speed was used (1-5), Rotation = which of rotations (1-3) is reported, EMG2 = Vastus Medialis, EMG3 = Biceps Femoris, Baseline = average EMG activity during extension period, ARV = average rectified value of the baseline, 60-900 = time interval during flexion in milliseconds.)

Table 13 – State of VL muscle activity during knee flexion at different velocities

	Speed 1	Speed 2	Speed 3	Speed 4	Speed 5	Candidate
P3	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y	C
	Y:N	Y:Y	Y:Y	Y:Y	Y:Y	
P4	Y:Y	Y:Y	**N:N**	Y:Y	Y:Y	C
	Y:Y	Y:Y	Y:Y	**N:N**	Y:Y	
P6	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y	C
	Y:Y	Y:Y	Y:Y	Y:Y	Y:Y	
P7	**N:N**	Y:Y	**N:N**	**N:N**	Missing	UNDECIDED
	N:N	**N:N**	**N:N**	Y:Y	Missing	
P9	Y:Y	Y:Y	Y:Y	Y:Y	Y:N	C
	Y:Y	Y:Y	Y:Y	Y:Y	Y:N	
P12	**N:N**	N:Y	N:Y	N:Y	Y:N	C
	N:N	Y:N	N:Y	**N:N**	N:Y	
P13	N:Y	**N:N**	**N:N**	**N:N**	Y:N	C
	N:N	Y:N	Y:N	Y:N	Y:N	

(*The first letter (Y or N) in a pair, shows whether or not flexion of the knee joint resulted in increased muscle activation (reflex contraction) for at least 60 ms anytime during joint flexion (i.e., increased ARV above the threshold for at least one epoch of 60 ms). The second letter (Y or N) shows whether or not any such increased activity was followed by at least 60 ms of inhibition/deactivation. The two rows of results for each participant belong to the last two experimental rotations used in the analysis. Based on state of muscle activity during stretch, it was decided whether a participant was candidate for training (C). Participant 7 did not tolerate speed 5, and therefore data is missing for this condition.)*

Discussion

The purpose of this exploratory, non-confirmatory study (Scheel et al., 2021) was to examine whether the three presumed criteria for increasing muscle fascicle length in healthy muscles (Davis et al., 2020), associated with high-velocity eccentric training, could be met by undergoing passive stretching of spastic muscles at (appropriately) high velocities. This was done by examining the strain of the fascicles, blood markers of muscle

damage, and muscle electrical activity. The present study assumed passive stretching of the spastic MTU at an appropriately high velocity would induce (stretch) reflex contraction, and hence, with continuation of stretching throughout the available range of motion resemble eccentric exercise in healthy individuals. In a group of individuals with CP, the behaviour of the spastic muscle fascicles during one session of passive stretching, and variations in the muscle-CK over three days were monitored.

Based on arguments formed in the previous chapters of this thesis, it was expected that the experimental protocol would be associated with the following observations: Positive fibre strain (≥5 %) in all spastic muscles during exercise involving stretch of MTU at (appropriately) high velocity; Passive stretching of the MTU at (appropriately) high velocity would not be associated with increased EMG activity during the stretch of the healthy MTU, but would induce detectable stretch reflex contraction in the spastic MTU, which may resemble clasp-knife phenomenon.

Fascicle length strain of the MTU fascicles is an important factor predicting muscle fiber damage with stretching. Greater mechanical strain associated with high muscular contraction force, similar to the condition of exercise under eccentric regime, likely results in greater damage to contractile proteins (Morgan and Talbot, 2011, Proske and Morgan, 2004). Stretching of the MTU is commonly practiced within CP, to maintain or improve available range of motion and with the added assumption that maintaining stretch around the extreme of ROM of the joint may result in the increase of muscle fascicles in the long term (Theis et al., 2013, Theis et al., 2015, Holly et al., 1980, Coutinho et al., 2004a). All participants with CP who underwent stretching experienced positive fascicle strain to varying degrees (Table 5.5), suggesting the current experimental protocol may provide minimal requirement of regular stretching interventions for increasing ROM and FL with no adverse effect.

For obtaining higher strain in the MTU during flexion of the joint and creating larger possibility of inducing microscopic damage to the muscle during exercise, fascicle length was measured with the participants lying supine with the knee joint off the plinth of the dynamometry system, but ROM limited to maximum available or 90 °, whichever less. For procedural convenience, probe of ultrasound scanner attached to the middle of the thigh –

regardless of the person-specific shape and orientation of the VL. Strain was calculated with respect to the length of fascicle at the most extended position of the knee prior to stretching. Under this experimental condition, many of the muscles were either contracting slightly in expectation of the upcoming flexion movement or experiencing continued reflex contraction from previous stretch (pre-loading). Therefore, strain values, rather than reflecting maximum strain possible in individual muscles, reflected maximum determined using alteration in the length of fascicles within the viewing probe under the experimental condition of the present study. This is a limitation when using surface US, as it does not consider the 3-dimensional nature of this structure, and does not take into account the curvature of the fascicle. Strain calculations also require the complete relaxation of the muscle, i.e, no passive tension exerted on the muscle in question; we know this was not the case, as reflex contractions were apparent throughout the stretch, and participants lack selective muscle recruitment, therefore baseline resting measures of FL cannot be considered absent of tension or compression forces on the muscle.

As described in the Results section, there was variation in person-specific responses to stretching protocol. It was speculated that transient alterations in the state of muscle activity in participants with CP, also contributed to large within and between subject variations in the outcome (Khalili and Hajihassanie, 2008).

Our findings suggest that a single session of passive stretching, following protocol employed in the current study, may induce positive fascicle strain in spastic muscles, however there could be other methodological factors that dictate the amount of strain that muscles/fascicles experience. Level of spasticity, or length and relative compliance of the muscle compared to the tendon being stretched might have contributed to the observed values in CP (Trompetto et al., 2014). It has previously been reported that compliance of the spastic tendon may be higher than its corresponding muscle (Barrett and Lichtwark, 2010, Lorenzo et al., 2017), which may mean the relative stretch experienced by the spastic tendon could be higher than the spastic muscle/fascicles during exercise, and lead to less fiber strain and subsequent damage. Kalkman et al., (2019) reported that although the tendon lengthens more than the in-series muscle during stretching, combining resistance training with stretching can increase tendon stiffness, resulting in FL increases in spastic

muscles (Kalkman et al., 2019). The combination of passive stretching while a reflex contraction is ongoing, mimicking an ECC contraction without conscious participation, could mean that tendon stiffness is increasing, allowing the stretch to be transferred to the muscles. Positive fiber strain during stretching is assumed to be required for damage to a muscle and trigger sarcomerogenesis (Davis et al., 2020, Butterfield et al., 2005a, Wirtz et al., 1988). Observed alterations in FL during exercise, partially satisfy the first of three presumed criteria for increasing FL with training in this population (Davis et al., 2020), but it is possible that combined methodological and person-specific factors determine whether or not an individual with CP is responsive to passive stretching training at appropriately high velocity with time.

As presented previously in Chapter 4, it has been established that there is huge person to person variability in muscle-CK; factors such as gender, age, ethnicity, as well as activity level all cause baseline CK variation. Results in the present study to establish whether a single bout of passive stretching was sufficient to induce muscle microdamage were inconclusive. Some participants showed increases in blood-CK post stretching, while others did not; although there was a small group increase, this was not above the limits of agreement established in Chapter 4, and therefore it cannot be conclusively determined if the stimulus was sufficient to induce muscle microdamage.

As expected, individuals with CP experienced the occurrence of reflex contractions during stretching at the highest speeds. Such contraction is assumed to be a protective mechanism that occurs in spastic muscles to prevent muscle damage occurring due to overstretching (Goldspink et al., 1974, Tabary et al., 1972). The reflex contractions during passive stretching protocol throughout ROM imposed by the dynamometry system indicated that muscle was resisting the stretch, and therefore the appropriately high velocity passive stretching exercise protocol in CP could be considered similar to eccentric exercise in healthy participants or those who can voluntarily activate the stretching muscle to resist stretching. While eccentric training in spastic muscles may not be possible due to inability of the participants to selectively control the stretching muscle during exercise, the present study suggests an experimental paradigm for eccentric training within the CP population with no requirement for their active participation, within the limitations highlighted above. This

could be hugely beneficial to the population, as eccentric training may increase muscle fascicle length, and subsequently alter force-length and force-velocity characteristics of the MTU and lead to improved effectiveness and efficiency of muscle capacity for producing force during functional activities.

Reflex contraction which occurred during passive stretching at the relatively higher velocity (velocities 4 and 5) was followed by a drop in the activation of muscle in all but two participants (Table 13). Drop in the reflex muscle activation could be attributed to the clasp-knife phenomena, in which the muscle gives way to the stretch to prevent further damage to the muscle. This could indicate that the individuals who experienced reflex contraction followed by a drop in the activation may benefit from repeated sessions of passive stretching training, as the muscle would be sufficiently damaged with repeated episodes of eccentric-like stretch. Such training, could theoretically lead to sarcomerogenesis, and subsequently increases muscle FL, and induce functional adaptations in these individuals. Further investigation is required to establish if our proposed experimental paradigm, which may mimic eccentric training in typically developing population via long-term high velocity passive stretching could actually lead to increased muscle FL and result in functional adaptations. Moreover, it seems current experimental protocol can satisfy the third of suggested criteria, i.e., momentary drop in the level of activity of stretching muscle, for increasing FL with training in CP (Davis et al., 2020).

Conclusion

Data presented here partially supports that at least two of the three presumed criteria necessary to increase healthy muscle fibre length can be induced using a single bout of high velocity passive stretching in individuals with CP. Whether muscle micro-damage could occur as a result of exercise was inconclusive. We claim as muscle activation patterns observed in the present study showed consistent reflex contraction (activation) followed by activation inhibition, characterised by a drop in muscle activation, the employed experimental paradigm could be an effective substitute for eccentric training in individuals who lack selective muscle recruitment. The nature of spasticity and reflex contractions mean that even during passive stretching with no conscious input from the participant, the

muscle will still be contracting, making the movement eccentric. The outcome of this over time, however is not known. Future interventions in individuals with CP could use the present passive stretching protocol at appropriately high velocities as a replacement for eccentric training, with the view to increases muscle fibre length and improve function over time; this concept is presented in Chapter 6. The next chapter explores the inconsistencies in the effectiveness of eccentric training programs in typically developing individuals, with specific reference to Sharifnezhad et al., with the aim of establishing the repeatability of their findings, and determining whether their hypothesis with respect to outcomes not measured, are in fact true.

CHAPTER 6: THE EFFECTS OF HIGH VELOCITY ECCENTRIC TRAINING IN HEALTHY YOUNG ADULTS

Abstract

The present study aimed to replicate the results of a high-velocity eccentric training protocol in healthy young adults, as conducted by Sharifnezhad et al. (2014), which led to an increase in muscle fascicle length (FL). Importantly, the study examined the claim made by Sharifnezhad et al. (2014) that a decrease in peak eccentric torque toward the end range of joint movement during training—expected according to the torque-angle relationship—was associated with a decline in electrical activity of the prime mover muscle. This decline is one of the presumed criteria necessary for increasing fascicle length in response to training, as proposed in previous chapters.

Twelve healthy participants underwent high-velocity eccentric training on their left leg, with their right leg acting as a control over a 10-week intervention period. Participants performed 5 sets of 16 repetitions of knee extensor eccentric contractions at 240 °/s, by resisting the flexing movement of an isokinetic dynamometer moving in passive mode. Pre- and post-intervention measurements included quadriceps maximal isometric strength at 4 different knee joint angles, vastus lateralis fascicle length changes over time and between legs, and changes in the muscle activity of the quadriceps and hamstrings during eccentric contraction using EMG. Results showed the difference between isometric torque changes of control and exercise legs was significantly larger at angle 3 (65 °) compared to angle 1 (15 °): Mean difference = 0.021; 95%CI [0.001 – 0.041]. There was a significant difference between pre-intervention (PRE) at follow-up (FUP) sessions for the FL ($t_{(11)}$ = -2.292; p = 0.043). A significant increase in the joint angle at which maximum ECC torque occurred was observed, and maximum ECC torque at 90 ° increased significantly between weeks 2 and 10 ($t_{(11)}$ = -3.49; p = 0.005).

Consistent with the results of Sharifnezhad et al. study, these findings suggest that high-velocity eccentric training may be a highly effective method for enhancing muscle strength, and promoting fascicle length adaptations in healthy young adults, and changing the length-tension characteristics of the muscle. This training modality could be particularly beneficial to those with markedly shorter FL such as those with spastic muscles. Future research should explore the long-term effects and potential applications in various populations and clinical settings.

Introduction

The use of eccentric training (ECC) in humans has successfully led to alteration in muscle strength and morphology, including increase in muscle fascicle length (FL)(Franchi et al., 2014, Franchi et al., 2017, Reeves et al., 2009a). The specific training conditions which are needed to consistently increase FL however, however have been difficult to establish due to variations in reported methodologies.

As stated in the previous chapters, Sharifnezhad et al. (2014), was one of the first groups to test the effects of different ECC protocols on muscle morphological changes. This group found that even with a consistently high load, the only training condition that resulted in a significant increase in VL muscle FL was the high (240 °/s) lengthening velocity. Importantly, the researchers also reported a drop in the knee joint moment during muscle lengthening only in this condition (Sharifnezhad et al., 2014b). The group attributed the drop in moment to a momentary deactivation of the VL muscle during the lengthening contraction which led to the assumption that in order to successfully increase muscle FL in healthy muscles, the velocity needs to be (appropriately) high enough to elicit a drop in muscle activation during the lengthening contractions. Despite the claim, Sharifnezhad et al. did not directly measure muscle activity during their training intervention.

More recently however, Marzilger et al. (2019), investigated how different lengthening velocities affect muscle morphological changes over an 11-week ECC training period. The program involved training at 4 different angular velocities, and measured FL before and after the training period. This group found that there was no significant difference in the increase in FL observed among groups, meaning that ECC training lengthening velocity did not affect observed increases in muscle FL. They concluding that lengthening velocity was not the modulating factor with regard to increases in muscle FL, but the high mechanical demand of eccentric loading (Marzilger et al., 2019). This directly contradicts the findings of Sharifnezhad et al., with respect to the effect on FL, and therefore, the ECC training conditions necessary to successfully increase FL consistently warrant further investigations.

A reason for the inconsistency in the conclusions made by the authors of these two studies, on the velocity-dependency or independency of increase in FL with ECC training, could be due to the selection bias (depletion of the susceptible) in Sharifnezhad et al. (2014), where a significant number of participants (approximately 40 %) did not complete the training period. Marzilger et al. (2019) did not provide any further explanation on the possible reasons underlying differences between the results of the two studies which belonged to the same research group.

Since its publication in 2014, Sharifnezhad et al. Protocol 4 has not been replicated. The purpose of this study was to not only establish the repeatability of Sharifnezhad's findings that maximal effort high velocity ECC leads to increases in FL, but also to verify their suggestion of momentary muscle deactivation during the lengthening contraction of the quadriceps MTU, using electromyography. As a continuation from the previous experimental chapters (Chapters 4 and 5) the present study aimed to establish if an increase muscle FL and strength could be achieved over a prolonged ECC training period, as opposed to a single-bout of high velocity stretching which was previously presented. It was hypothesized that ECC training, using a protocol similar to Protocol 4 in Sharifnezhad et al., (2014), study will increase VL FL, and there will be deactivation/inhibition of the VL muscle post ECC peak torque during training as proposed by them.

Methods

The experimental procedure for all the measures in this chapter can be found in the General Methods section (Chapter 3).

Participants

Twelve healthy adults (6 female, 6 male, mean (SD) age 26 +/- 8, mean (SD) mass (kg) 66.85 +/- 14.5) were recruited from the students and staff of Brunel University London. All participants were free from any lower limb musculoskeletal injury, and were asked to refrain from participating in any other form of lower limb strength training during the duration of their participation. Ethical approval for conducting this study was obtained from

Brunel University London College of Health, Medicine and Life Sciences Research Ethics Committee (Appendix E).

Experimental Design

All participants attended the laboratory at Brunel University London for the pre-training session 5-7 days before the training began. For all participants the pre-training session involved baseline measures of isometric strength of the quadriceps at four different angles (15 °, 40 °, 65 ° and 90 °) on each leg. Each participant was instructed to push as hard as they can to their maximum force for two seconds and maintain this maximal contraction for three seconds; they had three trials at each angle followed by a three-minute rest period. The order of the angles was randomized in excel. During the three-minute rest period, VL FL measures were taken using ultrasonography at each angle to establish pre-training FL at each angle, and to determine if changes occur between legs as a result of training.

All participants then underwent three training sessions per week for ten weeks. Electromyography measures were taken on the VM and BF every 6th session to establish muscle activation during one training set. An isometric maximal voluntary contraction (iMVC) strength retest was undertaken every 7th session on the training leg to determine strength improvements as a result of training, and to ensure the load increases appropriately. Maximum torque measured during the iMVC was then used as a target for training; participants were all encouraged to hit this target during every repetition of the training period. If maximum torque did not increase during an iMVC retest, the highest torque measured during the training period was used as the target for training until the next retest.

After the 10-week training period was completed, participants were invited back 7-10 days after the last training session to undergo a follow up session. During this, iMVC measures were taken on both the training and control legs at the aforementioned angles, with FL measures being taken using US at each angle during the rest period between sets. This was done to establish if any strength or FL increases occurred as a result of training by comparing the training leg with the untrained (control) leg.

Data Processing: Electromyography

EMG was recorded during one training set every two weeks (weeks 2, 4, 6, 8 and 10) in order to establish whether a drop in muscle activation occurred during training, and whether any angle dependent changes in torque occurred. Five channels recorded VM and BF activity (EMG2 and EMG3 respectively), position of the movement arm of the dynamometer, velocity of the movement arm, and torque produced during the movement. This data was DC removed (by using a moving average of every 0.005 s), rectified, and smoothed before being exported as a spreadsheet text file from Spike2. This was then opened in Excel and split into columns for each channel recorded. The excel files were then opened in MATLAB (R2013b, MathWorks, USA) where a custom written code (Appendix F) was written to split the data into 16 repetitions recorded, and extract each flexion period for each channel. This was then written back into an Excel spreadsheet. The spreadsheet contained the data from the five channels in different sheets from the flexion period of the 16 repetitions; this was then averaged to give one column of data for each EMG session for each participant. This was then put back into MATLAB (R2013b), where a custom written code (Appendix G) extracted the averages from the EMG2, EMG3 and Position sheets, to find peak torque for each session. The code then calculated how many data points from peak torque to the end of the repetition, and calculated the average rectified value for both EMG signals for the same number of data points before and after peak torque. This was then exported to a new Excel spreadsheet. As this was done for every participant, it resulted in a single spreadsheet with average rectified values for before and after peak torque for each participant for each time-point. This then allowed us to establish whether a drop in EMG activation occurred after peak torque, as hypothesised by Sharifnezhad et al., 2014, but not measured.

Eccentric torque measurements were also taken using the Spike2; this data allowed us to establish any differences between isometric and eccentric peak torque, as well as any changes in the angle at which peak eccentric torque occurred throughout training. The average of 16 repetitions during flexion for each session was calculated, and maximum eccentric toque for each session was established, as well as what angle this occurred at. This was done in Excel by putting position and torque outputs together. Eccentric torque at each

of the iMVC angles (0 °, 15 °, 40 °, 65 ° and 90 °) was also established, and put in a separate spreadsheet to allow comparison to iMVC torques measured during training. These torque measures were normalised to participants' weight before statistical analysis was undergone.

Data Processing: Isometric torque

Isometric torque was measured pre-training, at follow up, and every 7th session during training to allow us to establish any strength changes as a result of training, and to increase the target load for training appropriately. These values were put into an Excel spreadsheet to allow comparison over the 10-week period. The isometric torque values were normalised to participants' weight. The percentage change was calculated from pre-training to follow up, to establish any isometric strength changes as a result of training. This was also compared to isometric torque measured on the control leg, to ensure changes occurred due to training specifically.

Data analysis

Initially, to assess the effect of training on strength (normalised iMVC), a 2 (time) x 4 (angle) within-subjects ANOVA was considered and used to analyse differences between the two legs (Δscore) from pre-training to follow up at different joint angles (15 °, 40 °, 65 °, and 90 °). After completion of the study, to further investigate and support our claim of increase in FL in the experimental leg in response to training, we conducted two further analyses: 1) a 2 (week) x 5 (angles) within-subjects ANOVA with repeated measures on normalised peak ECC torque. By conducting this analysis, we could compare the maximum eccentric torque at weeks 2 and 10, as these were the closest time to pre-training and follow up, at five different joint angles (0 °, 15 °, 40 °, 65 ° and 90 °); 2) a t-test on the angle at which peak normalised ECC occurred between weeks 2 and 10. For these further two analyses, no data from the control leg was available.

Results

Isometric Torque Measures (change score)

The change score, i.e. difference between the left (experimental) and right (control) legs (Table 14), in isometric peak torque normalised to participants' body weight, and for different joint angles at pre-training and follow up for the experimental leg can be seen in Table 15. A within-subjects ANOVA with time (2 levels) and angle (4 levels) was used to analyse change in iMVC between the two legs from pre-training to follow up. There was a significant angle effect, and the interaction between angle and time was also significant (Table 16). Difference in iMVC between the left and right legs increased with training in favour of the left leg which had undergone training. Pairwise comparisons showed that the difference between the two legs was significantly larger at angle 3 (65 °) compared to angle 1 (15 °): Mean difference = 0.021; 95%CI [0.001 – 0.041].

Table 14 – Change in normalised peak torque between left and right legs at different joint angles at pre-training and follow-up

CHANGE SCORE IN NORMALISED ISOMETRIC PEAK TORQUE (N·m) (LEFT-RIGHT)					
Participant	Angle (°)				
	15	40	65	90	Timepoint
1	-0.030	-0.024	-0.020	0.006	PRE
2	-0.002	0.000	-0.042	-0.021	PRE
4	-0.013	-0.030	0.018	-0.003	PRE
5	-0.007	-0.032	0.011	0.009	PRE
6	0.033	0.072	0.087	0.061	PRE
7	0.009	0.050	0.066	0.002	PRE
8	-0.019	-0.027	-0.019	-0.037	PRE
9	0.046	0.042	0.040	-0.037	PRE
10	-0.016	-0.032	-0.032	-0.002	PRE
11	0.023	0.053	0.067	-0.044	PRE
12	-0.022	-0.030	-0.046	-0.046	PRE
13	-0.002	0.012	0.010	0.007	PRE
1	-0.022	-0.020	0.009	-0.002	FUP
2	0.035	0.037	0.112	0.133	FUP
4	-0.015	-0.026	0.009	0.081	FUP
5	-0.012	-0.018	-0.046	0.047	FUP
6	0.004	0.000	-0.030	-0.120	FUP
7	-0.018	-0.044	0.004	0.002	FUP
8	0.006	0.037	0.039	0.068	FUP
9	0.009	0.059	0.114	0.091	FUP
10	0.018	0.034	0.043	0.138	FUP
11	-0.026	-0.023	0.040	0.014	FUP
12	-0.015	0.003	-0.022	0.045	FUP
13	-0.003	0.007	0.056	0.116	FUP

(*Participant = participant number, Angle = fixed angle of iMVC measurement in degrees*; PRE = pre-training; FUP = follow-up)

Table 15 – Change in normalised peak torque between pre-training and follow-up at different joint angles

CHANGE IN NORMALISED ISOMETRIC PEAK TORQUE (PRE-FUP)				
Participant	Angle (°)			
	15	40	65	90
1	-0.002	0.000	-0.042	-0.021
2	-0.013	-0.029	0.018	-0.003
4	-0.007	-0.032	0.011	0.009
5	0.033	0.072	0.087	0.061
6	0.009	0.050	0.066	0.002
7	-0.019	-0.027	-0.019	-0.037
8	0.046	0.042	0.040	-0.037
9	-0.016	-0.032	-0.032	-0.002
10	0.023	0.053	0.067	-0.044
11	-0.022	-0.030	-0.046	-0.046
12	-0.002	0.012	0.010	0.007
13	0.000	0.004	0.011	-0.009

(*Participant = participant number, Angle = fixed angle of iMVC measurement in degrees*)

Table 16 – A one-way repeated measures ANOVA on normalised peak ECC torque for time-angle effect

Tests of Within-Subjects Effects				
	F	df	Sig.	partial eta squared
Time (s)	1.28	11	0.28	0.10
Angle (°)	3.24	33	0.04	0.23
Time*Angle	5.79	33	0.00	0.35
Error		11		

(*Time = shows main effect of time, Angle = shows main effect of knee joint angle at which peak torque was measured, Week*Angle = shows the interaction between Week and Angle)*

Eccentric Torque Changes

ECC torque of the left (experimental) leg was measured every two weeks (weeks 2, 4, 6, 8 and 10) at different (0 °, 15 °, 40 °, 65 °, and 90 °) joint angles. These were then normalised to participants' body weight. Normalised eccentric peak torques can be seen in Table 17. A within-subjects repeated measures ANOVA with time (2 levels) and angle (5 levels) was run

on the maximum eccentric torque at weeks 2 and 10 as these were the closest time to pre-training and follow up.

There was a significant main effect of angle on maximum ECC torque: ECC peak torque significantly increased as flexion of the knee increased. There was also a significant interaction effect between time and angle on maximum ECC torque; maximum ECC torque at 90 ° significantly increased at week 10 compared with week 2 (Table 18). The main effect of time (week 2 vs. week 10) was not significant.

Table 17 – Normalised maximum eccentric peak torque at each angle for weeks 2 and 10

NORMALISED PEAK ECCENTRIC TORQUE										
	Week 2					Week 10				
	Angle (°)									
Participant	0	15	40	65	90	0	15	40	65	90
1	0.006	0.064	0.160	0.233	0.177	-0.010	0.024	0.073	0.123	0.210
2	0.005	0.064	0.137	0.215	0.108	0.010	0.058	0.140	0.247	0.343
4	0.003	0.071	0.148	0.228	0.296	-0.014	0.037	0.119	0.197	0.240
5	0.021	0.054	0.089	0.171	0.260	0.036	0.065	0.086	0.178	0.299
6	0.023	0.076	0.135	0.230	0.341	-0.014	0.041	0.122	0.240	0.408
7	0.013	0.057	0.152	0.283	0.307	-0.021	0.024	0.112	0.253	0.353
8	0.004	0.058	0.131	0.217	0.303	-0.015	0.038	0.119	0.195	0.290
9	-0.004	0.044	0.099	0.065	0.047	-0.014	0.033	0.100	0.138	0.123
10	-0.017	0.000	0.048	0.123	0.228	-0.026	-0.012	0.010	0.079	0.203
11	-0.015	0.047	0.132	0.231	0.228	-0.024	0.012	0.063	0.162	0.304
12	-0.011	0.042	0.125	0.203	0.164	-0.024	0.034	0.125	0.257	0.339
13	-0.026	-0.009	0.031	0.089	0.147	-0.019	0.018	0.078	0.135	0.205

(*Participant = participant number, Angle = the angle at which ECC peak torque was measured)*

Table 18 – A one-way repeated measures ANOVA on normalised peak ECC torque for time-angle effect

Tests of Within-Subjects Effects				
	F	df	Sig.	partial eta squared
Week	0.01	11	0.93	**0.001**
Angle	104.34	44	<.001	**0.905**
Week*Angle	8.63	44	<.001	0.44
Error		11		

(*Week = shows main effect of time, Angle = shows main effect of knee joint angle at which peak torque was measured, Week*Angle = shows the interaction between Week and Angle)*

A paired-sample t-test was run on the angle at which maximum ECC torque occurred during flexion of the knee joint between Weeks 2 and 10 (Table 20). A significant increase in the joint angle at which maximum ECC torque occurred was observed (Table 19). For participant 10, peak ECC torque occurred at an angle of 44 ° and 76 °, in Weeks 2 and 10, respectively. A sensitivity analysis was conducted with exclusion of this participant, but the outcome remained the same.

Table 19 – Angle of maximum normalised peak ECC torque

NORMALISED PEAK ECCENTRIC TORQUE-ANGLE			
Week 2		Week 10	
Angle (°)	Peak Torque (Nm)	Angle (°)	Peak Torque (Nm)
66	0.24	105	0.25
75	0.23	87	0.34
89	0.30	89	0.24
94	0.27	95	0.32
90	0.34	101	0.47
84	0.34	86	0.37
91	0.30	102	0.35
44	0.10	76	0.14
95	0.24	103	0.28
78	0.26	106	0.39
73	0.21	83	0.36
90	0.15	92	0.21

Table 20 – A paired-sample t-test on angle at which maximum ECC torque occurs at weeks 2 and 10

Paired-Sample t-test								
					t	df	Significance	
Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference				One-Sided p	Two-Sided p
			Lower	Upper				
-13.08	12.99	3.75	-21.34	-4.83	-3.49	11	0.003	0.005

Muscle activation changes

Muscle activation was measured on the left (experimental) leg throughout one training set every two weeks. The average rectified values for EMG2 (VM) and EMG3 (BF) across

different weeks can be seen in Table 21 and Table 22 respectively. A 2 (pre peak torque and post peak torque) x 2 (week 2 and week 10) within-subjects with repeated measures ANOVA tested change in EMG activity before and after peak torque for EMG2 and EMG3 between Week 2 and Week 10. Results for the EMG2 and EMG3 muscles are shown in Tables 23 and 24, respectively. As amplification factor for some of the measurements was different, these values were excluded and are shown as missing in the tables below.

Results show that for both EMG2 and EMG3 there was a significant difference between EMG level when the peak torque was the point of reference: ARV for EMG2 dropped significantly after peak ECC torque compared with pre ECC peak torque. For EMG2 There was also a significant time effect, showing a significant increase in ARV from week 2 to week 10 (Table 23). For EMG3 There was no significant time effect, showing no significant increase in ARV from week 2 to week 10; ARV for EMG3 a significant difference after peak ECC torque compared with pre ECC peak torque (Table 24).

Table 21 – EMG2 ARV for pre and post peak ECC peak torque (mV)

AVERAGE RECTIFIED VASTUS MEDIALIS MUSCLE ACTIVITY						
	Participant	Week 2	Week 4	Week 6	Week 8	Week 10
Pre Peak Torque	1	0.007	0.007	0.008		0.009
	2	0.015	0.017	0.019		0.022
	4	0.008	0.012	0.013		0.016
	5	0.010	0.014		0.013	0.012
	6		0.010		0.013	0.011
	7	0.015		0.018	0.024	0.017
	8	0.009	0.013	0.012	0.013	0.012
	9	0.007	0.005	0.005	0.005	0.005
	10	0.003	0.005	0.007	0.007	0.007
	11	0.015	0.021	0.021	0.013	0.020
	12	0.010	0.020	0.013	0.016	0.017
	13	0.002	0.003	0.003	0.003	0.003
Post Peak Torque	1	0.005	0.006	0.008		0.006
	2	0.005	0.015	0.018		0.022
	4	0.007	0.012	0.011		0.017
	5	0.008	0.011		0.012	0.008
	6		0.008		0.011	0.010
	7	0.005		0.012	0.013	0.006
	8	0.008	0.011	0.011	0.011	0.009
	9	0.003	0.003	0.003	0.002	0.003
	10	0.003	0.005	0.007	0.007	0.005
	11	0.014	0.022	0.021	0.013	0.020
	12	0.001	0.006	0.004	0.004	0.004
	13	0.002	0.002	0.002	0.003	0.003

Table 22 – EMG3 ARV for pre and post peak ECC peak torque

AVERAGE RECTIFIED BICEPS FEMORIS MUSCLE ACTIVITY						
	Participant	Week 2	Week 4	Week 6	Week 8	Week 10
Pre Peak Torque	1	0.04	0.02	0.04	0.03	0.03
	2	0.03	0.03	0.06	0.02	0.04
	4	0.03	0.03	0.03	0.03	0.03
	5	0.04	0.06	0.04	0.04	0.03
	6	0.03	0.02	0.02	0.03	0.07
	7	0.05	0.05	0.04	0.03	0.03
	8	0.03	0.02	0.03		
	9	0.03	0.02	0.02	0.02	0.02
	10	0.02	0.02	0.01	0.05	0.04
	11	0.04	0.06	0.05	0.06	0.05
	12		0.03	0.03	0.04	0.04
	13	0.03	0.03	0.03	0.05	0.05
Post Peak Torque	1	0.03	0.03	0.05	0.03	0.03
	2	0.01	0.02	0.05	0.02	0.04
	4	0.03	0.02	0.03	0.02	0.02
	5	0.03	0.04	0.11	0.04	0.03
	6	0.02	0.02	0.03	0.02	0.07
	7	0.05	0.03	0.03	0.02	0.02
	8	0.03	0.02	0.03		
	9	0.02	0.02	0.02	0.02	0.01
	10	0.02	0.02	0.02	0.05	0.03
	11	0.04	0.06	0.05	0.08	0.06
	12		0.02	0.03	0.02	0.02
	13	0.02	0.03	0.02	0.05	0.05

Table 23 – Within-subjects ANOVA with repeated measures on ARV pre-post peak ECC torque values between Weeks 2 and 10 for EMG2

Tests of Within-Subjects Effects			
	F	df	Sig.
EMG ARV	9.48	10	0.01
Time	9.85	10	0.01
EMG ARV*Time	0.06	10	0.82

(*EMG ARV = ARV of EMG2, Time = week of training, EMG ARV*Time = the effect of EMG ARV and time)*

Table 24 – Within-subjects ANOVA with repeated measures on ARV pre-post peak ECC torque values between Weeks 2 and 10 for EMG3

Tests of Within-Subjects Effects			
	F	df	Sig.
EMG ARV	6.05	9	0.04
Time	1.29	9	0.29
EMG ARV*Time	2.42	9	0.15

(*EMG ARV = ARV of EMG3, Time = week of training, EMG ARV*Time = the effect of EMG ARV and time)*

FL changes

It was expected that fascicles would be most stretched at 90 ° of flexion of the knee joint (Table 25). Accordingly, a paired samples t-test was run on the differences in FL between the left and right legs, between PRE and FUP sessions (Table 26).

Table 25 – Fascicle length measurements at 90 ° for control and experimental leg at PRE and FUP

	LL - PRE	LL - FUP	RL - PRE	RL-FUP	Δscore-PRE	Δscore-FUP
P1	9	11	9	11	0	0
P2	7	9	8	9	-1	0
P4	11	13	11	11	0	2
P5	8	12	9	11	-1	2
P6	11	9	9	11	2	-2
P7	9	10	10	10	0	0
P8	9	12	10	10	-1	2
P9	11	13	10	11	0	2
P10	7	10	8	10	-1	0
P11	10	11	11	10	-1	1
P12	10	11	12	10	-2	1
P13	8	11	8	8	0	3

(*P = Participant number, LL – PRE = left leg FL measurement at pre-training, LL – FUP = left leg FL measurement at follow-up, RL – PRE = right leg FL measurement at pre-training, RL – FUP = right leg FL measurement at follow-up, Δscore-PRE = difference between left and right legs FL at pre-training, Δscore-FUP = difference between left and right legs FL at follow-up)*

There was a significant difference between PRE at FUP sessions for the FL ($t_{(11)}$ = -2.292; p = 0.043); training resulted in a significant increase in FL of the experimental leg.

Table 26 – Results of the t-test on change in FL between the experimental and control legs at PRE and FUP

Paired-Sample t-test					t	df	Significance	
Mean	Std. Deviation	Std. Error Mean	95% CI of the Difference				One-Sided p	Two-Sided p
			Lower	Upper				
-1.33	2.02	0.58	-2.61	-0.05	-2.29	11	0.021	0.043

Discussion

The purpose of this study was to replicate Sharifnezhad's study which found that only high velocity eccentric training resulted in changes in muscle FL over a ten-week period.
This was done as previous chapters report that the effects of a single-bout of passive stretching was not sufficient to induce muscle microdamage, resulting in no increases in FL, therefore a prolonged training period using the protocol previously established by Sharifnezhad and colleagues, was necessary. They hypothesized that a drop in torque which occurs towards the end of the flexion period, is due to a drop in muscle activation, however they did not measure EMG, and therefore this study aims to confirm or refute this hypothesis.

Measures of isometric torque, which show a significant time-angle interaction, support the findings of Sharifnezhad et al., as they reported an increase in the maximum resultant knee joint moment towards an increased knee angle, meaning that isometric contractions occur at a longer muscle length at FUP compared with PRE. This supports the principle that eccentric training results in alterations in the moment-angle relationship, which in turn affects the length-tension characteristics of the muscle (Brown and Donnelly, 2011). This is also an indication of serial sarcomere adaptations as a result of training, as it has been reported that an increase in serial sarcomere number affects the length tension characteristics of the muscle, increasing force producing capabilities, which are a common outcome measure in many, if not all, strength training interventions.

Force producing capacity differs depending on the contraction type; it has been reported that eccentric torque values are higher compared to isometric and concentric torque values (Griffin, 1987). The use of EMG during a complete training set enabled us to establish the peak ECC torque changes throughout the training period, and the angle these occurred at. Peak ECC torque increased significantly as the joint angle increased. Interestingly, the angle at which peak torque occurs increased significantly from week 2 to week 10 towards a more flexed knee joint angle, further supporting the presence of adaptations in the moment-angle characteristics of the muscle, and therefore alterations in the length-tension characteristics (Brown and Donnelly, 2011).

The group (Sharifnezhad et al., 2014) reported a drop in knee joint moment toward the end of the flexion phase, when muscle fascicle shortening occurs. They hypothesized that this was due to a momentary drop in muscle activation during the movement, however this was not measured. The addition of EMG within the present study allows for the measurement of muscle activation patterns during the flexion phase; muscle activation patterns measured before and after peak torque clearly show a drop in muscle activation of the VM after peak torque, which occurs during the final third of the flexion phase. This supports Sharifnezhad et al.,'s hypothesis that a drop in joint moment towards the end of the flexion phase is in fact due to a decrease in muscle activation during that period. This drop in joint moment during lengthening of the muscle fiber, as a result of a drop in VL muscle activation, has been reported as the best predictors for inducing sarcomerogenesis, in addition to the magnitude of the joint moment generated during eccentric loading (Butterfield and Herzog, 2006). Results also show an increase in muscle activation at FUP compared with PRE, indicating that the muscle is not only operating maximally at different lengths, but also showing an increased force producing capacity. This could be due to contractions occurring at a more optimal angle for producing force; this is a common outcome measure, especially for those with reduced force producing capacity due to structural changes within the muscle, such as those with spastic CP. Such training, if undertaken, could result in increased contractile efficiency, leading to an improvement in functional skills, which are impaired within the population (Bar-On et al., 2014).

A significant increase in muscle FL was found PRE-FUP, therefore training directly resulted in increased FL. This, coupled with increases in ECC and isometric torque is indicative of changes in the joint moment-angle relationship, and in support the notion of sarcomerogenesis being induced as a result of training, which would result in an increase of sarcomeres in series. Experimenter reliability was previously established in Chapter 4, however due to the nature of the measurement, there are many factors that could associated with this finding, or lack thereof. Measurements were taken during iMVC measurement at rest periods, meaning participants legs were held at a fixed angle by the dynamometer during measurements, but participants were performing maximal exercise between measurements. Therefore, changes in the orientation of muscle between measurements was entirely possible. This impacted the ability to clearly frame the full muscle, including both aponeuroses, resulting in difficulty calculating muscle FL manually.

Conclusion

The present study replicated an established ECC training protocol, which reported a drop in knee joint moment and an increase in FL when training at high lengthening velocities. With the addition of EMG, it can be confidently established that the hypothesized reason for this drop in knee joint moment is in fact due to a drop in muscle activation during the last third of the extension phase. The results presented above were in support of Sharifnezhad et al's finding; an increase in muscle FL as a result of training, increased muscle strength, and a drop in joint moment as a direct result of a drop in muscle activation towards the end of flexion. It has previously been established in both human and animal models, that ECC training results in increases in strength, as well as structural changes at muscular level (Butterfield and Herzog, 2006, Butterfield et al., 2005a, Franchi et al., 2014, Franchi et al., 2017, Reeves et al., 2009a); these outcomes would be extremely useful to those with spasticity. As it was established in Chapter 5 that at least two of the three proposed criteria for increasing muscle fascicle length using high velocity passive stretching can be met, and an ECC training intervention study was undertaken, which produced increases in angle specific joint moment, as well as increases in the angle at which peak force production occurs, it is clear that ECC training would be beneficial to those with impaired function of

the muscles. Due to the lack of selective muscle recruitment within the population, ECC is not feasible, therefore HVPS could be used instead. This could determine if the same outcomes occur in spastic muscles as do in typically developing muscles after the training period, which in turn, could direct future interventions that aim to improve muscle function.

CHAPTER 7: THE EFFECT OF A 10 WEEK HIGH VELOCITY PASSIVE STRETCHING INTERVENTION IN INDIVIDUALS WITH CEREBRAL PALSY - A CASE STUDY

Abstract

This case study investigated the effects of a 10-week high-velocity passive stretching of the quadriceps muscles on vastus lateralis fascicle length, knee joint range of motion, and functional outcomes in an individual with cerebral palsy (CP). While traditional passive stretching is commonly used in CP management, based on information provided in the previous chapters, we claim that high-velocity passive stretching via inducing reflex contraction, with or without active contribution from the spastic participant to resist stretching, may mimic eccentric training and lead to increase in fascicle length and associated improved functional abilities.

One adult male participant with spastic hemiplegic CP underwent a 10-week intervention consisting of high-velocity passive stretching sessions targeting the quadriceps, three times per week. The participant was instructed to resist passive stretching wherever possible. Outcome measures included muscle fascicle length (assessed via ultrasonography), isometric and eccentric torque, EMG, and functional assessments in terms of knee angular velocity during gait and COP excursion while maintaining standing balance.

All measurements were taken at baseline and follow up (7 days post intervention), but EMG, FL and isometric torque measures were taken every 6^{th} and 7^{th} session respectively. Peak isometric torque increased at all angles, with the greatest increase (39 %) occurring at the greatest angle (75 °). Knee angular velocity increased by 116 % post training compared to pre in the affected leg, and walking speed increased 2.3 % at FUP. Total COP excursion decreased by 13 % post training, and changes in the length-tension relationship indirectly suggested the notion that FL was possibly increased as a result of training. FL measures were not reported due to limitations with the scanning depth of the probe; both aponeuroses were not visible at any point during scanning, and therefore FL could not be reliably measured.

This case study provides preliminary evidence that a 10-week high-velocity passive stretching intervention may lead to favorable changes in muscle architecture, joint flexibility, and functional outcomes in individuals with CP. The findings highlight the potential of high velocity passive stretching as a novel therapeutic tool in CP management. However, larger controlled studies are necessary to confirm these results and explore the mechanisms underlying the observed changes.

Introduction

Individuals with CP often present with spasticity, muscle contractures, lack of selective muscle recruitment and reduced functional abilities. Rehabilitation approaches in CP typically involve stretching exercises aimed at improving muscle-tendon unit (MTU) flexibility and reducing contractures to improve function (Bar-On et al., 2018). The implicit assumption underlying stretching is that it maintains or increases length of the shorter than normal muscle to combat contracture and reduce spasticity. If the reduced length of the spastic muscle fascicles, or overly stretched sarcomeres of the fascicle length, are kept within normal values(Pontén et al., 2005), none of the effects and associated functional improvements could be achieved. Therefore, FL is not increased in response to stretching or led to increase in the number of in-series sarcomeres.

Specific protocols of eccentric training which may lead to increase in FL, however, require active input from participants, and it is not feasible to undertake this mode of training in individuals who lack selective muscle recruitment. It is likely that the right training adaptation environment could be created in order to successfully increase muscle fascicle length in spastic muscles by high velocity passive stretching of the muscle.

It was established in Chapter 5 of this thesis, that at least two of the three criteria which may be required to increase FL could be met in a heterogeneous group of individuals with CP using a single bout of high velocity passive stretching (HVPS). It was established in Chapters 4 and 5 that momentary deactivation of the muscle occurs during high velocities of stretching, both passively and eccentrically in spastic and typically developing muscles respectively, however there was a lack of convincing evidence for the occurrence of sufficient muscle damage shown by an increase in blood-CK measures. It was observed that the occurrence of reflex muscle contraction during the HVPS (at maximum angular velocity recorded during self-selected walking) meant that the muscle was active during passive lengthening, which resembled eccentric contraction in typically developing muscles. This could be extremely beneficial to individuals with CP, as it presents an accessible mode of training which mimics eccentric exercise. Importantly, it does not require active participant's

input if they are incapable of contributing to the eccentric exercise, and can potentially result in all the benefits of eccentric training.

The present study has explored this novel strategy that may simulate the benefits of eccentric training—a modality known to induce muscular adaptations - by stimulating the stretch reflex and promoting lengthening of the muscle-tendon unit (Theis et al., 2015) – in individuals with CP. The aim of inducing such reflex response to stretching is to elicit neuromuscular adaptations, thus enhancing motor function in individuals with CP (Gough and Shortland, 2012). Eccentric training (longer term eccentric exercise to differentiate it from one-off sessions of eccentric contractions) or variations of it similar to the one employed here, when induce the right training adaptation environment for the muscle, holds theoretical promise in promoting muscle lengthening, improving muscle tone, and enhancing functional capabilities in individuals with CP (Kalkman et al., 2019). Therefore, this study aimed to delve into the potential of HVPS as a therapeutic modality in individuals with CP. This single-case study examined the effects of a structured HVPS training program on muscle strength (force-producing capacity), functional outcomes, and muscular adaptations. The aim was to pilot the feasibility and efficacy of HVPS in inducing the potential effects of eccentric exercise in individuals with CP as suggested in the previous chapters.

Methods

Participant

One individual with CP (male, aged 24, right hemiplegic, GMFCS level 1, height 191 cm, mass 128.8 kg) was recruited from advertisements within the local area. The individual had not undergone any lower limb botulinum toxin injections in the last 6 months, or surgery within the last year of participation, and was also asked to refrain from any lower limb stretching or exercises that were outside of their normal day to day routine for the period of their participation. Ethics approval for conducting this study was obtained from Brunel University London College of Health, Medicine and Life Sciences Research Ethics Committee (Appendix F).

Experimental overview

The experimental overview followed the protocol detailed above in Chapter 6. The participant attended the laboratory at Brunel University London for the pre-training session 7 days before the training began. The pre-training session involved baseline measurement of isometric strength of the quadriceps at four different angles (19 °, 38 °, 54 °, and 75 °) on each leg which were determined by the participants' ROM.

The individual also underwent gait analysis during the pre-training session: reflective markers were mounted the lower limb following Helen Hayes marker set to establish baseline kinematic and kinetic data using inverse dynamics. The participant was instructed to look straight ahead and walk as he normally would for approximately 10 meters from one end of the laboratory to the other end. A force plate was flushed with the floor of the laboratory within the walkway; this was done five times, with the most successful trial with a clean heel strike on the force plate then used to determine knee angular velocity during gait. Measures of balance were taken by instructing participants to stand stationary on the force plate for 30 seconds while measuring the center of pressure excursion.

As the individual with CP exhibited appropriate selective muscle recruitment during the pre-training test of iMVC, and had a maximum walking knee angular velocity higher than that of the isokinetic dynamometer (413 °/s), the individual underwent a HVPS protocol with the velocity of training (240 °/s) which remained consistent throughout the 10-week period.

After the 10-week training period was completed, with the exception of three sessions that were missed due to illness in week 2, the participant was invited back within 7-10 days after the last training session (which was initially decided) to undergo a follow up session. During this follow up session, iMVC measures were taken on both the training and control leg at the aforementioned angles, with FL measures being taken using USat each angle during the rest period between sets. This was done to establish if any strength or FL increases occurred as a result of training by comparing the training leg with the untrained (control) leg. Gait analysis was also undergone during the follow-up session, using the same protocol as the

pre-training session, to establish if any changes in gait and balance occurred as a result of training.

Experimental Procedure

The experimental procedure for all the measures in this chapter can be found in the previous chapter (Chapter 6).

Electromyography

EMG was measured every session to monitor the activation patterns of the VM and BF muscles during eccentric contractions at high velocities. Electrode placement was adjusted where appropriate for the individual, as muscle structure and placement can be different due to a decrease in muscle size and volume.

Ultrasonography

Ultrasonography was undergone on the training leg every 7th session during the 10-week training period; scans were taken during rest at maximum extension angle, maximum flexion angle, and at each angle that the iMVC was undergone.

Data Processing

Electromyography

A custom written code in MATLAB (R2013b) (Appendix G) was used to calculate the average rectified value (ARV) of EMG2 and EMG3 for the first (50%) and second (50%) halves of the flexion period.

Torque measurements allowed us to establish any changes between the experimental and control legs for the iMVC and eccentric peak torques, as well as any changes in the angle at which peak eccentric torque occurred throughout training. Eccentric torque at each of the iMVC angles (0 °, 19 °, 38 °, 54 °, and 75 °) was also established, and compared to iMVC

torques measured during training. These torque measures were normalised to the participant's weight before statistical analysis was completed.

Ultrasonography

Irregularities in the form of the muscle meant that deep aponeurosis was not visible for any of the recorded FL measures at any time-point, therefore while FL estimation was attempted both manually and by the use of auto-tracking software (Chapter 4), no FL measures could be determined and will not be reported.

Isometric torque

Isometric torque (iMVC) was measured pre-training, at follow up, and every 7th session during training to allow us to establish any strength changes as a result of training, and to increase the target load for training appropriately. The isometric torque values were normalised to participants' weight. The percentage change was calculated from pre-training to follow up, to establish any isometric strength changes as a result of training. This was also compared to isometric torque measured on the control leg, to ensure changes occurred due to training specifically.

Gait and balance measures

Five walking trials and three balance trials were collected during pre-training and follow up sessions. An AIM model was created previous to the pre-training session to allow for automatic allocation of markers during the data collection. Any markers not automatically allocated were assigned manually after the sessions. Traces of each marker during each trial were gap filled using a polynomial and smoothed, and data was exported as .c3d file to Visual3d (Version 6.01.08, C-Motion, Germantown, MD). Force plate data was exported from QTM as a spreadsheet text file, and opened in Excel to allow the calculation of the centre of pressure excursion. Test gait files were collected prior to the session and opened in Visual3d, which were used to create a model using the same marker set used for data collection to save time between the pre-training and first training sessions. A static calibration file was taken at each gait analysis data collection session to input into Visual3d,

which allowed the model to be applied to the data collected. For the pre-training session, one dynamic and one walking file were then added to the workspace, and the model was applied to the data. For the follow up session, two walking files and one dynamic file were uploaded, one self-selected walking trial and one fast walking trial. A pipeline was then created to plot the right and left knee joint angle and velocity during walking. This signal was then low pass filtered using a 6^{th} order Butterworth filter, and the data was exported as text and opened in Excel. This allowed for the maximum knee angular velocity during walking to be established. This was done for pre-training and follow up sessions to establish any changes in knee angular velocity during walking, and differences between right and left legs. Gait events and walking speed were established by linking heel and toe markers to the pelvis in Visual3d to establish heel strike and toe off. As walking speeds were similar for pre-training and the fast-walking trial at follow-up, those are the trials that will be presented.

In order to measure balance, participant was instructed to stand as still as possible on the force plate for 30 seconds, while staring at a fixed point directly in front of them. This was done in order to measure changes in the centre of pressure (COP) of the participant during standing. Force plate data was also recorded in QTM and exported as a TSV file, which was then processed in Excel. Out of the 30 seconds of standstill data that was recorded, the first 10 seconds were disposed to allow time for the participant to settle on the force plate and focus on the target. Prieto et al., was followed to calculate the excursion of the centre of pressure (COP) in the medio-lateral, anterio-posterior and total excursion (*Equation 4*).

Equation 4:

ML excursion = sum of ML movement / 1000

AP excursion = sum of AP movement / 1000

Total excursion = (ML excursion + AP excursion) / 1000

Results

As this is a case study with a singular participant, results will be presented descriptively.

Muscle activation changes

EMG was measured for VM and BF in every session, time normalized and averaged to create a grand mean for each week. EMG data were split into the first and second half of the flexion phase to establish any changes in muscle activation during lengthening. ARV values for the first and second 50 % of data was then calculated. Results can be seen in Table 27.

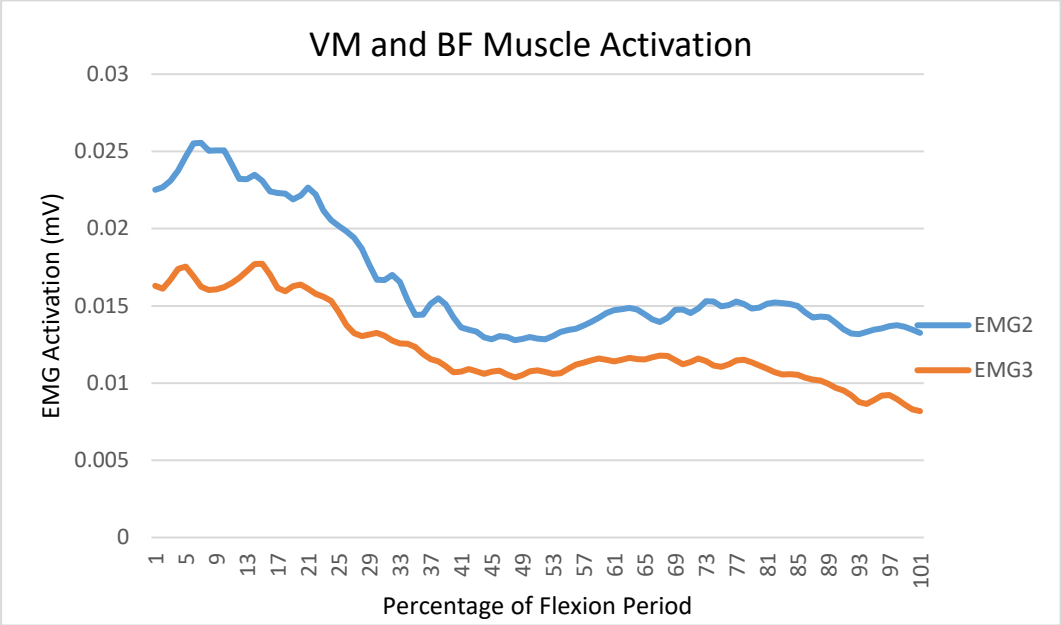
Table 27 – ARV of EMG2 and EMG3 during first and second half of flexion

VASTUS MEDIALIS AND BICEPS FEMORIS MUSCLE ACTIVATION PATTERNS						
Week	ARV EMG2 First 50%	ARV EMG2 Second 50%	EMG2 %change	ARV EMG3 First 50%	ARV EMG3 Second 50%	EMG3 %change
1	0.001	0.001	-12.98	0.025	0.016	-37.67
3	0.001	0.001	-6.86	0.060	0.040	-33.19
4	0.001	0.001	-18.27	0.029	0.026	-8.46
5	0.002	0.001	-43.75	0.021	0.013	-39.23
6	0.001	0.001	-31.91	0.020	0.014	-28.31
7	0.001	0.001	-32.34	0.038	0.016	-57.98
8	0.001	0.001	-32.69	0.176	0.122	-30.57
9	0.001	0.001	-22.56	0.011	0.010	-17.05
10	0.019	0.014	-25.52	0.014	0.011	-24.66

(*Week = week of training, ARV EMG2 first 50% = ARV of VM EMG signal during first 50 % of flexion, ARV EMG2 second 50% = ARV of VM EMG signal during second 50 % of flexion*, *EMG2 %change = change in VM EMG ARV first50%-second50% of flexion, ARV EMG3 first 50% = ARV of BF EMG signal during first 50 % of flexion*, *ARV EMG3 second 50% = ARV of BF EMG signal during second 50 % of flexion, EMG3 %change = change in BF EMG ARV first50%-second50% of flexion*)

Similar to activation patterns presented in Chapter 6, ARV decreased in both EMG2 (VM) and EMG3 (BF) toward the second half of the flexion phase, showing a drop in muscle activation during lengthening. This is clearly illustrated in Figure 3, which can be seen below. ARV values also increased from week 1 to week 10, showing increased muscle activation over time.

Figure 3 – A graph showing VM and BF muscle activation during the flexion phase



(EMG Activation = muscle activity in mV, Percentage of Flexion Period = percentage of knee flexion from peak extension to peak flexion, EMG2 = VM, EMG3 = BF)

Eccentric and isometric torque changes

As previously mentioned, torque was measured for every session, so weekly means of ECC torque produced by the participant were established. As the participant presented with spasticity, although he was instructed to resist the motion of the dynamometer, selective muscle recruitment may have been limited. Measures of maximum ECC torque can be seen below in Table 28. ECC torques was normalised to participants body weight, and percentage change each week, and difference between the first and last measurements (PRE-FUP) were calculated. A slight increase in ECC torque was observed for weeks 8 and 10 of 12 % and 3 % respectively; the greatest week to week increase occurred between weeks 1 and 3, and was of 68 %, which should be considered with caution (see Discussion). Week 2 data is missing because three sessions were missed during training due to illness, as stated previously. The change in ECC torque from week 1-week 10 increased by 73 %, which was due to a low level of torque measured in week 1; as stated above, this increase should be considered cautiously.

Table 28 – Changes in ECC torque over the 10-week training period

MAXIMUM ECCENTRIC TORQUE AT ISOVELOCITY					
Week	Angle (°)	Torque (Nm)	Normalised Torque	Δ in Torque	Δ in Torque Pre-FUP
1	61	66.60	0.05		
3	57	112.08	0.09	68%	
4	60	107.59	0.09	-4%	
5	60	107.87	0.09	0%	
6	61	102.71	0.08	-5%	
7	60	100.25	0.08	-2%	
8	59	112.72	0.09	12%	
9	59	111.99	0.09	-1%	
10	60	115.08	0.09	3%	73%

(*Week = training week, Angle = angle at which peak ECC torque occurred, Torque = ECC torque measured, Normalized Torque = ECC torque measured normalized to participants body weight in Newton-meters, Δ in Torque = the difference in normalized ECC torque between the current and previous weeks, Δ in Torque PRE-FUP = the change in ECC torque between weeks 1 and 10)*

Angles at which ECC peak torque occurred, ranged between 57 and 61 °. Isometric torque was measured during the iMVC every two weeks, to ensure the training load increased appropriately. If iMVC did not increase fortnightly, the maximum isometric torque measured during the whole training period was used as a target for training. Changes in isometric torque values normalized to the participants' body weight, at termination of training can be seen in Table 29.

Table 29 – Changes in isometric torque measured across training period

ISOMETRIC TORQUE (Nm)							
Angle (°)	PRE	Week 3	Week 5	Week 7	Week 9	FUP	Δ in Torque
19	0.04	0.05	0.06	0.05	0.05	0.05	20%
38	0.07	0.08	0.08	0.09	0.08	0.08	5%
54	0.11	0.14	0.12	0.16	0.14	0.12	7%
75	0.14	0.19	0.20	0.22	0.18	0.19	39%

(*Angle = fixed angle measurements were obtained at, PRE = iMVC at pre-training time-point, Week 3 = training week 3 measurements, Week 5 = training week 5 measurements, Week 7 = training week 7 measurements, Week 7 = training week 7 measurements, Week 9 = training week 9 measurements, FUP = iMVC at follow-up time-point, Δ in Torque = changes in iMVC between PRE-FUP expressed as a percentage)*

Peak isometric torque (PRE-FUP) increased at all angles, but the biggest increases can be seen at 19 ° and 75 °, which were the smallest and greatest knee angles, respectively. Although the increases at other angles were small, strength increases of 5-7 % follows the general pattern of increase in iMVC observed in response to training.

Functional outcomes: gait kinetics and kinematics

Gait analysis was undertaken during pre-training and follow-up sessions to establish safe knee angular velocities for training, but also to establish if any changes in gait occur as a result of training. Measures of angular knee velocity of both knees (Table 30), walking speed (Table 31), and right knee joint moment (Table 32) were done at pre-training and follow up for the fastest walking trial recorded. Changes in these measures are commonly considered as changes in function and therefore are often outcome measures of interventions within the population.

Table 30 – Knee joint angular velocity changes at PRE and FUP

	ANGULAR VELOCITY		
	RK ω	LK ω	Δ in ω
PRE	413.36	841.32	51 %
FUP	895.65	728.68	-23 %

(RK ω = right knee velocity, LK ω = left knee velocity, Δ in ω = change in angular velocity between legs)

Pre-training angular knee velocity for the right (affected) leg was 51 % less than the left (non-affected) leg. This increased at FUP, where right knee angular velocity was in fact 23 % higher than left knee velocity.

Table 31 – Changes in walking speed PRE-FUP

WALKING SPEED		
Walking Speed (m/s)		Δ in Speed
PRE	1.27	
UP	1.30	2.30%

(Walking speed = self-selected fast walking speed at PRE and FUP, Δ in Speed = changes in walking speed PRE-FUP expressed as a percentage)

From the data presented above in Table 31, it is clear that self-selected walking at a fast speed increasing from pre-training to follow-up, however this increase was only 2.3 %, and therefore may not be a meaningful change.

Table 32 – Right knee joint moment changes PRE-FUP

RIGHT KNEE JOINT MOMENT (Nm)		
	RK Moment	Δ in Moment
PRE	7.45	
FUP	48.79	554.60%

(RK Moment = joint moment of the right knee at PRE and FUP, Δ in Moment = change in right knee joint moment PRE-FUP)

Right knee joint moment also increased as seen above in Table 32, by more than five times, which could very much be determined as a meaningful change as a result of training.

Functional outcomes: balance measures

COP excursion values for antero-posterior excursion, medio-lateral excursion and total excursion can be seen in Table 33; three balance trials were undertaken at each time-point, which were then averaged to give one measure for each time-point. Changes in COP excursion were then calculated PRE-FUP using the average of the three trials (Table 34).

Table 33 – Measures of COP excursion (m) at PRE and FUP

CENTRE OF PRESSURE					
		Trial			
		1	2	3	Average
	ML	1.03	0.93	1.02	0.99
PRE	AP	1.78	1.90	1.78	1.82
	TOTAL	2.25	2.29	2.24	2.26
	ML	0.78	0.85	0.91	0.85
FUP	AP	1.57	1.59	1.60	1.59
	TOTAL	1.90	1.96	2.00	1.96

(PRE = pre-training time-point, FUP = follow-up time-point, ML = COP movement in the medio-lateral direction, AP = COP movement in the antero-posterior direction, TOTAL = total excursion calculated)

Table 34 – Changes in average COP excursion PRE-POST

AVERAGE Δ IN COP EXCURSION			
	PRE	FUP	Δ in COP Excursion
EXCURSION ML	0.99	0.85	-15%
EXCURSION AP	1.82	1.59	-13%
TOTAL EXCURSION	2.26	1.96	-13%

(Total excursion ML = total excursion in the medio-lateral direction, Total excursion AP = total excursion in the antero-posterior direction, Total excursion = total excursion measured in both ML and AP directions, Δ in COP Excursion = change in COP excursion PRE-FUP expressed as a percentage)

COP excursion decreased at FUP when compared to PRE. The biggest decrease was of ML excursion, which was a decrease of 15 % in side-to-side sway during standing. AP and total excursion both decreased by 13 % from PRE to FUP. The participant was more stable and balance improved as a result of training based of the results presented above.

Discussion

The purpose of this study was to establish if a 10-week HVPS program (rationale of which was established in Chapter 5) would be feasible and mimic the effects of eccentric exercise, observed in healthy individuals and reported in Chapter 6 of this thesis, in an individual with

spastic CP where selective muscle recruitment was expected to be limited, and therefore participant involvement in training would be minimal. Whether any changes in functional abilities such as walking and balance would be observed in response to HVPS training were also determined.

No adverse effect was reported by the participant and he successfully completed the study apart from sessions lost to an illness. It is likely that a meaningful increase in postural stability, isometric muscle strength, knee angular velocity and joint moment during walking occurred after 10 weeks of training with HVPS. Similar to the healthy participants (Chapter 6), HVPS was associated with a drop in muscle activity at later stages of muscle-tendon stretching. However, we could not be confident in the accuracy of FL measures confidently to report any possible adaptation in length in this study.

The muscle activation patterns clearly showed a drop in activation during the second 50 % of the flexion period for VM and BF during all weeks. This is in agreement with the data presented in Chapter 6, as well as in other studies, which hypothesized (without including EMG in the results) that a drop in knee joint moment during the final flexion phase was due to a drop in muscle activation (Sharifnezhad et al., 2014b, Davis et al., 2020), supporting the idea that HVPS in spastic muscles may have similar characteristics to ECC training.

Eccentric and isometric changes in muscle torque showed increase across weeks and from pre-training to follow-up, although the increase in ECC was driven by a very low level of maximum torque during isovelocity recorded in week 1; If results of week 1 for maximum ECC torque are ignored, then alteration in maximum ECC throughout the remaining of training period and follow-up was minimal. Maximum ECC torque during isovelocity was not very different from max ECC torque recorded throughout the flexion phase, as peak ECC torque occurred just after isovelocity, just as deceleration had begun. Considering the drop in EMG of both VM and BF muscles, it could be argued that the observed maximum ECC torque, which did not alter during training, represented resistance to fast stretching of the quadriceps muscle-tendon unit and did not reflect resistive torque which could have been actively produced by the participant in the current experimental context. In other words, application of the HVPS resulted in the measurement of maximum resistive torque of the

spastic MTU to stretching. As the participant possessed some selective muscle recruitment, and was instructed to resist the movement of the dynamometer where possible, it is not known whether the ECC torque measured was the result of voluntary contractions or reflex contractions, or a combination of both. The fact that peak torque occurred during deceleration just after the isovelocity period, could be indicative of the muscle activation being a reflex contraction followed by a clasp-knife response, or the inability of the participant to continue selectively recruiting the quadriceps. However, as the muscle activation was minimal, it could be argued that the observed resistance was primarily due to resistance of the spastic MTU, without the influence of a reflex contraction.

Increased muscle strength isometrically as a result of training was functionally beneficial to individuals with spastic muscles. These muscles have a reduction in force producing capacity, as well as alterations in muscle structure, making muscular contractions less efficient than their typically developing peers (Barber et al., 2011c). This increase in force producing capacity could result in improvements in functional abilities such as walking and balance, as stronger muscles will be less prone to fatigue, and therefore will be able to function more efficiently when performing functional skills (Gillet et al., 2015).

Pre-training measures of knee joint angular velocity differences between legs were consistent with literature, FUP measures however, were not; although there is limited literature on the effects of strength training on knee angular velocity during walking in typically developing adults, in individuals with CP, knee angular velocity during walking after a strength training interventions is largely inconsistent; Damiano et al., (2010) reported some changes in gait kinematics after an 8-week resistance training program in CP, but concluded that while strength training may improve walking abilities in some individuals, this was largely determined by muscle weakness as a main contributor to their gait deficiencies (Damiano et al., 2010). This indicates that not all individuals with CP will experience functional improvements as a result of strength training, and that there may be specific characteristics that determine whether someone will be a high or low responder to training. This could be determined by spasticity levels, or the presence of consistent reflex contractions as a protective mechanism in response to stretch. This supports the findings in Chapter 5, where candidates for training were established as suitable or unsuitable based

off muscle activation patterns during a single bout of HVPS. Whether these participants possessed differences in muscle weakness, however, is not known, as strength measures were not taken during that protocol.

What is clearer is that the participant experienced an improvement in walking and balance as a result of training; while walking speed only increased slightly, knee joint moment increased notably at FUP when compared with PRE, and measures of balance (based on COP excursion which decreased by 13-15 %) improved. This decrease in COP excursion was highest in the medio-lateral direction, showing that the side-to-side sway of the participant during standing decreased notably; as the participant was hemiplegic, this potentially shows an increase in the functional ability of the affected (right) leg, and less reliance on the left leg to provide stability during standing.

It is unfortunate that fascicle length data could not be used. There are a number of possible contributors to the inability to measure or estimate the length of the fascicle lengths with available data confidently. The participant in this study had a large volume of subcutaneous tissue which made focusing of the scanner to the depth required for observing the deep aponeurosis difficult. Length of the probe (5 cm) was also not the best for scanning long fascicles such as those of VL muscle. Furthermore, it should be noted that the atypical shape of the muscle would have caused different orientations at different joint angles, and getting out of the view of the scanner head.

Recruiting more participants for this study within the timeframe of this doctoral work proved very difficult. This was partly due to the nature of the HVPS training which places significant time constraints on potential participants' schedule. In particular, younger age participants may find this difficult to mix training with their education, or parents'/guardians' other life commitments. However, no adverse effect was reported by the participant and he successfully completed the training sessions.

Conclusion

There is lack of agreeability amongst researchers on the possibility of employing ECC training within the CP population due to lack of selective muscle recruitment. Results of the present study suggest that HVPS could be possible in spastic CP and mimics characteristics of the ECC training in healthy muscles by showing drop in the EMG activity at longer lengths of the stretched MTU. At high velocities of stretching, such drop in muscle activity (which could initially be either reflexively triggered or voluntarily produced) provides the opportunity of measuring spastic muscles resistive torque which is not contaminated by muscle activity due to the presence of reflex contraction. Notable improvement in strength represented by increase in iMVC at different joint angles, and functional improvements in a commonly used measure of postural stability as a result of the 10-week training program suggest HPVS could be beneficial to individuals with CP, as currently there is no consistent agreement on the most effective intervention strategies at providing functional benefits. The present study, being the first to determine whether HVPS could mimic ECC training and result in functional improvements, could be used to inform future interventions that aim to increase functional abilities in spastic muscles.

As the study presented was undertaken on only one individual, who was in fact relatively high functioning (GMFCS Level I), further investigation is warranted in individuals who have more limited selective muscle recruitment. FL measurements were not possible on the US data collected. Therefore, the effect of training on fascicle response in the present study is not known. This was the first study of its kind, and lessons taken from the implantation of this novel approach provides many areas of further investigation for those interested in increase functional abilities using resistance training.

CHAPTER 8: GENERAL DISCUSSION

Overview

The series of theoretical and empirical work presented in this thesis, describe development of a line of research which examined the possibility of increasing length of the VL muscle fascicles (FL) in individuals with spastic CP using high velocity passive stretching (HVPS). Relative shortness of spastic muscle fascicles, compared to the healthy muscles, might be a contributing factor to the observed contracture and spasticity, and reduced functional abilities in individuals with CP. Subsequently, increasing length of the spastic fascicles is considered as a treatment goal in this population.

Theoretical underpinning of this doctoral work and application of variations of high velocity ECC and HVPS in healthy and CP individuals which was followed were decided based on the outcome of a comprehensive review of literature on the past and present techniques used to increase muscle fascicle length in healthy human and animal models. Rehabilitation techniques used to improve function, but led to increase in muscle fascicle length in spastic muscles were also explored to find commonality amongst different approaches and techniques. Chapter 2 and paper published in Medical Hypotheses (Appendix A) argue that while techniques were varied, and literature was widely inconsistent at what conditions need to be met in order to successfully increase FL in healthy and spastic muscles, it is likely that muscles respond to eccentric contractions (ECC) which are delivered 1) at a high enough velocity which could induce microscopic damage to the muscle, and 2) are associated with positive fibre strain and 3) a momentary drop in muscle activation during stretching, by adding sarcomeres in series. Thereafter, the major issue was whether such potentially effective environment for triggering sarcomerogenesis with training could be created for individuals with CP, and whether spastic muscles would indeed respond to training in such environment with either increasing length of the fascicles, or by adding sarcomeres in series which may not be associated with increase in FL as could be measured with ultrasonography, but could be indirectly understood by other means.

Chapter 5 of this thesis established that presumed prerequisites 1-3 above for increasing FL could be induced in individuals with CP using HVPS. More specifically, it was established that positive fascicle strain could be observed using HVPS which were associated with reflex

muscle contraction and momentary deactivation or inhibition of the muscle similar to clasp knife phenomenon. We concluded this would open a window of opportunity for individuals with CP, who were unable to selectively recruit their spastic muscles, to get trained in a context which mimicked ECC with the view of increasing FL. Therefore, the presence of reflex contraction in response to stretching of the muscle at fast velocities, followed by the observed drop in muscle activation, if done over a long training period, would yield similar improvements in muscle functioning and architecture to that of healthy muscles after undertaking high velocity ECC training.

The effect of high velocity ECC training and its variation in the form of HVPS were tested, as it was argued that this training mode would be suitable for individuals with CP, in studies reported in Chapters 6 and 7, respectively. Chapter 6 was a cohort study involving a 10-week ECC training program at high velocity in healthy muscles, informed by the exploratory study in Chapter 5. It can be confidently concluded that the presumed criteria for increasing FL could be satisfied in healthy individuals after conducting the study reported in Chapter 4. Importantly, the results of a previous study were investigated, which was integral in shaping the rationale for the use of HVPS for increasing FL; this was replicated while addressing one of its key limitations. Sharifnezhad et al., (2014) had shown high velocity ECC training could lead to increases in FL, but they reported a drop in joint torque at long muscle lengths during training, that could not be justified based on the muscle force-length relationship. They suggested the drop in joint torque would be possible if contracting muscles experienced deactivation despite fascicles being stretched. With the addition of EMG during replication of Sharifnezhad et al., (Protocol 4) in this study, we were able to measure muscle activation patterns during training, to establish whether the drop in muscle activation occurred as proposed by them (Sharifnezhad et al., 2014b). The study was done concurrently with a 10-week HVPS program in an individual with spastic hemiplegia as presented in Chapter 6.

In Chapter 6, we concluded that magnitude of muscle activation did in fact drop towards the end of the flexion phase in healthy muscles, and muscle FL increased significantly in the experimental leg as a result of training. This is supported by the finding that peak ECC torque occurred at longer lengths of the muscle-tendon unit which indirectly suggested

possibility of addition of sarcomeres in series with training, under a condition that satisfied our proposed three criteria for increasing FL. The pilot study in Chapter 7, which was based on a single participant with spastic hemiplegia, supported that HVPS did not have any adverse effect and improved aspects of function and strengths of the individual, however, we could not support or refute its effect on altering fascicle length. This was the first study to undertake a HVPS protocol at a predetermined velocity (not based on maximum angular velocity of the knee joint during baseline assessment), on a single participant with the aim of determining the effect of passively induced ECC training at high velocity (HPVS) on FL and functional abilities. An important ancillary finding of the study was the observed drop in muscle activity at longer lengths of the muscle-tendon unit associated with recordings of a consistent maximum ECC Torque over several weeks. It was concluded that the recorded maximum ECC torque reported showed the resistive torque of the spastic MTU with minimal interference of reflex contraction, and hence the technique could be used for objective assessments of neural hypertonicity in this population and its alteration with interventions in future studies.

At conclusion of this series of studies, the outcome of high velocity ECC in healthy individuals and high velocity passive stretching training of individuals with CP could be summarized as follows: the presumed prerequisites for inducing sarcomerogenesis based on healthy human and animal models can be met in spastic CP using HVPS; high velocity ECC training at 240 °/s can lead to increase in SSN in TD individuals; the contracting muscle is momentarily deactivated or inhibited during high velocity ECC training or HVPS in both TD and CP individuals; and HVPS in spastic CP, as employed here, could be used to objectively assess spastic MTU stiffness, and improve functional abilities.

CHAPTER 9: GENERAL CONCLUSION

Overview

This thesis has explored the responses of typically developing and spastic muscles to two variations of stretching at high velocities, with specific focus on whether these trainings provided similar adaptations in muscle fascicle length and functional outcomes. Through a multidimensional examination, incorporating physiological and biomechanical analyses, we sought to further understand the adaptive responses within muscle architecture following targeted ECC/HVPS training interventions, comparing typically developing and spastic muscle responses. The findings of this research illuminate key insights into the similarities in responses of spastic and typically developing muscles, providing a novel finding, that while the two are structurally different and under different neural control, responses to the experimental protocols presented – when high velocity passive stretching was employed - may in fact be similar: fascicles showed positive strain, and muscles were deactivated and/or inhibited during stretching at longer lengths. In contrast, while taking part in the ECC training did lead to increase in FL at the predetermined knee angular velocity of 240 °/s in the healthy individuals, the same velocity delivered using HVPS, did not lead to longitudinal adaptation of muscle fascicle length in CP.

In individuals with spasticity, specifically those with CP, the final study revealed need for tailored approaches in resistance training interventions to optimize outcomes in this population. Moreover, the comparative analysis with typically developing counterparts served as a benchmark, providing a valuable perspective on the unique challenges and opportunities presented by spasticity. Adopting HVPS intervention to mimic ECC training within the population was a novel idea, which warrants further investigation, as the potential improvements to function appear very promising for the population, which require minimal, if any, contribution from the participant.

The implications of this research extend beyond the realm of biomechanics, offering valuable contributions to the design of rehabilitation strategies and training protocols, both eccentrically and passively. By comprehensively addressing the intricacies of muscle responses, this study contributes to the vast body of research interventions aiming to enhance functional outcomes in individuals with spasticity. The synthesis of biomechanical

insights and clinical considerations presented in this thesis lays a foundation for future investigations, fostering a deeper understanding of muscle adaptation and ultimately advancing the field of neuromuscular rehabilitation.

References

ABEL, M. F., JUHL, G. A., VAUGHAN, C. L. & DAMIANO, D. L. 1998. Gait Assessment of Fixed Ankle-Foot Orthoses in Children With Spastic Diplegia. *Archive of Physical Medicine and Rehabilitation*, 126-133.

ACCARDO, P. 1982. Freud on Diplegia: Commentary and Translation. *American journal of diseases of children*, 452-456.

AMELINK, G. J., KAMP, H. H. & BAR, P. R. 1988. Creatine kinase isoenzyme profiles after exercise in the rat: sex-linked differences in leakage of CK-MM. *Pflugers Arch,* 412**,** 417-21.

AMELINK, G. J., KOOT, R. W., ERICH, W. B., VAN GIJN, J. & BAR, P. R. 1990. Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an in-vitro rat skeletal muscle preparation. *Acta Physiol Scand,* 138**,** 115-24.

BAIRD, M. F., GRAHAM, S., BAKER, J. & BICKERSTAFF, G. 2012a. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *Journal of Nutrition and Metabolism*, 1-13.

BAIRD, M. F., GRAHAM, S. M., BAKER, J. S. & BICKERSTAFF, G. F. 2012b. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *J Nutr Metab,* 2012**,** 960363.

BAR-ON, L., AERTBELIËN, E., MOLENAERS, G. & DESLOOVERE, K. 2014. Muscle activation patterns when passively stretching spastic lower limb muscles of children with cerebral palsy. *PLoS ONE*, 1-13.

BAR-ON, L., KALKMAN, B. M., CENNI, F., SCHLESS, S. H., MOLENAERS, G., MAGANARIS, C. N., BASS, A., HOLMES, G., BARTON, G. J., O'BRIEN, T. D. & DESLOOVERE, K. 2018. The relationship between medial gastrocnemius lengthening properties and stretch reflexes in cerebral palsy. *Frontiers in Pediatrics*, 1-11.

BARBER, L., BARRETT, R. & LICHTWARK, G. 2011a. Passive muscle mechanical properties of the medial gastrocnemius in young adults with spastic cerebral palsy. *Journal of Biomechanics*, 2496-2500.

BARBER, L., BARRETT, R. & LICHTWARK, G. 2011b. Validity and reliability of a simple ultrasound approach to measure medial gastrocnemius muscle length. *Journal of Anatomy*, 637-642.

BARBER, L., BARRETT, R. & LICHTWARK, G. 2012. Medial gastrocnemius muscle fascicle active torque-length and Achilles tendon properties in young adults with spastic cerebral palsy. *Journal of Biomechanics*, 2526–2530.

BARBER, L., HASTINGS-ISON, T., BAKER, R., BARRET, R. & LICHTWARK, G. 2011c. Medial gastrocnemius muscle volume and fascicle length in children aged 2 to 5 years with cerebral palsy. *Developmental Medicine and Child Neurology*, 543-548.

BARBER, L., HASTINGS-ISON, T., BAKER, R., BARRETT, R. & LICHTWARK, G. 2011d. Medial gastrocnemius muscle volume and fascicle length in children aged 2 to 5years with cerebral palsy. *Developmental Medicine and Child Neurology*, 543-548.

BARRETT, R. S. & LICHTWARK, G. A. 2010. Gross muscle morphology and structure in spastic cerebral palsy: a systematic review. *Developmental Medicine & Child Neurology,* 52**,** 794-804.

BARTLETT, D. & PALISANO, R. 2000. A Multivariate Model of Determinants of Motor Change for Children With Cerebral Palsy. *Physical Therapy*, 598-614.

BECKERS, L. & BASTIAENEN, W. 2015. Application of the Gross Motor Function Measure-66 (GMFM-66) in Dutch clinical practice: a survey study. *BMC Pediactrics*, 146.

BLACK, H. R., QUALLICH, H. & GARELECK, C. B. 1986. Racial differences in serum creatine kinase levels. *The American Journal of Medicine,* 81**,** 479-487.

BLAZEVICH, A. J., CANNAVAN, D., COLEMAN, D. R. & HORNE, S. 2007a. Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. *Journal of Applied Physiology*, 1565-1575.

BLAZEVICH, A. J., GILL, N. D., DEANS, N. & ZHOU, S. 2007b. Lack of human muscle architectural adaptation after short-term strength training. *Muscle Nerve*, 78-86.

BONOW, R. H., COLLINS, K. L., ENE, C. & BROWD, S. R. 2018. Spasticity: Classification, Diagnosis, and Management. *Principles of Neurological Surgery (Fourth Edition).* Philadelphia: Elsevier.
BRANCACCIO, P., MAFFULLI, F. & LIMONGELLI, F. 2007. Creatine kinase monitoring in sport medicine. *British Medical Bulletin*, 209-230.
BREWSTER, L. M., CORONEL, C. M., SLUITER, W., CLARK, J. F. & VAN MONTFRANS, G. A. 2012. Ethnic differences in tissue creatine kinase activity: an observational study. *PLoS One,* 7, e32471.
BROWN, S. J. & DONNELLY, A. 2011. Changes in human skeletal muscle length during stimulated eccentric muscle actions. *J Physiol Sci,* 61, 31-6.
BURKE, D., KNOWLES, L., ANDREWS, C. & ASHBY, P. 1972. Spasticity, decerebrate rigidity and clasp-knife phenomenon: an experimental study in the cat. *Brain*, 31-48.
BURKE, D., WISSEL, J. & DONNAN, G. A. 2013. Pathophysiology of spasticity in stroke. *Neurology.*, S20-6.
BUTTERFIELD, T. A. & HERZOG, W. 2006. Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage. *J Appl Physiol (1985),* 100, 1489-98.
BUTTERFIELD, T. A., HERZOG, W. & LEONARD, T. R. 2005a. Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *Journal of Applied Physiology*, 1352-1358.
BUTTERFIELD, T. A., LEONARD, T. R. & HERZOG, W. 2005b. Differential serial sarcomere number adaptations in knee extensor muscles of rats is contraction type dependent. *Journal of Applied Physiology*, 1352-1358.
CABANISS, C. D. 1990. Creatine Kinase. *In:* RD, WALKER, H. K., HALL, W. D. & HURST, J. W. (eds.) *Clinical Methods: The History, Physical, and Laboratory Examinations.* Boston.
CANS, C. 2000. Surveillance of cerebral palsy in Europe: a collaboration of cerebral palsy surveys and registers. *Developmental Medicine and Child Neurology*, 816–824.
CDC. 2022. *What is Cerebral Palsy?* [Online]. Centres for Disease Control and Prevention. Available: https://www.cdc.gov/ncbddd/cp/facts.html#:~:text=CP%20is%20the%20most%20common,control%20his%20or%20her%20muscles. [Accessed 22/06/2022 2022].
CLARKSON, P. M. & HUBAL, M. J. 2002. Exercise-induced muscle damage in humans. *American Journal of Physical Medicine and Rehabilitation*, S52-S69.
CONDIE, D. N. & MEADOWS, C. B. 1993. Ankle-Foot Orthosis. *Biomechanical basis of orthotic management.* Oxford: Buttweworh-Heinmann.
COUTINHO, E. L., GOMES, A., FRANÇA, C., OISHI, J. & SALVINI, T. F. 2004a. Effect of passive stretching on the immobilized soleus muscle fiber morphology. *Brazilian Journal of Medical and Biological Research*, 1953-1861.
COUTINHO, E. L., GOMES, A. R. S., FRANCA, C. N., OISHI, J. & SALVINI, T. F. 2004b. Effect of passive stretching on the immobilized soleus muscle fiber morphology. *Brazilian Journal of Medicine and Biological Research*, 1853-1861.
CRAIG, J., HILDERMAN, C., WILSON, G. & MISOVIC, R. 2016. Effectiveness of stretch interventions for children with neuromuscular disabilities: Evidence-based recommendations. *Pediatric Physical Therapy*, 262-275.
DAMIANO, D. L., ARNOLD, A. S., STEELE, K. M. & DELP, S. L. 2010. Can strength training predictably improve gait kinematics? A pilot study on the effects of hip and knee extensor strengthening on lower-extremity alignment in cerebral palsy. *Phys Ther,* 90, 269-79.
DAMIANO, D. L., MARTELLOTTA, T. L., SULLIVAN, D. J., GRANATA, K. P. & ABEL, M. F. 2000. Muscle force production and functional performance in spastic cerebral palsy: relationship of cocontraction. *Arch Phys Med Rehabil,* 81, 895-900.
DAMIANO, D. L. & WILEY, M. E. 1998. Lower-extremity strength profiles in spastic cerebral palsy. *Developmental Medicine & Child Neurology*, 100-107.
DAVIS, J. F., KHIR, A. W., BARBER, L., REEVES, N. D., KHAN, T., DELUCA, M. & MOHAGHEGHI, A. A. 2020. The mechanisms of adaptation for muscle fascicle length changes with exercise: Implications for spastic muscle. *Med Hypotheses,* 144, 110199.

DAYANIDHI, S., DYKSTRA, P. B., LYUBASYUK, V., MCKAY, B. R., CHAMBERS, H. G. & LIEBER, R. L. 2015. Reduced satellite cell number in situ in muscular contractures from children with cerebral palsy. *J Orthop Res,* 33**,** 1039-45.

DE DEYNE, P. G. 2001. Application of Passive Stretch and Its Implications for Muscle Fibers. *Physical Therapy***,** 819-827.

DUNN, P. M. 1995. Dr William Little (1810-1894) of London and cerebral palsy. *Archives of Disease in Childhood. Fetal and Neonatal Edition***,** F209–F210.

ELIAKIM A, N. D., SHENKMAN L 1995. Serum enzyme activities following long-distance running: comparison between Ethiopian and white athletes. *Isr J Med Sci,* 31**,** 657-659.

ERSKINE, R. M., JONES, D. A. & WILLIAMS, A. G. 2010. Inter-individual variability in the adaptation of human muscle specific tension to progressive resistance training. *European Journal of Applied Physiology***,** 1117–1125.

EVANS, P., ALBERMAN, E., JOHNSON, A. & MUTCH, L. 1987. Standardization of recording and reporting cerebral palsy. *Developmental Medicine and Child Neurology***,** 272.

FARRIS, D. J. & LICHTWARK, G. A. 2016. UltraTrack: Software for semi-automated tracking of muscle fascicles in sequences of B-mode ultrasound images. *Computer Methods and Programs in Biomedicine,* 128**,** 111-118.

FIELDING, R. A., VIOLAN, M., SVETKEY, L. & ABAD, L. W. 2000. Effect of prior exercise on eccentric exercise-induced neutrophilia and enzyme release. *Medicine & Science in Sports & Exercise***,** 359-364.

FRANCHI, M., ATHERTON, N., REEVES, N., FLÜCK, M., WILLIAMS, J., BELTRAN VALLS, R. & NARICI, M. 2014. Architectural, functional and molecular responses to concentric and eccentric loading in human skeletal muscle. *Acta Physiologica***,** 642-654.

FRANCHI, M. V., ATHERTON, P. J., MAGANARIS, C. N. & NARICI, M. V. 2016. Fascicle length does increase in response to longitudinal resistance training and in a contraction-mode specific manner. *Springerplus***,** 94.

FRANCHI, M. V., REEVES, N. D. & NARICI, M. V. 2017. Skeletal Muscle Remodeling in Response to Eccentric vs. Concentric Loading: Morphological, Molecular, and Metabolic Adaptations. *Frontiers in Physiology***,** 1-16.

FRIDÉN, J. & LIEBER , R. L. 2003. Spastic muscle cells are shorter and stiffer than normal cells. *Muscle and Nerve***,** 157-164.

FUKUNAGA, T., KUBO, K., KAWAKAMI, Y., FUKASHIRO, S., KANEHISA, H. & MAGANARIS, C. N. 2001. In vivo behaviour of human muscle tendon during walking. *Proceedings of the Royal Society B: Biological Sciences***,** 229–233.

FUKUTANI, A. & KURIHARA, T. 2015. Comparison of the muscle fascicle length between resistance-trained and untrained individuals: cross-sectional observation. *Springerplus***,** 341.

GILLET, J. G., BARRETT, R. & LICHTWARK, G. 2013. Reliability and accuracy of an automated tracking algorithm to measure controlled passive and active muscle fascicle length changes from ultrasound. *Computer Methods in Biomechanics and Biomedical Engineering***,** 678-687.

GILLET, J. G., LICHTWARK, G. A., BOYD, R. N. & BARBER, L. A. 2015. FAST CP: protocol of a randomised controlled trial of the efficacy of a 12-week combined Functional Anaerobic and Strength Training programme on muscle properties and mechanical gait deficiencies in adolescents and young adults with spastic-type cerebral. *BMJ Open*.

GLANTZ, S. A. 1974. A CONSTITUTIVE EQUATION FOR THE PASSIVE PROPERTIES OF MUSCLE. *Journal of Biomechanics***,** 137-145.

GOLDSPINK, G., TABARY, C., TABARY, J. C., TARDIEU, C. & TARDIEU, G. 1974. Effect of denervation on the adaptation of sarcomere number and muscle extensibility to the functional length of the muscle. *The Journal of Physiology***,** 733-742.

GOUGH, M. & SHORTLAND, A. P. 2012. Could muscle deformity in children with spastic cerebral palsy be related to an impairment of muscle growth and altered adaptation? *Developmental Medicine and Child Neurology***,** 495-499.

GRACIES, J. M. 2005. Pathophysiology of spastic paresis. II: Emergence of muscle overactivity. *Muscle and Nerve*, 552-571.
GRIFFIN, J. W. 1987. Differences in elbow flexion torque measured concentrically, eccentrically, and isometrically. *Phys Ther,* 67, 1205-8.
GROSSET , J. F. & ONAMBELE-PEARSON, G. 2008. Effect of Foot and Ankle Immobilization on Leg and Thigh Muscles' Volume and Morphology: A Case Study Using Magnetic Resonance Imaging. *The Anatomical Record*, 1673-1683.
HERRIN, K. & GEIL, M. 2016. A comparison of orthoses in the treatment of idiopathic toe walking: A randomized controlled trial. *Prosthet Orthot Int,* 40, 262-9.
HOLLY, R. G., BARNETT, J. G. & ASHMORE, C. R. 1980. Stretch-induced growth in chicken wing muscles: a new model of stretch hypertrophy. *American Journal of Physiology*, C62–C71.
HONG, A.-R., HONG, S.-M. & SHIN, Y.-A. 2014. Effects of Resistance Training on Muscle Strength, Endurance, and Motor Unit According to Ciliary Neurotrophic Factor Polymorphism in Male College Students. *Journal of Sports Science and Medicine*, 680-688.
HOSL, M., BOHM, H., ARAMPATZIS, A. & DODERLEIN, L. 2015. Effects of ankle-foot braces on medial gastrocnemius morphometrics and gait in children with cerebral palsy. *J Child Orthop,* 9, 209-19.
HOSL, M., BOHM, H., ECK, J., DODERLEIN, L. & ARAMPATZIS, A. 2018. Effects of backward-downhill treadmill training versus manual static plantarflexor stretching on muscle-joint pathology and function in children with spastic Cerebral Palsy. *Gait Posture,* 65, 121-128.
HUSSAIN, A. W., ONAMBELE, G. L., WILLIAMS, A. G. & MORSE, C. I. 2013. Passive stiffness of the gastrocnemius muscle in athletes with spastic hemiplegic cerebral palsy. *European Journal of Applied Physiology*, 2291–2299.
HUTTON, J. L. & PHAROAH, P. O. D. 2002. Effects of cognative, motor, and sensory disabilities on survival in cerebral palsy. *Archives of Disease in Childhood*, 84-89.
IWASE, D., METOKI, Y., AIKAWA, J., UCHIDA, K., FUKUSHIMA, K., MATSUO, T., MATSUO, A., INOUE, G. & TAKASO, M. 2023. A Modified Sliding-Lengthening Approach to Tendon Lengthening with a Locking Mechanism Suture: A Technical Note. *Indian J Orthop,* 57, 505-509.
J. C. COOK, E. W., L. J. HAYWOOD 1990. Creatine kinase: race-gender differences in patients hospitalized for suspected myocardial infarction. *J Natl Med Assoc.,* 82, 249–254.
JEFFRIES, L., FISS, A., MCCOY, S. W. & BARTLETT, D. 2016. Description of Primary and Secondary Impairments in Young Children With Cerebral Palsy. *Pediatric Physical Therapy*, 7-14.
JONES, D., ROUND, J. & DE HANN, A. 2004. Recruitment And Control Of Motor Units. *Skeletal Muscle From Molecules To Movement.* London: Churchill Livingstone.
KALKMAN, B., HOLMES, G., MAGANARIS, C., BARTON, G., BAR-ON, L., BASS, A., WRIGHT, D., WALTON, R. & O'BRIEN, T. 2019. Resistance training combined with stretching increases tendon stiffness and is more effective than stretching alone in children with cerebral palsy: A randomized controlled trial. *Frontiers in Pediatrics*, 1-10.
KARAMIZRAK, S. O., ERGEN, E., TORE, I. R. & AKGUN, N. 1994. Changes in serum creatine kinase, lactate dehydrogenase and aldolase activities following supramaximal exercise in athletes. *The Journal of sports medicine and physical fitness* 141-146.
KAWAKAMI, Y. 2005. The Effects of Strength Training on Muscle Architecture in Humans. *Internation Journal of Sport and Health Sciences*, 208-217.
KHALILI, M. A. & HAJIHASSANIE, A. 2008. Electrical simulation in addition to passive stretch has a small effect on spasticity and contracture in children with cerebral palsy: a randomised within-participant controlled trial. *Australian Journal of Physiotherapy,* 54, 185-189.
KOMI, P. V. 2011. NEUROMUSCULAR ASPECTS OF SPORT PERFORMANCE. *THE ENCYCLOPEDIA OF SPORTS MEDICINE.* Chichester: Blackwell Publishing.
KOMI, P. V., KANEKO, M. & AURA, O. 1987. EMG activity of the leg extensor muscles with special reference to mechanical efficiency in concentric and eccentric exercise. *Int J Sports Med,* 8 Suppl 1, 22-9.

KRUSE, A., SCHRANZ, C., TILP, M. & SVEHLIK, M. 2018. Muscle and tendon morphology alterations in children and adolescents with mild forms of spastic cerebral palsy. *BMC Pediatrics*, 156.
KUBO, K., KANEHISA, H. & FUKUNAGA, T. 2002. Effects of resistance and stretching training programmes on the viscoelastic properties of human tendon structures in vivo. *The Journal of Physiology*, 219–226.
KUMBHARE, D., PARKINSON, W. & DUNLOP, B. 2008. Validity of Serum Creatine Kinase as a Measure of Muscle Injury Produced by Lumbar Surgery. *Journal of Spinal Disorders & Techniques*, 49-54.
KWAH, L. K., PINTO, R. Z., DIONG, J. & HERBERT, R. D. 2013. Reliability and validity of ultrasound measurements of muscle fascicle length and pennation in humans: a systematic review. *Journal of Applied Physiology*, 761-769.
LANCE, J. W. 1980a. Symposium. *Spasticity: Disordered Motor Control.* Chicago: Year Book Medical Pubs.
LANCE, J. W. 1980b. Symposium synopsis. *Spasticity: Disordered Motor Control*, 485–494.
LEE, J. H., SUNG, I. Y. & YOO, J. Y. 2008. Therapeutic effects of strengthening exercise on gait function of cerebral palsy. *Disability and Rehabilitation*, 1439-1444.
LEE, S. J., SUNG, I. Y., JANG, D. H., YI, J. H., LEE, J. H. & RYU, J. S. 2011. The effect and complication of botulinum toxin type a injection with serial casting for the treatment of spastic equinus foot. *Ann Rehabil Med,* 35, 344-53.
LIEBER, R. L. & FRIDEN, J. 1993. Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology*, 520–526.
LIEBER, R. L. & FRIDÉN, J. 2000. Functional and clinical significance of skeletal muscle architecture. *Muscle & Nerve*, 1647–1666.
LIEBER, R. L. & THEOLOGIS, T. 2021. Muscle-tendon unit in children with cerebral palsy. *Dev Med Child Neurol,* 63, 908-913.
LIU, M. Q., ANDERSON, F. C., SCHWARTZ, M. H. & DELP, S. L. 2008. Muscle contributions to support and progression over a range of walking speeds. *J Biomech,* 41, 3243-52.
LORENZO, T. M., ROCON, E., CABALLERO, I. M., BARRAGÁN, A. R. & LARA, S. L. 2017. Prolonged stretching of the ankle plantarflexors elicits muscle-tendon adaptations relevant to ankle gait kinetics in children with spastic cerebral palsy. *Medical Hypotheses,* 109, 65-69.
LYNN, R. & MORGAN, D. 1994a. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *Journal of Applied Physiology*, 1439-1444.
LYNN, R. & MORGAN, D. L. 1994b. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *Journal of Applied Physiology*, 1439-1444.
MALAIYA, R., MCNEE, A. E., FRY, N. R., EVE, L. C., GOUGH, M. & SHORTLAND, A. P. 2007. The morphology of the medial gastrocnemius in typically developing children and children with spastic hemiplegic cerebral palsy. *Journal of Electromyography and Kinesiology*, 657-663.
MARZILGER, R., BOHM, S., MERSMANN, F. & ARAMPATZIS, A. 2019. Effects of Lengthening Velocity During Eccentric Training on Vastus Lateralis Muscle Hypertrophy. *Front Physiol,* 10, 957.
MATHEWSON, M. A. & LIEBER, R. L. 2015. Pathophysiology of Muscle Contractures in Cerebral Palsy. *Physical Medicine and Rehabilitation Clinics of North America*, 57-67.
MCNEE, A. E., GOUGH, M., MORRISSEY, M. C. & SHORTLAND, A. P. 2009. Increases in muscle volume after plantarflexor strength training in children with spastic cerebral palsy. *Developmental Medicine and Child Neurology*, 429-435.
MENTIPLAY, B. F., BANKY, M., CLARK, R. A., KAHN, M. B. & WILLIAMS, G. 2018. Lower limb angular velocity during walking at various speeds. *Gait and Posture*, 190-196.
MINEAR, W. L. 1956. A classification of cerebral palsy. *Pediatrics*, 841-852.
MOHAGHEGHI, A. A., KHAN, T., MEADOWS , T. H., GIANNIKAS, K., BALTZOPOULOS, V. & MAGANARIS, C. N. 2007. Differences in gastrocnemius muscle architecture between the

paretic and non-paretic legs in children with hemiplegic cerebral palsy. *Clinical Biomechanics.*, 718-724.
MOHAGHEGHI, A. A., KHAN, T., MEADOWS, T. H., GIANNIKAS, K., BALTZOPOULOS, V. & MAGANARIS, C. N. 2008. In vivo gastrocnemius muscle fascicle length in children with and without diplegic cerebral palsy. *Clinical Biomechanics.*, 44-50.
MOREAU, N. G., SIMPSON, K. N., TEEFEY, S. A. & DAMIANO, D. L. 2010. Muscle architecture predicts maximum strength and is related to activity levels in cerebral palsy. *Phys Ther,* 90, 1619-30.
MORGAN, D. L. & TALBOT, J. A. 2002. The addition of sarcomeres in series is the main protective mechanism following eccentric exercise. *Journal of Mechanics in Medicine and Biology*.
MORGAN, D. L. & TALBOT, J. A. 2011. The Addition of Sarcomeres in Series Is the Main Protective Mechanism Following Eccentric Exercise. *Journal of Mechanics in Medicine and Biology,* 02, 421-431.
MORRIS, C. 2007. Definition and classification of cerebral palsy: a historical perspective. *Developmental Medicine and Child Neurology*, 3-7.
MORRIS, C. 2009. Definition and classification of cerebral palsy: a historical perspective. *Developmental Medicine and Child Neurology*, 3-7.
MOUGIOS, V. 2007. Reference intervals for serum creatine kinase in athletes. *Br J Sports Med,* 41, 674-8.
MUNDKUR, N. 2005. Neuroplasticity in children. *Indian J Pediatr,* 72, 855-7.
MUTCH, L., ALBERMAN, E., HAGBERG, B., KODAMA, K. & PERAT, M. V. 1992. Cerebral palsy epidemiology: where are we now and where are we going? *Developmental Medicine and Child Neurology*, 547-551.
NIILER, T. A. 2018. Assessing Dynamic Balance in Children with Cerebral. *Cerebral Palsy*.
NOBLE, J. J., CHRUSCIKOWSKI, E., FRY, N. R. D., LEWIS, A. P., GOUGH, M. & SHORTLAND, A. P. 2017. The relationship between lower limb muscle volume and body mass in ambulant individuals with bilateral cerebral palsy. *BMC Neurol,* 17, 223.
NOBLE, J. J., FRY, N. R., LEWIS, A. P., KEEVIL, S. F., GOUGH, M. & SHORTLAND, A. P. 2014. Lower limb muscle volumes in bilateral spastic cerebral palsy. *Brain and Development*, 294-300.
OSTENSJO, S., CARLBERG, E. B. & VOLLESTAD, N. K. 2003. Everyday functioning in young children with cerebral palsy. *Developmental Medicine and Child Neurology*, 603-612.
PALISANO, R., ROSENBAUM, P., WALTER, S., RUSSELL, D., WOOD, E. & GALUPPI, B. 1997. Gross Motor Functioning Classification System. *Developmental Medicine and Child Neurology*, 214-223.
PAVAO, S. L., DOS SANTOS, A. N., WOOLLACOTT, M. H. & FERREIRA ROCHA, N. A. C. 2013. Assessment of postural control in children with cerebral palsy: A review. *Research in Developmental Disabilites*, 1367–1375.
PERLSTEIN, M. A. 1952. Infantile cerebral palsy; classification and clinical correlations. *Journal of the American Medical Association*, 30-34.
PIN, T., DYKE, P. & CHAN, M. 2006. The effectiveness of passive stretching in children with cerebral palsy. *Developmental Medicine and Child Neurology*, 855-862.
PINGEL, J., BARTELS, E. M. & NIELSEN, J. B. 2016. New perspectives on the development of muscle contractures following central motor lesions. *Journal of Physiology*, 1027-1038.
PONTÉN, E., FRIDÉN, J., THORNELL, L. E. & LIEBER, R. L. 2005. Spastic wrist flexors are more severely affected than wrist extensors in children with cerebral palsy. *Dev Med Child Neurol,* 47, 384-9.
POON, D. M. & HUI-CHAN, C. W. 2009. Hyperactive stretch reflexes, co-contraction, and muscle weakness in children with cerebral palsy. *Dev Med Child Neurol,* 51, 128-35.
POTIER, T., ALEXANDER, C. & SEYNNES, O. 2009. Effects of eccentric strength training on biceps femoris muscle architecture and knee joint range of movement. *European Journal of Applied Physiology*, 939-944.

POWELL, M. M., SILVA, P. D. & GRINDELAND, T. 1989. Effects of two types of ankle- foot orthoses on the gait of children with spastic diplegia. *Developmental Medicine and Child Neurology*, 8-9.
PROSKE, U. & MORGAN, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol,* 537, 333-45.
PROSKE, U. & MORGAN, D. L. 2004. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of Physiology*, 333-345.
REEVES, N., MAGANARIS, C., LONGO, S. & NARICI, M. 2009a. Differential adaptations to eccentric versus conventional resistance training in older humans. *Experimental Physiology*, 825-833.
REEVES, N. D., MAGANARIS, C. N., LONGO, S. & NARICI, M. V. 2009b. Differential adaptations to eccentric versus conventional resistance training in older humans. *Experimental Physiology*, 825-833.
REID, S., HAMER, P., ALDERSON, J. & LLOYD, D. 2010. Neuromuscular adaptations to eccentric strength training in children and adolescents with cerebral palsy. *Dev Med Child Neurol,* 52, 358-63.
RILEY, D. A. & VAN DYKE, J. M. 2012. The Effects of Active and Passive Stretching on Muscle Length. *Physical Medicine and Rehabilitation Clinics of North America*, 51-57.
RIMMER, J. H. 2001. Physical fitness levels of persons with cerebral palsy. *Dev Med Child Neurol,* 43, 208-12.
ROSE, J. & MCGILL, K. C. 1998. The motor unit in cerebral palsy. *Developmental Medicine and Child Neurology*, 270-277.
ROSE, J. & MCGILL, K. C. 2005. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. *Developmental Medicine and Child Neurology*, 329-336.
ROY, D. S. & MCLAUGHLIN, J. F. 2009. Spasticity. *Finnie's Handling the Young Child with Cerebral Palsy at Home.* Oxford: Butterworth Heinmann Elsevier.
S., A. D., R., C. R., R., J. B. & S., R. R. 2020. The Effects of Spasticity on Motor Recovery After a Stroke. *Journal of Neuropsychiatry*.
SANGER, T. D., DELGADO, M. R., GAEBLER-SPIRA, D., HALLET, M. & MINK, J. W. 2003a. Classification and Definition of Disorders Causing Hypertonia in Childhood. *American Academy of Pediatrics*, e89-e97.
SANGER, T. D., DELGADO, M. R., GAEBLER-SPIRA, D., HALLET, M. & MINK, J. W. 2003b. Classification and Definition of Disorders Causing Hypertonia in Childhood. *Pediatrics*, e89–e97.
SCHEEL, A. M., TIOKHIN, L., ISAGER, P. M. & LAKENS, D. 2021. Why Hypothesis Testers Should Spend Less Time Testing Hypotheses. *Perspectives on Psychological Science,* 16, 744-755.
SCHOLTES, V. A., BECHER, J. G., COMUTH, A., DEKKERS, H., VAN DIJK, L. & DALLMEIJER, A. J. 2010. Effectiveness of functional progressive resistance exercise strength training on muscle strength and mobility in children with cerebral palsy: a randomized controlled trial. *Developmental Medicine and Child Neurology*, e107-e113.
SEWELL, M. D., EASTWOOD, D. M. & WIMALASUNDERA, N. 2014. Managing common symptoms of cerebral palsy in children. *BMJ,* 349, g5474.
SHARIFNEZHAD, A., MARZILGER, R. & ARAMPATZIS, A. 2014a. Effects of load magnitude, muscle length and velocity during eccentric chronic loading on the longitudinal growth of the vastus lateralis muscle. *The Journal of Experimental Biology*, 2726-2733.
SHARIFNEZHAD, A., MARZILGER, R. & ARAMPATZIS, A. 2014b. Effects of load magnitude, muscle length and velocity during eccentric chronic loading on the longitudinal growth of the vastus lateralis muscle. *Journal of Experimental Biology*, 2726-2733.
SMITH, L. R., LEE, K. S., WARD, S. R., CHAMBERS, H. G. & LIEBER, R. L. 2011a. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. *Journal of Physiology*, 2625-2639.
SMITH, L. R., LEE, K. S., WARD, S. R., CHAMBERS, H. G. & LIEBER, R. L. 2011b. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. *The Journal of Physiology*, 2625-2639.

TABARY, J., TABARY, C., TARDIEU, C., TARDEIU, G. & GOLDSPINK, G. 1972. Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *The Journal of Physiology*, 231-244.

TAYLOR, N. F., DODD, K. J., BAKER, R. J., WILLOUGHBY, K., THOMASON, P. & GRAHAM, H. K. 2013. Progressive resistance training and mobility-related function in young people with cerebral palsy: a randomized controlled trial. *Developmental Medicine and Child Neurology*, 806-812.

TE VELDE, A., MORGAN, C., NOVAK, I., TANTSIS, E. & BADAWI, N. 2019. Early Diagnosis and Classification of Cerebral Palsy: An Historical Perspective and Barriers to an Early Diagnosis. *J Clin Med,* 8.

THEIS, N., KORFF, T., KAIRON, H. & MOHAGHEGHI, A. A. 2013. Does acute passive stretching increase muscle length in children with cerebral palsy? *Clinical Biomechanics.*, 1-7.

THEIS, N., KORFF, T. & MOHAGHEGHI, A. A. 2015. Does long-term passive stretching alter muscle–tendon unit mechanics in children with spastic cerebral palsy? *Clinical Biomechanics*, 1071-1076.

THEIS, N., MOHAGHEGHI, A. A. & KORFF, T. 2016. Mechanical and material properties of the plantarflexor muscles and Achilles tendon in children with spastic cerebral palsy and typically developing children. *Journal of Biomechanics*, 3004-3008.

THOMAS, S. S., MAZUR, J. M. & WRIGHT, N. 1992. Quantitative assessment of ankle foot orthoses for children with cerebral palsy. *Developmental Medicine and Child Neurology*, 547-555.

TOIGO, M. & BOUTELLIER, U. 2006. New fundamental resistance exercise determinants of molecular and cellular muscle adaptations. *European Journal of Applied Physiology*, 643–663.

TROMPETTO, C., MARINELLI, L, MORI, L., CURRA, A., MOLFETTA, L. & ABBRUZZESE, G. 2014. Pathophysiology of Spasticity: Implications for Neurorehabilitation. *BioMed Research International*, 1-8.

TURPIN, N. A., LEVIN, M. F. & FELDMAN, A. G. 2016. Implicit learning and generalization of stretch response modulation in humans. *Journal of Neurophysiology*, 3186-3194.

WG, H. 2015. Spreadsheed for analysis of validity and reliability. 19, 36-42.

WHITEHEAD, N. P., WEERAKKODY, N. S., GREGORY, J. E., MORGAN, D. L. & PROSKE, U. 2001. Changes in passive tension of muscle in humans and animals after eccentric exercise. *The Journal of Physiology*, 593–604.

WILLIAMS, P. E. 1990. Use of intermittent stretch in the prevention of serial sarcomere loss in immobilised muscle. *Annals of Rheumatic Disease*, 316-317.

WIRTZ, P., LOERMANS, H. M. T., DE HAAN, A. F. J. & HENDRIKS, J. C. M. 1988. Early immobilization of hindleg muscles of dystrophic mice: short-term and long-term effects. *Journal of Neurological Sciences*, 293-307.

ZHAO, H., WU, Y. N., HWANG, M., REN, Y., GAO, F., GAEBLER-SPIRA, D. & ZHANG, L. Q. 2011. Changes of calf muscle-tendon biomechanical properties induced by passive-stretching and active-movement training in children with cerebral palsy. *J Appl Physiol (1985),* 111, 435-42.

ZHAO, X., XIAO, N., LI, H. & DU, S. 2013. Day vs. day-night use of ankle-foot orthoses in young children with spastic diplegia: a randomized controlled study. *American journal of physical medicine & rehabilitation,* 92, 905-911.

Appendix

A. Published journal article referenced in Chapter 1

Medical Hypotheses 144 (2020) 110199

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

The mechanisms of adaptation for muscle fascicle length changes with exercise: Implications for spastic muscle

J.F. Davis[a,*], A.W. Khir[b], L. Barber[c], N.D. Reeves[d], T. Khan[e], M. DeLuca[a], Amir A. Mohagheghi[a]

[a] Division of Sport, Health & Exercise Sciences, Brunel University London, UK
[b] Department of Mechanical Engineering, Brunel University London, UK
[c] School of Allied Health Sciences, Griffith University, Brisbane, Australia
[d] Research Centre for Musculoskeletal Science and Sports Medicine, Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, UK
[e] Royal National Orthopaedic Hospital, London, UK

ARTICLE INFO

Keywords:
Spastic muscles
Fascicle length
Exercise
Eccentric

ABSTRACT

We are proposing optimal training conditions that can lead to an increase in the number of serial sarcomeres (SSN) and muscle fascicle length (FL) in spastic muscles. Therapeutic interventions for increasing FL in clinical populations with neurological origin, in whom relative shortness of muscle fascicles contributed to the presentation of symptoms such as spasticity, contracture, and limited functional abilities, do not generally meet these conditions, and therefore, result in less than satisfactory outcomes. Based on a review of literature, we argue that protocols of exercise interventions that led to sarcomerogenesis, and increases in SSN and FL in healthy animal and human models satisfied three criteria: 1) all involved eccentric exercise at appropriately high velocity; 2) resulted in positive strain of muscle fascicles; and 3) momentary deactivation in the stretched muscle. Accordingly, to increase FL in spastic muscles, new exercise protocols in which the three presumed criteria are satisfied, must be developed, and long-term muscle architectural and functional adaptations to such trainings must be examined.

Introduction

Muscle morphology at the macro level can be described as the architecture of the muscle, and typically includes parameters such as the cross sectional area (CSA), fascicle length (FL) and pennation angle (PA) which are commonly assessed in vivo using ultrasonography. Muscle fascicle length has been suggested as the single most important architectural parameter of a muscle affecting its function [1]. Many motor disabilities with neurological origin which present clinically with spasticity and contracture (e.g. cerebral palsy and stroke) are associated with relatively shorter muscle fascicles (compared to matched controls) [2–5] that may contribute to the observed impaired movement and functional limitations. Relative shortness of the muscle fascicle can affect the joint angle where optimal force is produced during activity (i.e., shift in the muscle torque-angle relationship) [6,7], and reduce velocity of contraction (i.e., altering torque-angular velocity relationship) [8,9]. Muscle fascicle length and mechanical properties is a function of number and length of its constituent in-series sarcomeres [10]. Accordingly, increasing the number of in-series serial sarcomeres could be a target of therapeutic and rehabilitative interventions for these populations with short fascicle [9,11,12].

In general, there are at least two theoretical approaches to the rehabilitation of motor function in neurologically impaired individuals with spasticity: 1) by directly affecting the neural control mechanisms (e.g., rhizotomy [13], through mirror therapy [14], constraint-induced movement therapy [15]; and 2) by altering properties of the muscle-tendon unit (MTU) such as muscle fibre/fascicle length (FL) which can not only affect the functional outcome of muscle contraction, but may also induce neural plasticity at the segmental or supraspinal levels by affecting threshold of tonic stretch reflex [16].

Abbreviations: FL, fascicle length; SL, sarcomere length; ROM, range of motion; SSN, serial sarcomere number; LTC, length tension characteristics; RT, resistance training; DOMS, delayed onset muscle soreness; ECC, Eccentric contraction; CSA, cross sectional area; MVC, maximum voluntary contraction; EMG, electromyography; PSN, parallel sarcomere number; MTU, muscle tendon unit; VL, vastus lateralis; BF, biceps femoris; PA, pennation angle; 1RM, one repetition maximum; PKE, passive knee extension; CON, concentric contraction; MT, muscle thickness; CP, cerebral palsy

* Corresponding author at: College of Health, Medicine and Life Sciences, Department of Life Sciences, Division of Sport, Health & Exercise Sciences, Heinz Wolff Building, Room 270, Brunel University London, Kingston Lane, Uxbridge, Middlesex UB8 3PH, UK.
E-mail address: Jessica.Davis@brunel.ac.uk (J.F. Davis).

https://doi.org/10.1016/j.mehy.2020.110199
Received 16 June 2020; Received in revised form 10 August 2020; Accepted 15 August 2020
Available online 18 August 2020

B. Ethical approval letter for Chapter 3

University Research Ethics Committee
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

11 July 2019

LETTER OF APPROVAL

Applicant: Miss Jessica Davis

Project Title: Measurement reliability of muscle creatine kinease and fascicle length

Reference: 13621-A-Jul/2019- 19705-1

Dear Miss Jessica Davis,

The Research Ethics Committee has considered the above amendment application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed amendment. Approval is given on the understanding that the conditions of approval set out below are followed:

- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Kind regards,

Professor Peter Hobson

Chair of the University Research Ethics Committee

Brunel University London

C. Ethical approval letter for Chapter 4

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

18 December 2018

LETTER OF APPROVAL

Applicant: Miss Jessica Davis

Project Title: Muscle response to exercise in cerebral palsy

Reference: 13450-NHS-Dec/2018- 15512-2

Dear Miss Jessica Davis

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Christina Victor

Chair

College of Health and Life Sciences Research Ethics Committee (DLS)
Brunel University London

D. MATLAB code for calculating ARV of EMG

```
ARV_EMG2_60 = mean(EMG2(r_start:r_start+119));
ARV_EMG2_120 = mean(EMG2(r_start+120:r_start+239));
ARV_EMG2_180 = mean(EMG2(r_start+240:r_start+359));
ARV_EMG2_240 = mean(EMG2(r_start+360:r_start+479));
ARV_EMG2_300 = mean(EMG2(r_start+480:r_start+599));
ARV_EMG2_360 = mean(EMG2(r_start+600:r_start+719));
ARV_EMG2_420 = mean(EMG2(r_start+720:r_start+839));
ARV_EMG2_480 = mean(EMG2(r_start+840:r_start+959));
ARV_EMG2_540 = mean(EMG2(r_start+960:r_start+1079));
ARV_EMG2_600 = mean(EMG2(r_start+1080:r_start+1199));
ARV_EMG2_660 = mean(EMG2(r_start+1200:r_start+1319));
ARV_EMG2_720 = mean(EMG2(r_start+1320:r_start+1439));
ARV_EMG2_780 = mean(EMG2(r_start+1440:r_start+1559));
ARV_EMG2_840 = mean(EMG2(r_start+1560:r_start+1679));
ARV_EMG2_900 = mean(EMG2(r_start+1680:r_start+1799));
ARV_EMG2_910 = mean(EMG2(r_start+1800:r_start+1819));
ARV_EMG2_920 = mean(EMG2(r_start+1820:r_start+1839));
ARV_EMG2_930 = mean(EMG2(r_start+1840:r_start+1859));
ARV_EMG2_940 = mean(EMG2(r_start+1860:r_start+1879));
ARV_EMG2_950 = mean(EMG2(r_start+1880:r_start+1899));
ARV_EMG2_960 = mean(EMG2(r_start+1900:r_start+1920));
EMG2_ARV_cat = [Baseline_EMG2 ARV_EMG2 ARV_EMG2_60 ARV_EMG2_120
ARV_EMG2_180 ARV_EMG2_240 ARV_EMG2_300 ARV_EMG2_360 ARV_EMG2_420
ARV_EMG2_480 ARV_EMG2_540 ARV_EMG2_600 ARV_EMG2_660 ARV_EMG2_720
ARV_EMG2_780 ARV_EMG2_840 ARV_EMG2_900 ARV_EMG2_910 ARV_EMG2_920
ARV_EMG2_930 ARV_EMG2_940 ARV_EMG2_950 ARV_EMG2_960];
ARV_EMG3_60 = mean(EMG3(r_start:r_start+119));
ARV_EMG3_120 = mean(EMG3(r_start+120:r_start+239));
ARV_EMG3_180 = mean(EMG3(r_start+240:r_start+359));
ARV_EMG3_240 = mean(EMG3(r_start+360:r_start+479));
```

```
ARV_EMG3_300 = mean(EMG3(r_start+480:r_start+599));
ARV_EMG3_360 = mean(EMG3(r_start+600:r_start+719));
ARV_EMG3_420 = mean(EMG3(r_start+720:r_start+839));
ARV_EMG3_480 = mean(EMG3(r_start+840:r_start+959));
ARV_EMG3_540 = mean(EMG3(r_start+960:r_start+1079));
ARV_EMG3_600 = mean(EMG3(r_start+1080:r_start+1199));
ARV_EMG3_660 = mean(EMG3(r_start+1200:r_start+1319));
ARV_EMG3_720 = mean(EMG3(r_start+1320:r_start+1439));
ARV_EMG3_780 = mean(EMG3(r_start+1440:r_start+1559));
ARV_EMG3_840 = mean(EMG3(r_start+1560:r_start+1679));
ARV_EMG3_900 = mean(EMG3(r_start+1680:r_start+1799));
ARV_EMG3_910 = mean(EMG3(r_start+1800:r_start+1819));
ARV_EMG3_920 = mean(EMG3(r_start+1820:r_start+1839));
ARV_EMG3_930 = mean(EMG3(r_start+1840:r_start+1859));
ARV_EMG3_940 = mean(EMG3(r_start+1860:r_start+1879));
ARV_EMG3_950 = mean(EMG3(r_start+1880:r_start+1899));
ARV_EMG3_960 = mean(EMG3(r_start+1900:r_start+1920));
EMG3_ARV_cat = [Baseline_EMG3 ARV_EMG3 ARV_EMG3_60 ARV_EMG3_120
ARV_EMG3_180 ARV_EMG3_240 ARV_EMG3_300 ARV_EMG3_360 ARV_EMG3_420
ARV_EMG3_480 ARV_EMG3_540 ARV_EMG3_600 ARV_EMG3_660 ARV_EMG3_720
ARV_EMG3_780 ARV_EMG3_840 ARV_EMG3_900 ARV_EMG3_910 ARV_EMG3_920
ARV_EMG3_930 ARV_EMG3_940 ARV_EMG3_950 ARV_EMG3_960];
```

E. Ethical approval letter for Chapters 5 and 6

College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)
Brunel University London
Kingston Lane
Uxbridge
UB8 3PH
United Kingdom

www.brunel.ac.uk

24 November 2022

LETTER OF APPROVAL

APPROVAL HAS BEEN GRANTED FOR THIS STUDY TO BE CARRIED OUT BETWEEN 30/11/2022 AND 30/06/2023

Applicant (s): Miss Jessica Davis Dr Amir Mohagheghi

Project Title: Effects of High Velocity Eccentric Training in Individuals with Cerebral Palsy and Healthy Adults

Reference: 38390-MHR-Nov/2022- 41940-2

Dear Miss Jessica Davis

The Research Ethics Committee has considered the above application recently submitted by you.

The Chair, acting under delegated authority has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- **PIS -What if something goes wrong? - Please delete 'the Chair of the Brunel University London, ' so the sentence scans properly.**
- **PIS - Apologies, the comment from the previous submission was unclear - please add back your name as the researcher and your Brunel email address.**
- **The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee by way of an application for an amendment.**
- **Please ensure that you monitor and adhere to all up-to-date local and national Government health advice for the duration of your project.**

Please note that:

- Research Participant Information Sheets and (where relevant) flyers, posters, and consent forms should include a clear statement that research ethics approval has been obtained from the relevant Research Ethics Committee.
- The Research Participant Information Sheets should include a clear statement that queries should be directed, in the first instance, to the Supervisor (where relevant), or the researcher. Complaints, on the other hand, should be directed, in the first instance, to the Chair of the relevant Research Ethics Committee.
- Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.
- The Research Ethics Committee reserves the right to sample and review documentation, including raw data, relevant to the study.
- If your project has been approved to run for a duration longer than 12 months, you will be required to submit an annual progress report to the Research Ethics Committee. You will be contacted about submission of this report before it becomes due.
- You may not undertake any research activity if you are not a registered student of Brunel University or if you cease to become registered, including abeyance or temporary withdrawal. As a deregistered student you would not be insured to undertake research activity. Research activity includes the recruitment of participants, undertaking consent procedures and collection of data. Breach of this requirement constitutes research misconduct and is a disciplinary offence.

Professor Louise Mansfield

Chair of the College of Health, Medicine and Life Sciences Research Ethics Committee (DLS)

Brunel University London

F. MATLAB code for splitting 16 training repetitions recorded using EMG in Spike2

```
Data = table2array(Session1set1);
Position = Session1set1(:, 6); % Gets column 6 (dynamometry position)
plot (Position,1);
% Activates cursors to determine start/end of each flexion
[rownumber, Position] = ginput;
rownumber = int64(rownumber);
% Initialize variables to store results
EMG2 = [];
EMG3 = [];
Torque = [];
Angle = [];
Velocity = [];
% Process each repetition
for rep = 1:length(rownumber) - 1
   % Extract data for the current repetition
   RepData = Data(rownumber(rep):rownumber(rep + 1), :);
   Rep_pos = RepData(:, 6);
   % Find min and max positions
   [Minpos, Iminpos] = min(Rep_pos);
   [Maxpos, Imaxpos] = max(Rep_pos);
   % Extract the portion between min and max positions
   Rep = RepData(Iminpos:Imaxpos, :);
   % Process and interpolate data for the current repetition
   NoD = height(Rep);
   Count = (0:NoD - 1)';
   const = 100 / (NoD - 1);
   Time100 = Count * const;
   x_int = (0:1:100)';
   % Interpolate data and concatenate with previous results
   EMG2 = cat(2, EMG2, spline(Time100, Rep(:, 9), x_int));
```

```
    EMG3 = cat(2, EMG3, spline(Time100, Rep(:, 8), x_int));
    Torque = cat(2, Torque, spline(Time100, Rep(:, 4), x_int));
    Angle = cat(2, Angle, spline(Time100, Rep(:, 6), x_int));
    Velocity = cat(2, Velocity, spline(Time100, Rep(:, 5), x_int));
end
% Plot the results
figure;
plot(Torque);
hold on;
plot(Angle);
plot(Velocity);
plot(EMG2);
plot(EMG3);
legend('Torque', 'Angle', 'Velocity', 'EMG2', 'EMG3');
```

G. MATLAB code for calculating the ARV of EMG data before and after peak torque

```
% Define the file names and sheet names
inputFileName = 'P01_Week2_Set4.xlsx';  % Replace with your input file name
outputFileName = 'Book2.xlsx';  % Replace with your output file name
% Read specific columns (17th column) from EMG2, EMG3, and Torque sheets in the input spreadsheet
[~, ~, rawEMG2] = xlsread(inputFileName, 'EMG2');
[~, ~, rawEMG3] = xlsread(inputFileName, 'EMG3');
[~, ~, rawTorque] = xlsread(inputFileName, 'Torque');
% Extract the 17th column data
EMG2 = cell2mat(rawEMG2(:, 17));
EMG3 = cell2mat(rawEMG3(:, 17));
Torque = cell2mat(rawTorque(:, 17));
% Find the index of the peak torque value and total length of the signal
[peakTorque, peakIndex] = max(Torque);
totalLength = length(Torque);
% ... (Previous code remains unchanged)
% Calculate the window sizes based on the peak torque position
dataPointsAfterPeak = totalLength - peakIndex;
dataPointsBeforePeak = peakIndex - 1;
% Calculate the window sizes for the same number of data points before and after peak torque
windowSizeAfter = min(dataPointsAfterPeak, dataPointsBeforePeak);
windowSizeBefore = windowSizeAfter;
startIndexAfter = peakIndex + 1;
startIndexBefore = max(1, peakIndex - windowSizeBefore);
% Extract the EMG signal portions for the specified windows
EMG2_afterPeak = EMG2(startIndexAfter:startIndexAfter + windowSizeAfter - 1);
EMG3_afterPeak = EMG3(startIndexAfter:startIndexAfter + windowSizeAfter - 1);
```

```
EMG2_beforePeak = EMG2(startIndexBefore:peakIndex - 1);

EMG3_beforePeak = EMG3(startIndexBefore:peakIndex - 1);

% Calculate the Average Rectified Values for both portions

ARV_EMG2_afterPeak = nanmean(abs(EMG2_afterPeak));

ARV_EMG3_afterPeak = nanmean(abs(EMG3_afterPeak));

ARV_EMG2_beforePeak = nanmean(abs(EMG2_beforePeak));

ARV_EMG3_beforePeak = nanmean(abs(EMG3_beforePeak));

% ... (Remaining code remains unchanged)

% Create an array of results

outputData = [ARV_EMG2_beforePeak, ARV_EMG2_afterPeak, ARV_EMG3_beforePeak,
ARV_EMG3_afterPeak];

% Write the results to a new Excel file

xlswrite(outputFileName, outputData', 'P01W2');  % Writes to OutputSheet in the output
Excel file
```