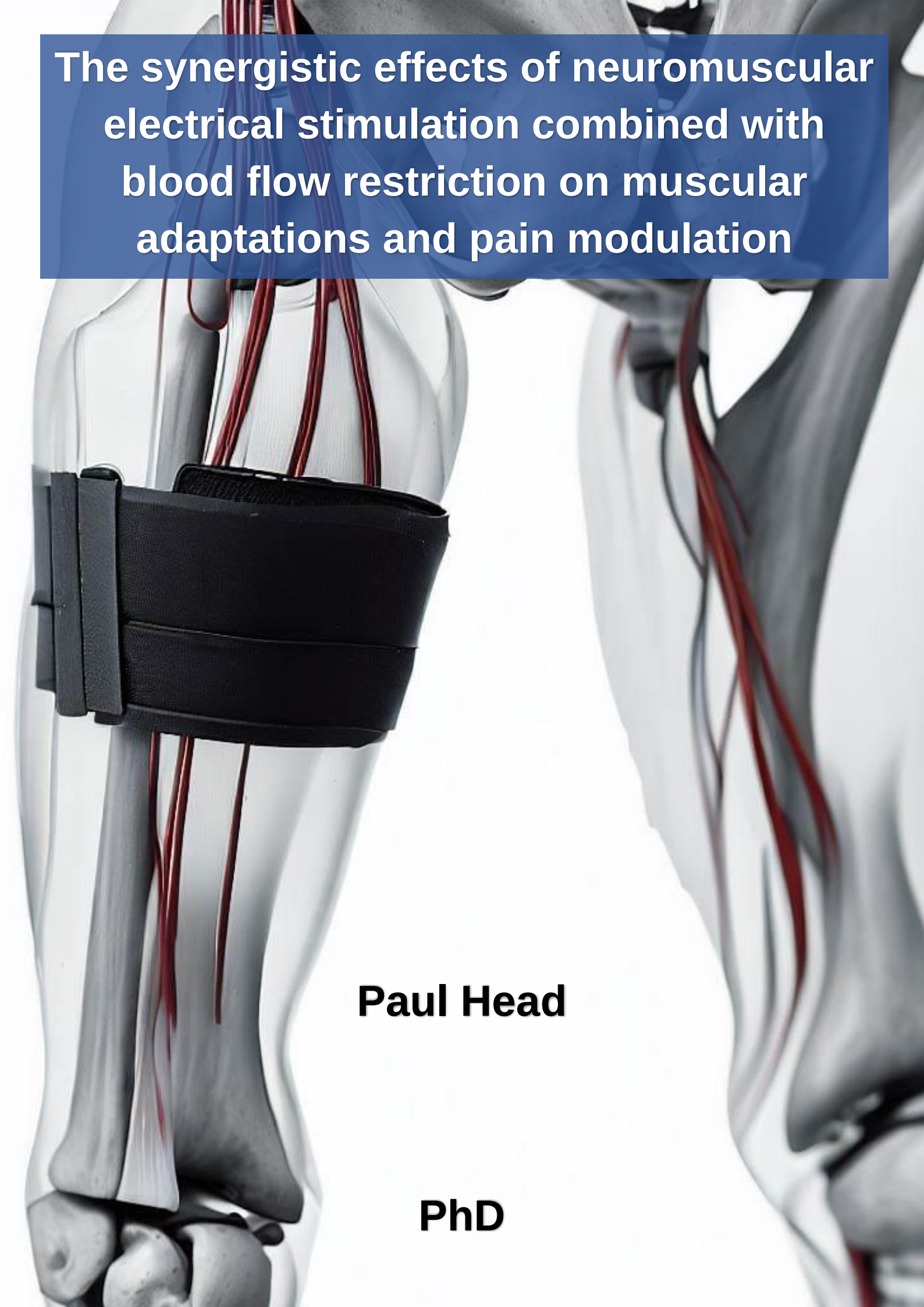
The synergistic effects of neuromuscular electrical stimulation combined with blood flow restriction on muscular adaptations and pain modulation



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PhD

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Abstract

This thesis conducts a thorough examination of the synergistic impact of neuromuscular electrical stimulation (NMES) combined with blood flow restriction (BFR) on muscular adaptations and pain modulation in healthy and knee osteoarthritis (OA) populations. The primary objective across subsequent chapters is to establish the superior efficacy of NMES+BFR over NMES alone, aiming to optimise muscular adaptations and modulate pain, potentially contributing to improved clinical outcomes and enhancing muscular adaptations without exercise. **Chapter 2** commences with a scoping literature review, identifying gaps and inconsistencies in existing research on NMES+BFR and drawing upon the wider literature bases of both to develop an evidence based methodology. Notably, methodological disparities in BFR and NMES application, namely not using arterial occlusion pressure (AOP) to prescribe the BFR stimulus and not using NMES frequencies and intensities found to enhance muscular strength, with these emerging as key contributors to the varying evidence base regarding their combined effectiveness in enhancing muscle strength and size outcomes. **Chapter 3** provides a comprehensive overview of the research methods employed, establishing a consistent framework for subsequent studies. **Chapter 4** optimises NMES+BFR methodologies, by using NMES recommended parameters and combining it with BFR using AOP (40-80%) to standardise the restrictive pressure. This study assessed acute measures of fatigue (surrogate marker for chronic training adaptations), muscle swelling, RPE, pain perception, and cardiovascular safety. Findings revealed increased fatigue after NMES+BFR (80%) compared to NMES alone. However, acute fatigue was observed after all NMES+BFR conditions, but greater perceptual pain and RPE reported after 60% vs. 40% AOP, therefore, eliminating the NMES combined with 60% condition from future investigation. Importantly, this chapter refines intervention parameters for a subsequent training study. **Chapter 5** focuses on a chronic training study to assess the effectiveness of NMES+BFR (40% and 80%) in increasing

muscle strength and size compared to NMES alone. A 6-week, 3-sessions-per-week randomised controlled trial was undertaken. Findings showed greater improvements in muscle strength (isometric and eccentric) and muscle size were observed in NMES+BFR groups, accompanied by greater NMES stimulation intensities tolerated during the training sessions compared to NMES alone. Due to the greater NMES stimulation intensity tolerated in Chapter 5 and the wider BFR evidence base reporting acute reductions in pressure pain and thermal pain thresholds, **Chapter 6** investigated the acute effects of pressure, thermal and temporal summation of pain (TSP) thresholds in healthy adults, revealing an acute increase in pressure pain thresholds immediately after NMES+BFR, explaining the greater tolerated currents observed in Chapter 5. **Chapter 7** replicates the methodology in a clinical population (knee osteoarthritis patients), due to this population demonstrating altered exercise induced hypoalgesia responses to healthy adults, which has been proposed as a main mechanism for reduced pain after BFR exercise. The results demonstrated acute increases in pressure pain thresholds, improvements in sit-to-stand performance, and reduced TSP after NMES+BFR (80%), with no effects observed after NMES alone. Cardiovascular safety is confirmed. **Chapter 8** synthesises and discusses the findings, emphasising the potential of NMES+BFR in enhancing strength and hypertrophy and modulating pain in healthy and knee OA patients without requiring exercise. In summary, this thesis offers a comprehensive exploration of NMES+BFR, showcasing its potential to enhance muscular adaptations and pain modulation in both healthy and clinical populations. The research underscores the promise of this intervention in improving clinical outcomes and providing a pre exercise intervention when exercise is not possible due to pain or contraindicated to enhance muscular adaptations and modulate pain.

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Conference and professional presentations

P. Head (2018). Acute Neuromuscular Electrical Stimulation (NMES) combined with Blood Flow Restriction (BFR) on physiological, perceptual and hemodynamic variables. Annual conference for the School of Sport, Health and Applied Sciences, St. Mary's University, London. **(Poster presentation).**

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P. Head (2019). Using Neuromuscular Electrical Stimulation (NMES) combined with Blood Flow Restriction (BFR) to optimise rehabilitation and return to play from rugby injuries. Annual conference for the England Rugby Football Union, Twickenham, London. **(Oral and practical presentation).**

P. Head (2019). Using Neuromuscular Electrical Stimulation (NMES) combined with Blood Flow Restriction (BFR) to optimise rehabilitation of musculoskeletal disorders. Chelsea and Westminster Hospital, London. **(Oral presentation).**

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P. Head (2020). Effect of Neuromuscular Electrical Stimulation (NMES) and Blood Flow Restriction (BFR) on acute muscle injury management. Sports MAP Muscle Injury Conference, Australia. **(Online oral presentation).**

P. Head (2021). Using Neuromuscular Electrical Stimulation (NMES) combined with Blood Flow Restriction (BFR) to optimise rehabilitation from acute injury management to return to play. Crystal Palace Football Club, London. **(Online oral presentation).**

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List of abbreviations

ABI: Ankle brachial index

AMI: Arthrogenic muscle inhibition

ANCOVA: Analyses of covariance

ANOVA: Analysis of variance

AOP: Arterial occlusion pressure

BFR: Blood flow restriction

BFRT: Blood flow restriction training

Bpm: beats/minute

CI: Confidence interval

CK: Creatine kinase

CPT: Cold pain thresholds

CSA: Cross-sectional area

CV: Coefficient of variation

DBP: Diastolic blood pressure

EIH: Exercise-induced hypoalgesia

EMG: Electromyography

ERK: Extracellular signal-regulated kinase

FMD: Flow mediated dilation

GP: General practitioner

HHb: Deoxyhemoglobin

HI-RE: High-intensity resistance exercise

HR: Heart rate

Hz: Hertz

ICC: Intraclass correlation coefficient

KOOS: Knee Injury and Osteoarthritis Outcome Score

mA: Milliamperes

MAPK: Mitogen-activated protein kinase

MDC: Minimum detectable change

mmHg: Millimetres of mercury

MPB: Muscle protein breakdown

MPS: Muscle protein synthesis

MRI: Magnetic resonance image

MTH: Muscle thickness

mTOR: mammalian target of rapamycin

MVC: Maximal voluntary contraction

MVIC: Maximum voluntary isometric contraction

mVO2: Muscle oxygen consumption

NIRS: Near-infrared spectroscopy

Nm: Newton metres

NMES: Neuromuscular electrical stimulation

NMES+BFR: Neuromuscular electrical stimulation combined with blood flow restriction

NMES+40% BFR: NMES combined with 40% BFR

NMES+60% BFR: NMES combined with 60% BFR

NMES+80% BFR: NMES combined with 80% BFR

NPRS: Numeric Pain Rating Scale

OA: Osteoarthritis

O2: Oxygen

PAR-Q: Physical activity and readiness questionnaire

PPT: Pressure pain thresholds

RPE: Rating of perceived exertion

SBP: Systolic blood pressure

SCI: Spinal cord injury

SD: Standard deviation

SEM: Standard error of mean

TESTEX: Tool for the assessment of study quality and reporting in exercise

TSP: Temporal summation of pain

US: Ultrasound

VA: Voluntary activation

VL: Vastus lateralis

VM: Vastus medialis

1RM: One repetition maximum

Chapter 1

Introduction

1.1 Introduction

Skeletal muscle mass plays a crucial role in overall health and well-being, as muscle tissue quantity directly affects muscle strength, functional capacity and physical performance[1,2]. Muscle size and strength are essential for physical function and quality of life as they enable us to perform activities of daily living with ease and independence, such as walking upstairs and maintaining balance[1,2]. Furthermore, they play a crucial role in preventing chronic diseases, such as osteoporosis and improving overall health and well-being[3]. Numerous situations, such as the recovery from illness or rehabilitation after injury, can necessitate a period of physical inactivity in otherwise healthy individuals. Periods of physical inactivity can result in muscle atrophy, which exacerbates negative health consequences[4,5]. During disuse and periods of immobilisation, skeletal muscle loss occurs at a rate of approximately 0.5% of total muscle mass per day, with strength declines between 0.3% and 4.2% each day[5]. Muscle atrophy and strength declines contribute to numerous negative health consequences including a loss of functional capacity and muscle strength[6–9], the development of insulin resistance[10,11], a decline in basal metabolic rate[12,13] and reductions in physical function[14].

Large reductions in muscular strength and muscle atrophy have also been associated with older age and have been observed in knee OA patients. Functional limitations associated with knee OA include muscle weakness and atrophy, particularly of the quadriceps, which play a key role in stabilising the knee joint[15]. These reductions in muscle strength and size knee OA are due to an inability to perform resistance exercise due to pain and disuse.

1.2 The complex interplay of pain and disuse on skeletal muscle function

Individuals with a history of joint injury or pathology, such as knee OA, commonly experience muscle weakness, activation failure and muscle volume loss[16–18]. The dynamic interplay between chronic pain and disuse poses a complex challenge to

understanding the advanced physiological mechanisms influencing skeletal muscle function.

Arthrogenic muscle inhibition (AMI), is a neuromuscular condition which leads to weakness and atrophy of the surrounding muscles, designed to protect the joint from further damage by inhibiting neural activation[19]. Multiple factors, including tissue damage, pain, inflammation and reduced proprioception contribute to AMI[16–22]. Tissue damage and inflammation activate immune cells and produce cytokines that impair muscle function, while pain reflexively inhibits the muscles, affecting their ability to contract effectively[23]. Altered movement patterns, muscle imbalances and decreased activation occur due to avoidance or modification of painful movements and reduced weight bearing, leading to muscle atrophy[24]. Chronic pain induces alterations in biomechanical parameters through a sophisticated network of molecular crosstalk. Inflammatory mediators, such as cytokines and prostaglandins, orchestrate complex signalling pathways influencing joint mechanics and muscle-tendon unit interactions[20,25]. Joint injury and pathology result in fewer motor neurons available to recruit and a diminished ability to voluntarily recruit motor neurons to a normal extent, due to a central protective mechanism, leading to central activation failure and lesser motor neuron pool excitability[19,26,27]. The muscle inhibition responsible for these observations is reflexive in nature and largely mediated by presynaptic mechanisms within the spinal cord[19]. These changes disrupt the normal neural signalling between joint receptors and the central nervous system, altering the sensory information transmitted to the spinal cord and brain[26]. Mechanisms contributing to AMI include alteration in muscle resting motor thresholds, changes in the discharge of articular sensory receptors, altered spinal reflex excitability and abnormal cortical activity[19,28].

Pain can limit an individual's ability to move and exercise, particularly weight-bearing activities and resistance training in knee OA patients[29]. This limitation affects exercise tolerance and hinders efforts to maintain or improve muscle strength[30]. Pain alters movement patterns, leading to compensatory strategies and alterations in biomechanics. Individuals with knee OA may unconsciously adopt abnormal gait patterns or modify their movements to minimise pain[25]. These altered biomechanics can disrupt the normal distribution of forces across the joint and surrounding muscles, leading to imbalances and increased stress on certain muscle groups, which may contribute to muscle fatigue and weakness over time[20]. Furthermore, disuse-related pain instigates a cascade of events influencing mitochondrial dynamics, subsequently impacting oxidative capacity and muscle endurance. Perturbations in the balance between mitochondrial fusion and fission processes result in impaired energy production, contributing to declines in muscle function[31]. Exercise-induced hypoalgesia (EIH), a phenomenon characterised by a reduction in pain perception following physical activity, is altered in individuals with knee OA [25]. Rather than experiencing reductions in pain sensitivity following exercise, knee OA patients often exhibit increases in pain and symptom flare ups[32,33]. The continuous joint pathology inherent in knee OA, marked by cartilage degeneration, inflammation, and structural alterations, often results in persistent nociceptive input that may override the potential pain-reducing effects of exercise[15]. Additionally, central sensitisation, a heightened responsiveness of central nervous system neurons to peripheral stimuli, is common in knee OA, potentially limiting the effectiveness of exercise-induced analgesia[20]. Psychological factors such as fear of movement and catastrophizing, which are prevalent in knee OA patients, can magnify the perception of pain, making it challenging for exercise to induce significant hypoalgesia. Reduced exercise tolerance due to pain during physical activity may lead to avoidance of exercises that could induce hypoalgesia, hindering the potential benefits of exercise-induced pain reduction. Moreover, the inflammatory milieu in knee OA may counteract the pain-modulating effects of exercise, and the presence of neuropathic pain components can introduce additional complexities in the responsiveness to exercise-induced hypoalgesia[20,25,29,34,35]. These negative effects

of exercise lead to the previously mentioned declines in muscle strength, size and function and interventions are warranted to enhance the clinical outcomes and quality of life for these individuals. Therefore, the impact of pain and pathology on muscle health underscores the critical importance of tailored rehabilitation strategies to prevent or alleviate muscle deterioration in individuals grappling with these complex issues, when exercise is too painful or contraindicated.

The present thesis will focus on developing exercise-mimicking interventional strategies to enhance muscular strength and hypertrophy without requiring voluntary movement to enhance outcomes and recovery during these periods. Finding interventions that positively influence muscle size and strength declines, and all of the physiological declines, discussed previously, due to pain, would potentially enhance functional outcomes and improve the quality of life for these individuals.

This thesis is structured to investigate various interventions that can improve muscle adaptations and alleviate pain in clinical populations. Two primary interventions will be explored: neuromuscular electrical stimulation (NMES) and blood flow restriction (BFR).

1.3 NMES

NMES has emerged as a pivotal tool for enhancing muscle strength in painful clinical populations by directly activating muscle fibres and facilitating essential physiological adaptations. This technique involves the precise application of electrical impulses to targeted muscles, achieved through surface electrodes. These electrical stimuli initiate muscle contractions, effectively replicating the benefits of traditional resistance exercise without requiring voluntary effort. Consequently, NMES recruits a broader spectrum of muscle fibres, including those typically underused during voluntary physical activity. This heightened recruitment fosters muscle strength as muscles adapt to the increased

demand[36]. However, it is worth noting that NMES has shown mixed results in terms of its ability to improve muscle hypertrophy or alleviate pain[37–40].

1.4 BFR

In contrast, BFR presents a distinctive approach to enhancing muscle size and strength in clinical populations while also managing pain[41–43]. BFR involves the application of a pneumatic cuff or inflatable tourniquet proximally on a limb, reducing arterial blood flow into the muscle while impeding venous return. This controlled restriction creates a unique physiological environment within the muscle, characterised by reduced oxygen supply and the accumulation of metabolites, inducing heightened metabolic stress[44]. Consequently, the body responds by releasing growth-promoting factors and recruiting a greater proportion of fast-twitch muscle fibres, typically activated during high-intensity resistance training[44]. Remarkably, even with lighter resistance loads, BFR exercises induce significant muscle fatigue and stimulate muscle hypertrophy. However, BFR requires being combined with voluntary resistance exercise to produce enhanced muscle size and strength, which may not be feasible for certain clinical populations, especially knee OA.

Recently, researchers have investigated the combination of NMES with BFR[45–47]. The scientific rationale is that combining BFR with low-intensity exercise has been found to increase muscle hypertrophy and strength compared to low-intensity exercise without BFR[48,49]. NMES can be classified as low-intensity isometric exercise, as it can evoke maximum voluntary contractions between 10% to 60%, which depends on the current intensity[38]. Thus, theoretically, the combination of NMES with BFR could potentially lead to enhanced muscular adaptations compared with NMES and BFR alone.

The results of trials using NMES and BFR in humans are varied, two studies reported increased muscle strength and hypertrophy compared with NMES and BFR alone in healthy

and spinal cord-injured adults[45,46] Other studies, however, found no added benefit to muscle strength and size[47]. Despite these mixed results, the clinical application for NMES and BFR in preventing atrophy and strength declines, as well as increasing muscle strength and size when in pain, post-surgery or during periods of immobilisation when voluntary exercise is contraindicated, is promising and will be explored in greater detail throughout this thesis.

Chapter 2

Review of the literature; NMES combined with BFR

2.1 Introduction

Skeletal muscle tissue requires a constant process of building up and breaking down of proteins to maintain its structure, with a turnover rate of approximately 1-2% daily[50]. The balance between MPS and MPB determines the net muscle protein balance and consequently, the amount of muscle mass in an individual. A stable muscle mass is maintained when MPS and MPB are in equilibrium[50]. However, disuse of human skeletal muscle alters the dynamics of muscle metabolism, leading to an increased rate of MPB and reduced rates of MPS[51]. For instance, Gibson et al. [9] showed that young men who were immobilised exhibited 30% slower rates of MPS compared to the non-immobilised limb. Further studies confirmed that disuse for 10-14 days caused reductions in MPS ranging from 27-50%[52–54]. Therefore, the available evidence strongly supports the idea that muscle atrophy in humans during a period of disuse is driven by the blunting of MPS[55], emphasising the critical role of physiological processes in shaping the fate of skeletal muscle under conditions of reduced activity.

In the realm of joint injury or pathology, such as knee OA or post-surgery, individuals commonly contend with AMI[19,56]. This neuromuscular condition, an intricate interplay of physiological and neural factors, manifests as muscle weakness, activation failure and volume loss[56]. AMI serves as a protective mechanism, inhibiting neural activation to safeguard the joint from further damage, but consequently leads to declines in muscle strength. Neural contributors to AMI include alterations in muscle resting motor thresholds, changes in articular sensory receptor discharge, modified spinal reflex excitability, and abnormal cortical activity[19,56]. This inhibitory neural response, combined with immune responses and cytokine-mediated impairments, results in altered movement patterns, muscle imbalances, and decreased muscle activation. This comprehensive understanding of both physiological and neural mechanisms in disuse atrophy lays the foundation for targeted interventions aimed at preserving muscle health and function in populations facing joint-related challenges.

Recently, the combination of NMES and BFR has emerged as a novel training method that can enhance muscle strength and hypertrophy without requiring volitional effort[45,46]. This technique involves applying electrical current to create action potentials and contract targeted muscles while simultaneously restricting the blood flow to the same muscle using an inflatable or pneumatic cuff[57]. Various studies have explored the effects of NMES combined with BFR (NMES+BFR) on muscle mass and strength in diverse populations, including healthy adults, obese adults, and patients with neurological disorders[45–47,58]. Despite the growing interest in NMES+BFR, the literature on this topic remains limited and inconclusive.

Therefore, this literature review aims to provide a comprehensive summary of the current state of knowledge on the effects and mechanisms of action of NMES+BFR on muscle mass and strength in different populations. By synthesising the available evidence, this review will contribute to a better understanding of the potential benefits and limitations of NMES+BFR as a training method. Additionally, the review will identify gaps in the existing literature, which will provide research questions that will be examined by this thesis.

2.2 Proposed mechanisms of action

The muscular adaptations resulting from exercise are triggered by the combined effect of mechanical tension, muscle damage, and metabolic stress[59]. However, in cases where voluntary exercise is not possible, such as after injury or surgery, NMES can be used as an exercise mimic, by causing involuntary muscle contractions and evoking mechanical tension[60]. NMES has recently been combined with BFR, owing to its ability to magnify metabolic stress[44]. Recent research has made progress in identifying the physiological mechanisms underpinning the observed muscular adaptations resulting from NMES combined with BFR, in both human and animal models[61–65]. Currently, the evidence suggests that NMES+BFR induces muscular adaptations via the indirect effects of

metabolic accumulation on neuromuscular fatigue and localised hypoxia, resulting in enhanced net protein balance. This is corroborated by the activation of the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) signalling pathways after NMES combined with BFR in animal model training studies[61,63–66].

The process of muscle growth is a complex and tightly regulated phenomenon that relies on the dynamic interplay between protein synthesis and degradation[67]. Protein synthesis refers to the process of creating new proteins from amino acids, while protein degradation refers to the breakdown of existing proteins[67]. The balance between the rate of protein synthesis and protein degradation ultimately determines skeletal muscle mass[67]. For example, a net decrease in protein synthesis and/or a net increase in protein degradation can lead to disuse atrophy[68]. Moreover, for muscle hypertrophy to occur, the intracellular environment should favour a positive protein balance, which can be achieved by increasing MPS, decreasing muscle protein breakdown, or both[67]. This is crucial for rehabilitation professionals due to the marked muscle wastage that occurs during periods of immobilisation after injury and surgery, leading to reduced levels of MPS[67,68].

Previous research has established that various stimuli, including nutrients, growth factors and NMES, can upregulate protein synthesis in skeletal muscle, primarily at the level of translation initiation[92,94]. A single session of NMES has been shown to increase protein synthesis rates in vivo in humans by up to 27% for a period of 4 hours[94]. This regulation of translation initiation by stimuli is primarily mediated by a protein kinase called mTOR[95]. When NMES is combined with BFR there is a positive muscle hypertrophy response[45,46,69]. Slysz et al. [70] examined the efficacy of NMES+BFR during 14 days of single-leg unloading. The researchers observed that applying NMES+BFR twice a day for 5-days a week prevented atrophy and resulted in mild hypertrophy (+5% [10%]) compared to BFR alone[98]. These results suggest that NMES+BFR can enhance protein

synthesis compared to BFR alone[94,97]. However, BFR applied on its own is insufficient to enhance rates of MPS[96,97].

Accumulation of metabolites during BFR exercise has been suggested as a stimulus of muscle adaptations, as it creates a localised environment of metabolic stress, which stimulates the activation of signalling pathways involved in protein synthesis and cellular adaptation[44]. Greater accumulation of metabolites has been observed after NMES+BFR compared with NMES alone[63]. Additionally, animal models have demonstrated an increase in lactate and H+ concentrations and a decrease in pH following NMES+BFR compared to either intervention on its own[71]. The greater accumulation of metabolites during NMES+BFR appears to induce neuromuscular fatigue earlier than NMES alone[61], through the metabolic stimulation of group III and IV afferent fibres[44]. Several human and animal model studies have shown greater neuromuscular fatigue with NMES+BFR than with NMES alone[61,65,72,73].

Murthy et al. [72] investigated the effect of NMES and varying degrees of BFR on evoked force and muscle oxygenation in the wrist extensors of healthy males using a crossover design. They observed reductions in twitch force when the applied BFR was ≥ 60 mmHg, while no effect was observed with lower BFR pressures of 20 and 40 mmHg[72]. In addition, Cole and Brown [73] found evoked force fatigue when NMES was combined with BFR pressures of 160 and 210 mmHg on the calf musculature of young adults, whereas BFR pressures below this did not result in evoked force fatigue[73]. These findings are supported by Wust et al. [65], who observed that evoked force fatigue increased from 33.8% to 68.2% with the addition of a thigh cuff inflated to 240 mmHg, to 5 minutes of NMES in healthy males and females.

In contrast, Satiago-Pescador et al. [74] did not find a difference in volitional fatigue between NMES and NMES+BFR groups. The authors measured volitional fatigue through knee

extension maximal voluntary contraction (MVC), unlike the previously mentioned studies, which assessed evoked force fatigue[65,72,73]. Nevertheless, Santiago-Pescador et al. [74] only applied one BFR pressure of 50% arterial occlusion pressure (AOP) which may explain the lack of effect observed, as fatigue only occurred in the higher BFR pressures used by others[72,73]. Animal model data also demonstrates that NMES+BFR induces evoked force fatigue compared to NMES alone[61]. Therefore, to maintain force output from the muscle when fatigued, the lower threshold motor units (Type I) may become insufficient to generate the necessary force. To compensate for this fatigue, the nervous system recruits higher threshold motor units (Type II), which have a greater force-generating capacity. The recruitment of higher threshold motor units is associated with a greater hypertrophic stimulus for muscle fibers. High-threshold motor units are typically associated with larger, fast-twitch muscle fibers, and their recruitment triggers a response that promotes muscle hypertrophy[75,76], resulting in a hypertrophic stimulus for a greater proportion of muscle fibres after NMES+BFR than NMES alone[45,46,61,69].

The recruitment of muscle fibres is a crucial factor in facilitating muscular adaptations as it determines the amount of tension applied to muscle fibres and triggers cellular processes necessary for muscle mass and strength gains[59,77]. Increased recruitment of muscle fibres is one of the underlying neural mechanisms that lead to muscle strength gains[59]. Motor units, consisting of a motor neuron and the muscle fibres it innervates, are the functional units of the neuromuscular system that generate muscle contractions[77]. During voluntary contractions, motor units are recruited according to the size principle, which proceeds from the smallest to the largest[78]. However, research on NMES has reported a non-selective, spatially fixed, and temporally synchronous motor unit recruitment pattern, or a complete reversal of the physiological voluntary recruitment order[36,79]. This means that type II muscle fibres are recruited even when using minimal NMES currents[80].

Li et al. [81] evaluated the effect of BFR, NMES and NMES+BFR during low-intensity squat training on muscle adaptations and muscle activation assessed through surface electromyography (EMG). They observed a greater increase in muscle activation following squats combined with NMES and BFR (NMES+BFR) and squats with NMES alone, compared to squats combined with BFR alone and the control group, who only performed the squatting intervention[81]. Their findings indicated that NMES and NMES+BFR induced greater strength and muscle activation than squats combined with BFR or done alone. Nonetheless, the fatigue mechanism that induces enhanced motor unit recruitment with NMES+BFR needs to be further assessed as a passive intervention. Factors associated with the onset of fatigue include failure of excitation of motor neurons, impairment of action potential propagation in the muscle membrane, conductivity of sarcoplasmic reticulum due to Ca2+ ion concentration, and changes in the concentration of catabolites and metabolites[82].

A mechanism for increased muscle strength and size with NMES+BFR, as opposed to NMES alone, is metabolic accumulation, which promotes a positive protein balance and indirectly induces neuromuscular fatigue[45,46,61,63,65,66]. The hypoxic environment associated with BFR may also promote anabolic signalling within the muscle and induce greater fatigue when combined with NMES[44,83]. Because the availability of oxygen is reduced during BFR, it is suggested that progressive recruitment of additional motor units may compensate for the force production deficit[44,83]. In a study by Nakajima et al. [61], NMES+BFR caused a 72.4% decline in muscle oxygenation levels. Moreover, Murthy et al. [72] demonstrated that reduced twitch force of the wrist extensors in humans was positively correlated with reduced muscle oxygenation in a dose-dependent manner.

Animal model studies indicate that protein synthesis pathways, including the phosphorylation of ribosomal protein rpS6, MAPK signalling pathways, mitochondrial biogenesis and glucose transporter, are enhanced with NMES+BFR compared to NMES

and BFR alone[61,64]. These increases are correlated with increased muscle hypertrophy after 3-weeks of training[61]. For instance, Nakajima et al. [61] provided the first animal model data demonstrating that the reduced partial pressure of oxygen (O2) saturation during NMES contractions with BFR enhanced the phosphorylation response of rpS6 in the mTOR signalling pathways, resulting in the observed muscle hypertrophy (6.2%) and increased mass (11%) after 3-weeks of training. Furthermore, NMES+BFR research observed an increase in Akt phosphorylation at Ser473, S6K1 at Thr389, and S6 at Ser235/236 following the application of NMES+BFR compared to NMES alone in rat gastrocnemius muscles[84]. Akt can activate S6K1 and S6 via mTOR phosphorylation[85]. S6K1 and S6 proteins can also regulate the translation initiation of skeletal muscle proteins[85]. S6K1 at Thr389 is a key regulator of muscle protein synthesis, which is closely associated with muscle hypertrophy after training[51,52]. Therefore, the phosphorylation of S6K1 at Thr389 is frequently used as a measure of mTOR activity[86,87]. Natsume et al. [64] observed that S6K1 and S6 were phosphorylated only when NMES was combined with BFR, suggesting that NME+BFR may enhance post-translational regulation of the mTOR pathway compared with either NMES or BFR alone.

Vascular adaptations have also been observed after NMES+BFR[45]. Arterial reactivity is typically measured as the ability of an artery to increase its size (diameter) in response to an increase in blood velocity, that is, flow mediated dilation (FMD)[88]. Gorgey et al. [45] found a 12% increase in FMD after NMES+BFR, compared to 6% after BFR alone. Previous BFR literature has shown enhancement of FMD[89]. Furthermore, FMD increased by 2.7-6.6% after NMES in the same clinical population[90]. Additionally, NMES training may improve muscle oxidative capacity and result in a fast-to-slow muscle fibre type transition and capillarisation of the stimulated muscles[91,92]. However, the application of NMES+BFR during a 2-week period of immobilisation was insufficient to mitigate artery structure impairments and FMD, suggesting the intervention-induced shear stress may not affect vascular function[93]. The evidence is currently mixed on NMES+BFR promoting

vascular adaptations and microvascular function, therefore remains inconclusive and warrants further investigation.

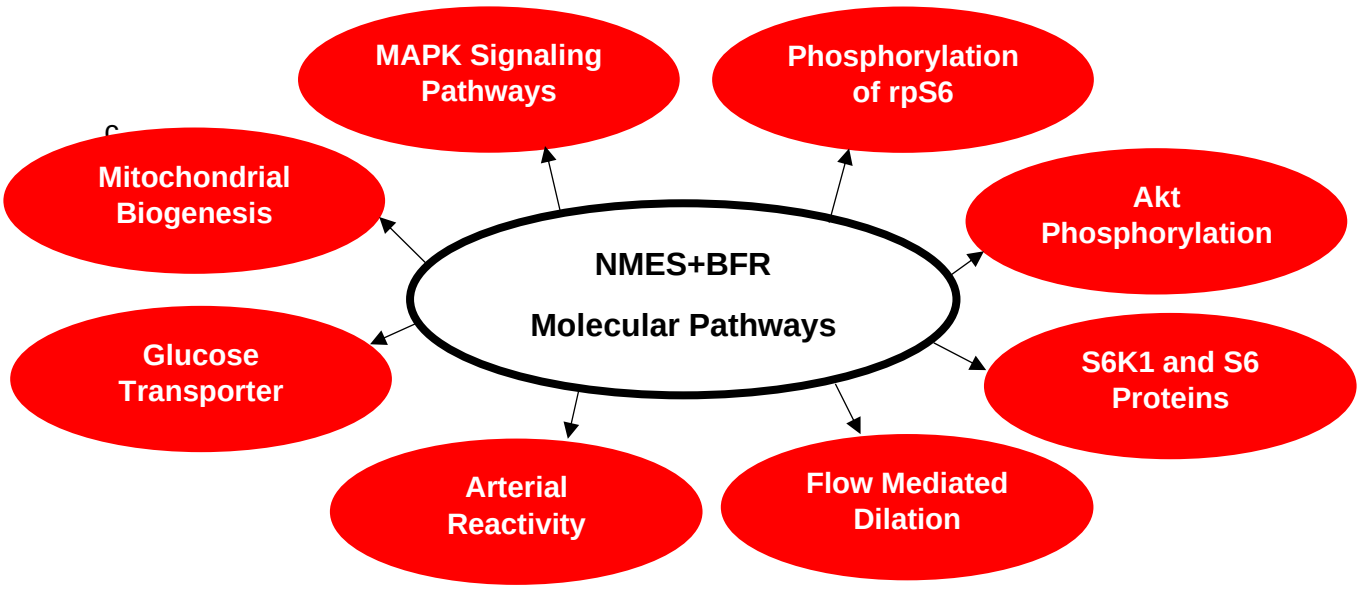

Fig 2.1. Diagram of NMES+BFR molecular pathways.

In conclusion, the existing research to date on NMES+BFR suggests that this combination can induce muscular adaptations through metabolic accumulation, hypoxia-induced fatigue, enhanced motor unit activation, vascular adaptations and increased protein synthesis, which is supported by the MAPK, ERK and mTOR signalling pathways[45,46,61–65]. However, these findings are not consistent across all studies, and further research is warranted.

2.3 Muscle strength

Muscle strength plays a vital role in human life, facilitating independent living by enabling us to perform essential activities[94]. To date, four human studies and two animal models have investigated the effectiveness of NMES+BFR in enhancing muscular strength compared to NMES alone, BFR alone[45–47,58,61,66,70], or a non-exercising control[47]. The current literature presents mixed findings on the superiority of NMES+BFR over NMES alone in humans for enhancing muscular strength[45–47,58]. Natsume et al. [46] where the

first to conduct a controlled trial and reported increased isometric and isotonic quadriceps strength after two weeks (20 sessions) of NMES+BFR training versus NMES alone. However, Slysz et al. [58] found differences in quadriceps strength improvements after NMES+BFR compared to a non-exercising control leg after a 6-week intervention, but no difference observed between NMES+BFR and NMES alone conditions. Gorgey et al. [45] observed similar improvements in evoked torque after 6-weeks of NMES+BFR and NMES in spinal cord injured patients, with no interaction observed. In a case study, a 15-week NMES+BFR intervention enhanced visually assessed neck stabilisation in a paediatric patient diagnosed with flaccid quadriplegia[22]. In contrast, Andrade et al. [47] reported no change in plantar flexor strength after 6-weeks of NMES+BFR training, potentially due to methodological inconsistencies to be discussed in greater detail below.

The NMES+BFR animal model data consistently shows greater force production after NMES+BFR interventions than NMES alone[61,62,64,66]. The training protocols for these animal model studies ranged from three to eight weeks[61,64,95,96]. Overall, the evidence base largely supports NMES+BFR's effectiveness at enhancing muscular strength, with one human and two animal models finding greater improvements than NMES alone[46,61,66]. However, Andrade et al. [47] reported no benefit, possibly due to the calf muscle group's training protocol and transverse placement of NMES electrodes, which has been shown to be less effective than longitudinal placement in enhancing muscular strength[38,47]. The observed diminished effectiveness of transverse placement of NMES electrodes, compared to longitudinal placement, in enhancing muscular strength is likely attributed to the suboptimal alignment with the natural direction of muscle fibers. This misalignment results in less synchronised and targeted recruitment of motor units and a dispersion of the electrical stimulus across various directions. These factors collectively reduce the effectiveness of NMES in enhancing muscular strength[38,97,98].

Slysz et al. [58] stimulated the same muscle groups as Natsume et al. [46] and Gorgey et al. [77] but obtained contrasting results. Slysz et al. [58] failed to report treatment session adherence, which was above 99.7% in the other studies[46,99], potentially compromising protocol follow-through and treatment tolerability. The BFR method used by Slysz et al. [58] was intermittent and arbitrary (220 mmHg) achieving 100% occlusion in most individuals, whereas the other studies employed a partial BFR stimulus[46,99]. Using a 100% BFR pressure is not effective for enhancing muscular strength and hypertrophy and is not supported by the broader BFR literature[41,48,100]. The different BFR protocols could have affected the results found, as Slysz et al. [58] used the same session frequency but obtained conflicting results to Gorgey et al. [99]. Additionally, Slysz et al. [58] did not report the contraction time, duty cycle used on the quadriceps, or the electrode size or placement for either their upper or lower limb NMES protocols. These elements are crucial for optimising NMES applications[38]. Another difference was that Gorgey et al. [99] used incomplete spinal cord injured patients instead of healthy subjects, which could explain the beneficial effects they found. Patients with neurological conditions tend to have greater muscular adaptations after NMES then non-neurological individuals[101].

The evidence base for NMES+BFR has limitations, as highlighted above and in various studies[46,47,99,102]. To assess the methodological quality of the NMES+BFR training studies, a previously validated assessment tool for the assessment of study quality and reporting in exercise (TESTEX) was used[103]. However, none of the trials used a separate control group, instead using different limbs to act as the control, and they failed to describe how randomisation was carried out. Using the same subject for both the intervention and control groups raises questions about the potential cross-transfer effect from the intervention or control limb, which could have affected the results[104,105]. It is well known that resistance training on one limb can cause increase in muscular strength not only in the trained limb but also in the contralateral, untrained limb due to the cross-education or cross-transfer effect[104,105]. This phenomenon is caused by both neural adaptation[104,105]

and muscular hypertrophy[106]. However, it has been suggested that the cross-transfer of strength increase is only due to neural factors[104,105]. This phenomenon has been suggested to occur through a different mechanism during BFR training (BFRT)[107]. Madarame et al. [107] showed that muscle cross-sectional area (CSA) and isometric peak torque of the trained arm muscles increased only when combined with resistance exercise with BFRT for the legs. They suggested that an increase in noradrenaline was involved in this remote effect of exercise on muscular size[107], but it was not different compared with no BFR and this finding has not been substantiated in further research. Furthermore, no instructions in the present NMES+BFR studies was given on what the participants should avoid during the training protocols. This confounding factor could have affected the results observed with activity undertaken away from the studies affecting the muscular adaptations observed. Using separate participants for both experimental and control groups is recommended in future research.

After BFRT, muscular strength and hypertrophy gains have been found both distal and proximal to the cuff location, suggesting systemic mechanisms may promote these adaptations[48,100]. Increased supraspinal activation has also been reported after BFR resistance exercise compared with conventional resistance exercise[108]. This phenomenon has also been found with NMES[109]. In rabbits, it was shown that repetitive unilateral muscle overuse caused by NMES overtime leads to both degenerative and regenerative tissue changes and myositis not only in the exercised muscles but also in the homologous non-exercised muscles of the contralateral leg[109]. Yong et al. [109] suggested that the nervous system is involved in the cross-transfer effects observed during NMES. This has also been supported in numerous other studies showing the spinal and supraspinal effects of muscular adaptations from NMES[39]. The cross-transfer effects observed in previous NMES and BFRT studies could have affected the NMES+BFR results observed to date[46,47,99,102].

The stimulation protocols employed in NMES+BFR training studies varied in frequency (ranging from 20-100 Hz), pulse width (400-450 μs), duty cycle (5-8 s on and 3-600 s off), and other parameters[46,47,99,102]. However only one study reported the ascent and descent phases of the stimulation cycle, pulse type and shape[47], and the number of stimulation repetitions[99]. Another limitation was the lack of reporting electrode size[46,99], which is crucial for replication, comfort, and evoked force production[38]. The methodological inconsistencies are discussed in greater detail below.

2.4 Muscle size

Muscle strength is crucial for human life and independence, while muscle size can also influence overall physical function by increasing strength and endurance[94]. The current evidence base for NMES+BFR again shows mixed and varied results regarding whether this combination is effective in producing muscular hypertrophy in humans in six human trials [46,47,58,70,99]. Gorgey et al. [77] discovered a between-group difference for extensor carpi radialis longus hypertrophy, which was 17% greater than NMES alone. The extensor carpi radialis longus is a forearm muscle positioned on the posterior (dorsal) aspect of the forearm, along the lateral (radial) surface. Originating from the lateral supracondylar ridge of the humerus to its distal attachment at the base of the second metacarpal bone in the hand, contributing to its role in extending and abducting the wrist joint[110]. Additionally, they found greater improvements in FMD changes compared to BFR alone, although no other between-group differences were observed. Notably, there was no extensor digitorum communis hypertrophy change. Since the forearm muscles affect various finger motions, previous NMES research has found it possible to selectively activate the extension/flexion of most fingers by stimulating the forearm muscles[111]. Thus, the physiological characteristics of each finger should be considered when placing the negative electrode for selective stimulation of individual fingers[111]. Therefore, electrode placement could have confined the stimulus to the extensor carpi radialis longus. Additionally, the extensor carpi radialis longus is a greater prime mover than extensor digitorum

communis[111], and thus the observed middle finger extension used as a point to cease increasing NMES current intensity could have stimulated the extensor carpi radialis longus but not promoted extensor digitorum communis hypertrophy.

Skiba et al. [69] assessed the effect of NMES+BFR vs. NMES alone on the quadriceps of complete spinal cord-injured patients. They observed greater quadriceps muscle thickness increases after eight weeks of NMES+BFR compared to NMES alone on the paralysed muscles[69]. The same result was observed by Gorgey et al. [45] in spinal cord-injured patients reporting a 17% greater increase in extensor carpi radialis longus CSA in the NMES+BFR forearm compared with NMES alone. Additionally, they reported that NMES+BFR induced a 15% increase in extensor carpi radialis longus CSA. In obese, but otherwise healthy adults, Natsume et al. [46] found quadriceps size increased from pre- to post-training after NMES+BFR, with no change observed for NMES alone. However, there was no between-group difference. More recently, Bergamasco et al. [112] studied the effect of NMES+BFR compared to low-intensity resistance exercise combined with BFR on vastus lateralis CSA, after a six-week intervention. They observed greater hypertrophy after low-intensity resistance exercises combined with BFR vs. NMES+BFR. However, the pre-to-post change observed after the six-week NMES+BFR intervention was (4.6%), indicating a hypertrophic response. In contrast, Slysz et al. [58] found neither NMES+BFR nor NMES alone enhanced quadriceps hypertrophy. Additionally, Andrade et al. [5] observed no change in soleus muscle thickness after six weeks of NMES+BFR training. This study is the only one to date that has found no beneficial within-subject effect of NMES+BFR on muscular adaptations. The authors state that this could have been because the soleus muscle is comprised primarily of slow-twitch fibres, which have a lower hypertrophic potential in comparison to type II fibres[47]. The previous statement is true[113]; however, BFRT has been shown to cause hypertrophy in both type I and II fibres[16–18], with some evidence showing preferential type I fibre hypertrophy[19]. Andrade et al. [5] may have seen an effect of the intervention if they utilised a larger sample size than seven, since they

showed a 15.2% change in muscle thickness. Additionally, their strength results show greater muscle size improvement in their control leg than what they observed in the intervention leg. The exercise and/or physical activities that the participants were undertaking away from the experimental sessions could have contributed to the results observed by Andrade et al. [47]. Slysz et al. [70] reported a mild increase in hypertrophy (+5% [10%], $p = 0.07$) over a 14-day period of unloading. This suggests that hypertrophy can occur even in the absence of voluntary motion during unloading.

BFR protocols also varied in the studies, with differences in the pressure applied, cuff size, and occlusion duration. quite largely with one study causing full occlusion[45–47,70,112,114]. One study induced full occlusion using a pressure of 130% of systolic blood pressure (SBP) for the upper limb[45]. SBP represents the pressure on arterial walls during heart contraction (systole) and indicates the maximum pressure during a heartbeat[99]. One study based their BFR pressure on thigh circumference[46], while one referenced previous BFRT research but used a different cuff width than the original study[47]. Additional studies employed an arbitrary pressure, applied intermittently[70,112]. Two studies did not report the cuff size used. When applying BFR the cuff width is an important consideration because using the same pressure with a wider cuff will apply a greater restrictive stimulus[115]. Standardising the BFR stimulus according to arterial occlusion pressure has been recommended in the wider BFR literature to enhance efficacy and safety during its application[57,116,117]. Furthermore, measuring every individual's AOP in the position that the intervention will be undertaken further enhances its accuracy and reliability[116] which to date has not been utilised in NMES+BFR research.

Human trials investigating muscular hypertrophy have produced inconsistent results[45–47,58,69,112]. While three trials found a between-group difference[45,46,69], one found within-group increases[58] and one observed no effect[47]. These discrepancies may be due to methodological differences between the studies, including variations in the

parameters of NMES and BFR, intervention lengths and methodological rigour previously discussed.

Overall, the current evidence suggests that the combination of NMES+BFR may be more effective than either intervention alone in inducing muscular adaptations. However, the studies conducted thus far have varied considerably in terms of methodology. Therefore, the later sections of this review with synthesise the methods employed in these studies and provide guidance for future research requirements.

2.5 NMES parameters

The NMES parameters employed in current NMES+BFR research are largely varied, unclearly reported, and not recommended by the wider NMES evidence base[37,38,118]. This may explain the lack of consistent muscular adaptations observed compared with previous NMES research. One of the main drawbacks of NMES treatments is the fact that they can be too painful, with increasing current creating further discomfort[38,119,120]. An ideal electrical stimulation application should be able to increase muscle strength with the least possible discomfort during stimulation[121]. However, the muscular adaptation observed is determined by the current intensity applied[84]. Therefore, to maximise adaptation, the methodology should focus on applying the greatest current intensity with the minimal amount of discomfort[38,84,121,122]. Just like any other form of training, appropriate programming is required to achieve the desired result. Using appropriate NMES parameters, electrodes and locating motor points is crucial for optimising muscular adaptations from its application[38].

2.5.1 Frequency

The frequency used during NMES interventions has been identified as a critical factor in determining its effectiveness on muscular adaptations[38]. Frequency refers to the pulses

produced per second during stimulation and is measured in Hz, where 40 Hz equates to 40 pulses per second[60]. Studies have shown that an optimal frequency range of 30-50 Hz is effective in enhancing muscular adaptations[118]. However, for the quadriceps, a frequency of 50 Hz has been recommended[123], due to this frequency minimising discomfort and increasing quadriceps strength compared to frequencies below this[123]. Moreover, tetanic muscle contractions are produced at a frequency rate of approximately 50 Hz in human muscles[36].

However, the NMES+BFR training and acute studies have used frequencies that ranged from 1-100 Hz[46,47,58,63,69,70,72–74,93,99,112] or have not been reported[65,124,125]. Slysz et al. [58] have reported using frequencies between 50-100 Hz, but it is unclear whether they ever used the recommended 50 Hz for the quadriceps[38,118]. No other NMES+BFR studies to date have utilised a 50 Hz frequency on the quadriceps[46,69]. The other NMES+BFR training studies used frequencies outside of what is recommended[45,69,70,112,114]. This may help explain the mixed effects observed.

In summary, frequency plays a crucial role in determining the effectiveness of NMES interventions on muscular adaptations. An optimal frequency of 50 Hz for the quadriceps, has been recommended by the wider evidence base[38,123,126]. However, the frequencies used in the NMES+BFR literature have not used this frequency, vary widely and are often not reported, which may explain the inconsistent results observed to date. It is essential to use appropriate frequencies, 50 Hz for the quadriceps, and report them clearly in future studies to maximise the effectiveness of NMES+BFR interventions.

2.5.2 Pulse width/duration

Electrical stimulation devices produce pulses in waveform patterns that are commonly represented by geometric shapes such as square, peaked, or sine waves[38]. These

shapes characterise electrical current that either rises above a zero baseline for the duration of the stimulation paradigm (uniphasic; e.g., direct current), or alternates above and below the baseline (biphasic or alternating current)[127]. The pulse width or pulse duration refers to the time span of a single pulse. In biphasic pulses, the pulse duration considers both phases[128]. A previous study compared pulse widths of < 200 μs vs. > 200 μs delivered to the soleus muscle and found that wider pulse widths produced stronger contractions of plantarflexion and augmented overall contractile properties[129]. Longer pulse durations typically penetrate more deeply into subcutaneous tissues, so these widths should be used when trying to impact secondary tissue layers[130]. Pulse widths between 400-600 μs are recommended in the wider NMES literature as they selectively target motor fibres without negatively impacting muscle fatigue or metabolic demands[131]. Kesar et al. [131] conducted a crossover study assessing pulse widths between 150-600 μs and observed greater evoked force, without fatigue, between 400-600 μs. Additionally, pulse durations closer to 400 μs produce greater quadriceps cross-sectional activation compared with 150 μs[132]. Natsume et al. [46] failed to report the pulse width applied and the other NMES+BFR training studies reported pulse widths in the recommended range (400-450 μs)[45,47,69,70,112,114]. The fact that the majority of NMES+BFR training studies used recommended parameters, pulse width does not seem to be a contributing factor to the mixed results currently observed.

However, we propose that pulse duration could be manipulated to fit with the recommendations in the wider strength and conditioning literature to optimise time under tension and rest periods of the interventions[133,134]. The current NMES+BFR training studies use contraction durations between 4-15 s[45–47,58,69,70,112], with rest periods after every NMES contraction ranging between 3-18 s or not reported[47,58]. Previous strength and conditioning research found greater levels of protein synthetic rates when implementing a time under tension of 6 s compared with 1 s[133]. The greatest muscle adaptations after NMES+BFR used contraction times of 5-15 s and rest between contractions of 3-5 s with 8-20 minutes stimulation periods each session[46,58,69,99]. The

work to rest ratio utilised by these studies were replicated in an animal model by Natsume et al. [64] to support greater muscle hypertrophy compared to NMES alone. This work period is equal to or greater than the rest period and differs from what is recommended in the wider NMES literature[38,123,135]. When applying NMES, it is generally recommended to have longer rest periods compared to the work periods, to reduce the effects of neuromuscular fatigue[38,123,135]. However, in the strength and conditioning evidence base, acute reductions in force output (i.e., fatigue) have been shown to correlate to increased chronic muscular adaptations[136,137]. This finding of acute fatigue leading to chronic muscle hypertrophy has been shown after NMES+BFR in rats[61]. Furthermore, Slysz et al. [58] reported elsewhere that they used a 600 s rest period between NMES contractions, making their ratio 1:150. The larger rest period used by Slysz et al. [58] may have contributed to their lack of effect compared with NMES alone. Therefore, the current NMES+BFR research suggests using a work ratio that is equal or greater to the rest period between stimulated contractions[45,46,61,64]. However, this approach differs from recommendations for NMES alone and warrants further investigation to optimise parameters for enhancing muscular adaptations.

2.5.3 Amplitude/ Intensity

A parameter that plays a role in inducing muscular adaptations is the strength of the current being administered, which is usually reported in milliamperes (mA). The intensity of electrical stimulation is commonly referred to as the current amplitude and is directly related to the amount of muscle activated during electrical stimulation[38]. Magnetic resonance imaging (MRI) has been used in previous studies to map the pattern of activation after repeated NMES evoked isometric contractions[138] These studies have found that increasing the intensity of electrical stimulation resulted in the activation of more motor units, which subsequently increased force output[36,84,138]. Therefore, increasing the current amplitude may lead to greater muscle recruitment and, thus, enhance the potential for muscular adaptations[84].

Higher intensities of electrical stimulation result in a stronger depolarising effect in the structures underlying the electrodes[139], and can lead to increases in strength[140–142]. However, patient comfort and tolerance must also be considered, as higher intensities are typically less tolerated. Nevertheless, both frequency and intensity play a crucial role in determining the quality of muscle contraction produced[143].

Table 2.1. NMES parameters for NMES+BFR training studies.

Author (year)	Frequency	Pulse width	Duty cycle	Intensity	Motor points located	Duration	Training frequency and duration
Natsume et al. (2015)	30 Hz	NR	8 s on / 3 s off	5-10% MVIC	Yes	20 mins, 4 x 5 mins	2 wks, 2 sessions a day (20)
Gorgey et al. (2016)	20 Hz	450 µs	5 s on / 5 s off	Wrist extension	No	7.5 mins	6 wks, 2 sessions a wk
Andrade et al. (2016)	35 Hz	400 µs	6 s on	20% MVIC	No	8 mins	6 wks, 3 sessions a wk
Slysz et al. (2018)	50-100 Hz	400 µs	NR	Max tolerable	No	12 mins, 3 x 4 mins	6 wks, 4 sessions a wk
Skiba et al. (2021)	20 Hz	400 µs	15 s on / 4 s off	Knee extension	No	12 mins, 3 x 4 mins	8 wks, 2 sessions a wk
Bergamasco et al. (2021)	75 Hz	400 µs	4 s on / 18 s off	8/10 pain	Yes	20 mins, 4 x 5 mins	6 wks, 3 sessions a wk (20)

NR: not reported; wks: weeks; s: seconds

The evidence base for NMES consistently demonstrates that the greater the muscular strength and hypertrophic adaptations observed, the higher the maximal tolerable intensities used[77,92,144,145]. However, to date, only Slysz et al. [58] have utilised max tolerable intensities in the NMES+BFR literature and has multiple methodological flaws which could have contributed to their lack of observable effect[102]. The other NMES+BFR training studies used a percentage of MVC to determine the current ranged from 5-20%[46,47] and others saying it was determined when movement occurred[45,69]. While beneficial muscular adaptations have been observed after intensities that elicited 5-10% MVC by Natsume et al. [46] is much lower than what is seen to cause muscular adaptations after NMES alone using maximal tolerable intensities[77,92,144,145]. Evoked force data

from Chapter 4 (Fig 4.3) found that NMES+BFR at maximal tolerable intensities produced 8-12% MVC, which could be attributed to the cuff placement for BFR, reducing the space between the anode and cathode electrodes causing less of the muscle to be stimulated, compared to NMES alone studies reporting >50% MVC's using max tolerable currents[77,92,144,145]. Therefore, basing the NMES intensity on the MVC produced could be a limiting factor for the current NMES+BFR literature[45–47]. Future NMES+BFR research should utilise max tolerable intensities to optimise potential benefits for enhancing muscular adaptations, regardless of evoked force, as recommend by the wider NMES literature.

2.5.4 Electrode placement and size

The success of NMES current in reaching underlying tissues is highly dependent on the size and placement of electrodes[111,146–148]. Electrodes placed on muscle motor points have an impact on muscle response and should be carefully considered[60]. The motor point is the specific location on the skin over a muscle where a contraction can be electrically induced with the lowest current amplitude[147]. As skin and tissue resistance to current is lowest at this point, patient discomfort is minimised, and evoked force is maximised[147,148]. Therefore, placing electrodes over motor points is crucial in enhancing the effectiveness of NMES[148]. Current intensity is associated with greater gains in muscle strength[84], thus, it is essential to use all possible techniques to maximise current and comfort[148].

A recent study comparing electrode placement using motor points of the muscle accurately located through stimulation compared with the recommended sites of several manufacturers showed differences in muscle performance outcomes in NMES delivered to the tibialis anterior and the vastus lateralis (VL) of the lower extremity[148]. The motor point placement not only produced higher torques but also increased blood flow and oxygen use,

making it more effective[147]. Electrode size is another essential variable, with larger electrodes (> 20 cm^2) promoting deeper current penetration and being more comfortable for the patient, leading to greater tolerance[60,97]. Using small electrodes (< 20 cm^2) can result in inadequate motor unit recruitment and reduced effectiveness[97].

In the current NMES+BFR literature, Natsume et al. [46] and Bergamasco et al. [112] are the only groups that located muscle motor points for electrode placement, which is a major limitation as previously mentioned. All NMES+BFR training studies used electrodes larger than 20 cm^2 in size[46,47,58,132]. However, for quadriceps, the greater muscle adaptations occur with one large proximal electrode and two smaller electrodes over the vastus medialis and vastus lateralis motor points[123]. Although Natsume et al. [46] and Bergamasco et al. [112] used this method, Slysz et al. [58] did not, which could explain the reduced effectiveness observed in their study[58].

2.6 BFR parameters

The vast majority of studies combining NMES and BFR have prescribed an arbitrary restrictive pressure[47,58,63,124] or based their occlusion pressure on systolic blood pressure (SBP)[99]. However, recent findings suggest that neither approach is effective for controlling the magnitude of BFR[57,117]. The current recommendation is to prescribe pressure via AOP[116]. Our group previously recommended determining AOP for prescribing BFR pressures[117], as it allows for standardised pressures regardless of equipment used or participant size. This is crucial since previous research shows that the restrictive stimulus produced is influenced by a variety of factors, including cuff width and the size of the participant's limb[115,149,150].

For the lower limb, AOP is determined using a handheld Doppler probe placed over the posterior tibial artery[151,152], with an inflatable or pneumatic cuff placed around the most proximal portion of a participant's thigh, and inflated until no auscultatory pulse is detected by the Doppler probe[153]. Other methods of determining BFR pressures used in the NMES+BFR literature include 130% SBP[45], basing the pressure on participant limb size characteristics[46] or arbitrary pressures[47,58]. Using 130% SBP as a BFR cuff pressure, creates full arterial occlusion of the upper limb[115]. Loenneke et al. [149] found that wide cuff inflation at 130% SBP (130% of ~120 = 156 mmHg) would exceed the necessary pressure for complete arterial occlusion (144 mmHg) of the lower limb in healthy individuals. Additionally, applying the same pressure and using a different cuff width or a larger participant will affect the BFR stimulus, creating methodological inconsistency that makes it challenging to analyse and compare the effectiveness of previous NMES+BFR trials[115].

Previous research suggests that the degree of BFR may be influenced by the amount of tissue surrounding the blood vessel, which affects the pressure exerted on the vasculature[115]. Limb circumference explains most of the variance in the cuff pressure required to occlude arterial flow[149]. Wide BFR cuffs require lower pressure to restrict arterial blood flow compared to narrow cuffs, with limb size and composition having a greater influence on the pressure[115]. The greatest muscular adaptations from NMES+BFR currently occurred when using a BFR pressure relative to the participant characteristics[46], 50% AOP[69] and 130% SBP[99]. Using AOP may help future research and may lead to greater muscular adaptations, as seen in the wider BFR literature[41,57].

Three NMES+BFR training studies[69,70,112] have used AOP to prescribe their BFR pressure. The NMES+BFR training studies showed beneficial effects when using AOP, although two of them used a 100% pressure[70,112] which is not recommended[57]. In animal models, Natsume et al. [71] reported using a cuff pressure approximately 40-60% of AOP, while Nakajima et al. [61] used a BFR pressure that considerably lowered O2 partial pressures but did not completely occlude blood flow. This may be interpreted as being

above 60% AOP but below 100% AOP, consistent with previous research on humans that found muscle oxygenation/deoxygenation during <60% AOP is not substantially different than during non-BFR exercise[154]. Reis et al. [154] concluded that 60% AOP appears to represent a threshold required to induce higher deoxygenation and decreased tissue oxygenation levels[154]. Skiba et al. [69] used 50% AOP combined with NMES and observed muscle growth compared with NMES alone. Therefore, current limited data supports the use of partial pressures of BFR when combined with NMES[57], although not conclusive.

During BFRT, cuff-related nerve injury is a potential complication that can be harmful[155,156]. The extent of nerve damage caused by cuff use can range from mild transient loss of function to permanent, irreversible damage[155]. Most cases of nerve damage occur beneath or near the edges of the cuff as shown by Ochoa et al. [156]. Despite the physiological and perceptual similarities to regular exercise, BFRT must be performed safely[157,158]. Rhabdomyolysis is another potential complication that has been reported after BFRT[159–162]. It involves the rapid dissolution of damaged or injured skeletal muscle, ranging from an asymptomatic illness with elevated creatine kinase (CK) levels to life-threatening conditions associated with extreme CK elevations, electrolyte imbalances, acute renal failure and disseminated intravascular coagulation[163]. All cases reporting rhabdomyolysis and acute vision loss after BFRT used arbitrary pressures and did not standardise the restrictive stimulus using AOP[160,161,164]. The protocols or cuff widths used were not reported in these cases, and this could have affected the applied restrictive stimulus, potentially contributing to these adverse events[159–162]. Furthermore, Martin-Hernandez et al. [124] observed syncopal episodes in three healthy young people during their NMES+BFR study. They also did not use AOP to determine their restrictive stimulus. Spranger et al. [165] proposed that BFRT, if performed incorrectly, may result in deleterious hemodynamic responses. Using AOP to determine BFR restrictive pressures may reduce the risk of adverse events from occurring and hemodynamic variables need to be assessed in NMES+BFR studies.

2.6.1 BFR frequency

The optimal training frequency for BFR is typically found to be between 2-3 sessions per week[48,57]. However, the current literature on NMES+BFR has employed various training frequencies, including 2 sessions per day, 5-days a week, for 2-weeks (20 sessions)[46]; 6-weeks with 3 sessions a week[47,112]; 6 weeks with 2 sessions per week[45]; 6 weeks with 4 sessions per week[58] and 8 weeks with 2 sessions per week[69]. Three beneficial and one non-effective study used the recommended training frequencies, however, the greatest effect was seen when using 2 sessions a day for a 2-week period.

In NMES research, training 2-3 times a week has also demonstrated beneficial effects on muscular adaptations[92]. Notably, Gorgey et al. [166] found muscular adaptations even with just one session a week. However, the twice-daily, 5-day-per-week protocol utilised by Natsume et al. [46] is not feasible for standard clinical practice. Therefore, future investigations should explore less frequent protocols that still yield muscular adaptations in order to determine the optimal training parameters.

2.7 Effect of NMES and BFR on pain

Pain modulation refers to the process by which the body alters a pain signal as it is transmitted along the pain pathway and involves interactions between local and central nervous system mechanisms[167]. NMES potentially alleviates pain through mechanisms such as activating large-diameter sensory fibers and triggering endorphin release. Its impact on pain modulation varies across conditions, including postoperative pain, musculoskeletal injuries, and chronic pain. While studies suggest positive effects, individual responses differ[126,168]. BFR training has been associated with higher levels of perceived muscle pain and RPE compared to traditional exercise without BFR[169,170]. The same higher perceptual ratings of pain and RPE have also been observed during NMES+BFR compared to NMES alone training sessions[46]. Furthermore, BFR, when combined with resistance and aerobic exercise, has shown reductions in pain intensity (pressure pain thresholds) in

healthy adults and patients with knee problems or lateral elbow tendinopathy[171]. As a result, it has been suggested that the BFR component may trigger a hypoalgesia response, via exercise-induced hypoalgesia (EIH), similar to high-load resistance exercise[171]. In pain-free individuals, the typical response to an acute exercise session is a phenomenon known as EIH, which is characterised by a temporary decrease in sensitivity to painful stimuli[172]. In research conducted in laboratory settings, EIH is commonly assessed by applying a painful stimulus to the body before and after a specific exercise regimen and evaluating changes in pain sensitivity, such as increased pain thresholds or reduced pain intensity in response to a standardised painful stimulus[173]. These methods have consistently demonstrated the presence of EIH in healthy individuals without pain[33]. Both aerobic and resistance exercise have shown to attenuate various aspects of pain sensitivity, including pressure, thermal and mechanical pain[33,174,175].

In contrast, the phenomenon of EIH exhibits greater variability and impairment in populations suffering from chronic pain, where pain and pain sensitivity may decrease, remain unchanged, or even increase in response to exercise[33]. Pain worsening during exercise can pose a barrier to adherence, potentially leading to a cycle of physical inactivity that can further exacerbate pain and disability in the long term. In individuals with knee OA, previous research has demonstrated evidence of nervous system sensitisation, which appears to exist on a spectrum[25]. Quantitative sensory testing in knee OA patients has identified features of pain sensitisation, such as heightened responsiveness to mechanical and thermal stimuli[176]. Moreover, direct measurements have revealed central hyperexcitability, as indicated by increased temporal summation suggesting spinal hyperexcitability, and a lack of conditioned pain modulation, indicative of abnormal endogenous pain regulation observed in individuals with knee OA[25,32]. Regarding EIH, researchers have discovered distinct responses to EIH among knee OA patient's, differentiating those with normal and abnormal endogenous pain inhibition from pain-free controls[32]. Knee OA patients exhibit heightened pain sensitivity (decreased pressure pain

thresholds) in response to exercise, suggesting dysfunction in EIH[32]. This implies that exercise interventions may induce increased pain in knee OA patient's, which could hinder muscle strength and function.

NMES has been explored as an alternative treatment for various chronic pain conditions[126]. Both experimental and clinical studies have provided evidence supporting the use of NMES in managing neuropathic pain, including cases involving traumatic nerve damage, neuroma, postherpetic neuralgia and diabetic neuropathy[168,177]. Moreover, there is moderate to strong evidence indicating the effectiveness of NMES in addressing post-stroke issues, as well as weakness and pain following ACL-R, total knee replacement and knee OA[126]. However, no research has been conducted thus far to examine the efficacy of NMES inducing EIH in musculoskeletal disorders.

BFRT has demonstrated pain reduction throughout training programs in various clinical conditions, such as knee pain, OA and post ACL-R[178–181]. Interestingly, low-load BFR appears to yield greater pain reduction than high-load resistance exercise. Existing literature suggests the presence of a hypoalgesia effect associated with BFR[178–181].

Recent evidence indicates that EIH occurs when low-intensity exercise (e.g., ≤ 30% of one repetition maximum [1RM]) is performed with BFR[182,183]. In individuals with knee pain, a single bout of low-intensity knee extension exercise with BFR was found to immediately reduce anterior knee pain compared to the same exercise without BFR[182,183]. This pain reduction lasted for at least 45 minutes and enabled the individuals to tolerate exercise with greater loads during the period[183], consistent with the findings of Giles et al. [179] in patellofemoral pain patients during a training intervention.

Hughes and Patterson [43] conducted a study to investigate the mechanisms underlying the hypoalgesia induced by BFR exercise. They employed a four-arm, crossover design involving low-intensity resistance exercise at 30% of 1RM with and without BFR, as well as high-intensity resistance exercise arm (HI-RE) at 70% 1RM. The duration of the HI-RE protocol matched that of the low-intensity portions of the trial, allowing for consistent pre- and post-exercise pain assessments across all arms. The authors observed that BFR resulted in greater hypoalgesia compared to free-flow low-intensity resistance exercise, both in the exercising limb and in remote muscles. The HI-RE group and both BFR groups exhibited similar reductions in pressure pain in remote muscles. In the exercising limb, BFR at 40% AOP produced inhibition comparable to HI-RE, while BFR at 80% AOP elicited the greatest increase in pressure pain threshold response[43]. Notably, pain inhibition was still present in the exercising limb 24 h after exercise in both arms. The authors observed increased plasma beta-endorphin concentration, a component of the endogenous opioid system, which contributed to the hypoalgesia effect[184].

The hypoalgesia effects of BFR and the potential impact of combining BFR with NMES have yet to be investigated. Such investigations could provide further insights and potential benefits of this combined intervention.

2.8 Is NMES combined with BFR tolerable?

In the past, both NMES and BFR have been described as painful interventions, which has been a primary limitation[122,169]. This is particularly evident in NMES interventions, where the pain induced by the electrical current has been identified as a major barrier to its use and effectiveness in enhancing muscle strength and function[38–40]. Available data shows that combining BFR with NMES results in greater pain and rating of perceived exertion (RPE) than NMES alone[185,186]. However, two studies reported adherence rates ranging

from 99.7% to 100% without any reported adverse events[46,99]. The other NMES+BFR training studies did not report adherence rates or adverse event occurrence[47,58,69,112], except for one participant withdrawal in the study by Slysz et al. [58]. The high adherence rates reported in studies showing benefits from combining NMES with BFR in both healthy and clinical populations on muscular adaptations could have contributed to their positive findings, contrasting with the lack of benefits found by others[47,58]. Natsume et al. [46] observed higher pain scores and RPE when NMES was combined with BFR compared to NMES alone. Despite the high pain and RPE ratings associated with the addition of BFR to NMES in previous reports, the extremely high adherence rate (> 99.7%) suggests that the NMES+BFR intervention was tolerable[46]. Slysz et al. [58] reported one participant withdrew due to reasons unrelated to the study. On the other hand, Andrade et al. [47] did not provide information on adherence rates, withdrawal rates, or adverse events. The lack of a mention of adverse events and the measurement of pain and RPE may have contributed to a more uncomfortable intervention in these two studies, potentially explaining the reduced effectiveness observed.

2.9 Conclusion

The evidence regarding the effect of NMES+BFR on muscular strength and hypertrophy is promising but inconsistent[46,47,58,99]. The mixed and varied responses observed could be attributed to methodological inconsistencies and the failure to apply appropriate parameters of NMES and BFR as determined in the broader literature for both interventions[47,102]. Limited mechanistic and chronic training study data suggest that the BFR stimulus should be between 40-80% AOP and the NMES parameters should include a frequency of 30-50 Hz, with the electrodes placed over the motor points of the muscle[45,61,64,66]. Future research should investigate the efficacy of NMES compared to NMES+BFR in separate subject groups, rather than using a contralateral limb, and determine the minimum AOP requirement for the BFR stimulus. The combined intervention

appears to be safe and tolerable, with no reported adverse events and high rates of intervention adherence documented in the literature to date.

BFRT has gained popularity as a rehabilitation method, especially when combined with light load resistance exercise[41,48,100]. However, its efficacy in reducing muscle atrophy and weakness without exercise is limited[187–190]. On the other hand, NMES has been effective in reducing muscle atrophy during immobilisation but has not consistently enhanced muscular size or strength in some clinical populations[191–193]. The combined use of NMES and BFR represents a new and novel rehabilitation approach. It has demonstrated greater effectiveness than NMES or BFR alone in enhancing muscular strength and hypertrophy in healthy and spinal cord injured populations[45,46]. However, the methodologies used are mixed and not well-defined, and conflicts with the broader NMES and BFRT evidence bases are evident. The hemodynamic safety of this combined intervention is uncertain, with reports of syncope episodes in the current literature, although this occurred when BFR was applied alone[124]. Further research is needed to investigate the mechanisms of action for NMES+BFR[61–66], but available data suggests similar effects as reported in the broader BFR literature[44,83]. However, the combined effect of both interventions could be synergistic or detrimental and requires further investigation to determine its potential for aiding clinical rehabilitation and muscular adaptations, especially in patients unable to perform volitional or light load resistance exercise. This thesis will help to establish the most beneficial intervention prescription, if any, for maximising muscular adaptations when voluntary exercise is not possible.

2.10 Thesis aims and research questions

2.11 Aims

1. To investigate and identify the most effective and safe methodologies when combining NMES and BFR.

2. To evaluate whether NMES+BFR is more effective in increasing muscular strength and hypertrophy compared to NMES alone.

3. To analyse the underlying mechanisms contributing to muscular strength and hypertrophy adaptations resulting from the application of NMES+BFR.

4. To determine whether NMES+BFR produces hypoalgesia effects similar to those observed when BFR is combined with traditional resistance training.

5. To assess the safety and efficacy of utilising NMES+BFR in clinical populations, with a specific focus on its potential to enhance muscular strength, hypertrophy, and pain.

2.12 Null Hypotheses

1. There is no significant difference in efficacy and safety between various methodologies of NMES and BFR when combined.

2. NMES+BFR does not lead to a greater increase in muscular strength and hypertrophy compared to NMES alone.

3. There are no specific mechanistic contributions to muscular strength and hypertrophy adaptations resulting from NMES+BFR.

4. NMES+BFR does not produce hypoalgesia effects similar to those observed with BFR combined with traditional resistance training.

5. The use of NMES+BFR in clinical populations does not result in significant improvements in muscular strength, hypertrophy, or pain when compared to standard rehabilitation protocols.

Chapter 3

General methods

This chapter will provide an overview of the research methodology, data collection procedures, participant selection, analysis techniques and ethical considerations employed throughout the studies of this thesis.

3.1 Health and safety

Full ethical approval for the experimental designs and procedures was obtained from the Ethics Subcommittee of St Mary's University, London (Appendices 1-4) before the start of each experimental study. Following the onset of the COVID-19 pandemic, all previously approved ethical approvals were updated in accordance with public health and University guidelines. All procedures were conducted in compliance with the Declaration of Helsinki (2013). The experimental sessions and outcome measurements took place at St Mary's University in a well-ventilated laboratory maintained at a temperature of 18-22 °C or at a private physiotherapy clinic. Throughout the experimental testing, strict measures were undertaken to ensure the cleanliness and safety of the laboratories and equipment used by human participants. Contaminated disposables were promptly disposed of in the appropriate disposal canister after use.

3.2 Participant recruitment

Participants for the experimental studies (Chapter 4, 5 and 6) were recruited from the student and staff population at St Mary's University through word of mouth. For Chapter 7, knee OA patients were recruited from private general practitioners (GP's) and consultants, who provided interested participants with study information and contacted the lead researcher. Two case studies involving clinical populations after surgery were also recruited in Chapter 6 using similar methods. A priori power calculations were conducted using G*Power software to determine the required number of participants for each experimental study.

Before the commencement of any experimental procedures, participants received both written and verbal explanations of the procedures, associated risks and benefits and the level of commitment required for each study. They completed a health and physical activity questionnaire. Inclusion criteria included the absence of cardiovascular or metabolic diseases, non-smoker status, resting systolic blood pressure below 140 mmHg and an ankle brachial index score between 0.9-1.4 to ensure stability for NMES and BFR exercise. Participants provided informed consent by signing a consent form, and they were informed that they could withdraw from the study at any time without having to provide a reason. They were also assured that their data would be coded to maintain anonymity, protected with passwords and treated confidentially.

Prior to data collection, two familiarisation sessions were conducted to ensure participant's comfort with the laboratory environment, equipment, protocols used, optimising maximal NMES current tolerated and while minimising potential learning effects. Participants were instructed to arrive at the laboratory well-hydrated and rested, refraining from strenuous exercises and alcohol within the previous 24 h, avoiding caffeine on the same day and avoiding food three hours before each test.

Descriptive data and anthropometric measurements, such as age, height, body mass and activity level, were recorded before the start of each experimental study. Height was measured to the nearest 0.01 m using a Harpenden Stadiometer (Holtain Ltd., Crymych, UK), while body mass was measured to the nearest 0.1 kg using calibrated laboratory scales (Seca GmbH & Co., Hamburg, Germany). These measurements were taken with participants standing without shoes and wearing their experiment clothing.

3.3 Ankle-brachial index

The ankle-brachial index (ABI) is a non-invasive diagnostic test used to assess blood flow to the legs and feet, serving as a screening tool for peripheral arterial disease and other vascular conditions[194]. Every participant's ABI was measured following published guidelines[194]. The measurement involved using a standard blood pressure cuff and a handheld Doppler probe (Hi-Dop, Ana Wiz Ltd, Surbiton, London, UK) to determine the systolic blood pressure (SBP) in the brachial artery (arm) and the posterior tibial artery (leg).

To measure the ABI with the Doppler method, participants were instructed to rest in a supine position for 5 minutes with their head and heels supported, at a comfortable room temperature. Cuffs were not placed on areas with a distal bypass or ulcer, and any open lesions were covered with an impermeable dressing to prevent contamination. Participants were asked to remain still during the measurement. The cuff was wrapped around the ankle with the straight wrapping method, as with brachial measurement, and the lower edge positioned 2 cm above the superior aspect of the medial malleolus. Gel was applied to the 8 MHz Doppler probe, which was then placed at a 45-60° angle to the skin surface in the pulse area. The probe was adjusted to obtain the clearest signal.

To detect pressure, the cuff was gradually inflated until the flow signal disappeared, and then slowly deflated to identify signal reappearance. If flow was still detected at the maximum inflation level (300 mmHg), the cuff was immediately deflated to prevent discomfort. Doppler was also used to detect brachial blood flow during arm pressure measurement. The same sequence of limb pressure measurement was followed for all participants. If the first arm measurement was 10 mmHg or more different than the other arm, the measurement was repeated at the end of the sequence, and the two numbers were averaged. However, if the difference between the two measurements exceeded 10 mmHg, only the second measurement was used for data collection. The ABI for each

participant was calculated by dividing the posterior tibial blood pressure by the arm systolic blood pressure[194].

3.3.1 Measurement reliability

Measurement reliability is of the utmost importance in research projects as it ensures consistent and accurate results. Reliability refers to the extent to which a measure produces consistent results over time and across different observers[195]. Unreliable measures can lead to inaccurate results, hindering the ability to draw valid conclusions and replicate studies for result validation. Test-retest reliability assesses the consistency of an outcome measure over time by administering the same measure to the same group of participants at different time points[195]. This reliability type helps determine if changes in outcome measures are due to actual participant change or measurement error. It is particularly crucial in evaluating intervention effectiveness, ensuring sensitivity changes and produce consistent and valid results[196].

The measurements in this PhD research were conducted by the same researcher (PH). Test-retest (intra-session) reliability of the measures was assessed using the coefficient of variation (CV) and minimum detectable change (MDC). The CV is a statistical measure that indicates the variability of a measurement as a percentage of its mean and is used as an indicator of the degree of variability in the data, relative to the mean. A lower percentage reflects greater reliability. CV was calculated by dividing the standard deviation by the mean and multiplying by 100:

CV (%) = (standard deviation / mean) x 100

The MDC determines the smallest change observed in a measurement with a given level of confidence. Changes exceeding the MDC indicate true change. MDC for measurements was calculated using the standard error of the mean and the 95% confidence interval (CI):

MDC = 1.96 x √ 2 x standard error of the mean

The test-retest reliability of measurements used in this PhD research is presented in this Chapter.

The test–retest (intra-session) reliability of ABI was assessed by conducting three sessions spaced 7 days apart on a sample of 20 adults. The results showed a CV of 0.9% and an MDC of 0.02.

3.4 Neuromuscular electrical stimulation

NMES is a therapeutic technique that utilises electrical impulses to stimulate nerves and muscles, inducing muscle contraction without voluntary effort (Chapter 2.1-2.5)[197]. In Chapter 4, participants underwent 8 minutes and 10 s of NMES at a fixed knee joint angle of 90° while seated on a custom-made strength chair equipped with a strain gauge. This duration was based on the minimal effective training dose in NMES+BFR studies conducted by Gorgey et al. [45] in spinal cord injured patients. Starting from Chapter 5, participants remained seated at a fixed knee joint angle of 90° and received 20 minutes of NMES divided into four sets of 5 minutes each. The duration was modified from Chapter 4 for the subsequent studies based on a NMES+BFR training study by Andrade et al. [47], which reported no effect on muscular strength or hypertrophy in healthy adults with NMES stimulus durations of less than 10 minutes. Subsequent studies adopted the stimulation duration of 4 x 5 minutes employed by Natsume et al. [46], who observed muscular strength and hypertrophy improvements after 2-weeks of NMES+BFR in healthy adults. The stimulation parameters used across all studies included a frequency of 50 Hz, duty cycle of 5 s of stimulation followed by a 5 s pause, pulse width of 400 µs, and intensity set at the

maximum tolerated for each participant[38,45,46,198]. The parameters were chosen based on previous research indicating their optimal effectiveness in promoting quadriceps muscle adaptations[38,60,123,147,198]. During all experimental studies, the quadriceps muscles were stimulated using three self-adhesive electrodes (Axion Medical, Axion GMBH, Villengen-Schwennigen, Germany) (2 mm thick) connected to a portable battery-powered neuromuscular electrical stimulator (Mi-Theta 600; Cefar Compex; Medicompex, Ecublens, Switzerland). The negative electrode (10 x 5 cm) was positioned approximately 13.2-13.4 cm below the inguinal crease (equal to the BFR cuff width), while the two positive electrodes (5 x 5 cm) were placed over the motor points of the vastus medialis (VM) and VL muscles. Muscle motor points were identified by gradually increasing the stimulatory current using a pen electrode (Compex; Medicompex, Ecublens, Switzerland) until a clear muscle twitch was observed[147]. The pen electrode was moved slowly over the skin, with the stimulatory current gradually increased until a clear muscle twitch was observed[38]. The electrode was placed at the point that elicited the largest visible twitch, and its location was recorded, marked, and consistently applied throughout each session. Participants were instructed to relax their thigh muscles during the stimulation period.

3.5 Determination of blood flow restriction pressure

BFR is a modality that involves partially restricting blood flow to target muscle groups using an inflatable cuff[117]. Several variables, such as cuff width, participant anthropometrics and muscle size, can influence the specific BFR pressure applied[57]. Therefore, the most reliable way to standardise BFR pressure regardless of participant characteristics and cuff width is by measuring each individual's AOP[57].

In Chapter 4, AOP was determined using a handheld Doppler probe (8 Hz) placed 2 cm proximal to the end of the medial malleolus, over the posterior tibial artery[151,152]. A pneumatic cuff (PTS tourniquet system, Delfi Medical Innovations, Vancouver, Canada)

with a width of 13.4 cm and a length of 58 cm was placed around the most proximal portion of each participant's dominant thigh. The cuff pressure was increased in stepwise increments by the pneumatic system connected to the tourniquet cuff. AOP was determined when no auscultatory pulse was detected by the Doppler probe[153]. BFR pressures used during the experimental conditions were 0%, 40%, 60% and 80% of AOP in a resting condition (seated with knees flexed to 90°), matching the body position during the intervention[116].

The test–retest (intra-session) reliability of measuring AOP via handheld Doppler by conducting three sessions spaced 7 days apart on a sample of 10 adults was CV of 2.1%, indicating good reliability, and an MDC of 7.2 mmHg.

For Chapter 5, 6 and 7, an automatic AOP determining pneumatic cuff (PTS ii tourniquet system, Delfi medical innovations, Vancouver, Canada) with the same dimensions as before was placed around the most proximal portion of each participant's dominant thigh. The pneumatic system connected to the tourniquet cuff increased the cuff pressure in stepwise increments, and AOP was automatically detected without the need for handheld Doppler measurement[153]. This device has been determined to have clinically acceptable accuracy and high reliability by our research group[153]. In each experimental study, AOP was measured for each individual in the position where the interventions took place (i.e., seated with the knee at 90°)[116]. The BFR pressure was maintained throughout the protocol of Chapter 4 and during each 5 minutes NMES set, released during the 1-minute rest periods, and immediately upon completion of the fourth 5 minutes set for the protocol of Chapter 5 and onwards[46].

3.6 Maximal voluntary isometric contraction

Maximum voluntary isometric contraction (MVIC) is a standardised method of measuring muscle strength by assessing the maximum force produced during an isometric contraction[199]. In Chapter 4 and 5 (MVIC, 5 minute all-out test), knee extension MVIC was measured using a custom-made strength chair and a digital strain gauge (Interface SSM-AJ-500 Force Transducer, Interface, Scottsdale, USA). Peak torque production during both voluntary and evoked NMES contractions was evaluated. Prior to testing, the strain gauge was calibrated with a known mass to convert voltage to Newtons. Participants were seated with the backrest at an 80˚ angle, and straps were used to stabilise the torso and hips to prevent unwanted movement. The load cell was fixed at a 90º angle of knee flexion (goniometer), and the resistance pad was secured 2 cm above the lateral malleolus. The chair set-up was recorded and standardised for each session. However, the custom-made strength chair design did not allow for a 60-75° knee angle, which is optimal for assessing quadriceps peak torque during voluntary and evoked contractions[199,200].

The MVIC protocol consisted of a warm-up phase followed by maximal contractions. The warm-up phase included three sets of 5 s submaximal contractions at 25%, 50% and 75% of each participant's voluntary maximal effort, with 30 s of rest between repetitions. Participants were instructed to exert maximum force as quickly as possible and peak torques were defined as the highest MVIC value observed during the peak 0.5 s of each contraction, multiplied by shank length and presented in newton metres (Nm)[199,201]. Verbal encouragement was provided throughout the testing. Initially, three contractions were performed, and if two measurements differed by more than 5%, an additional contraction was performed[201]. Post-intervention MVIC measurements were conducted 60 s after the NMES intervention and cuff deflation throughout Chapter 4. All raw MVIC signals were low-pass filtered using a zero-lag fourth-order Butterworth filter with an 11 Hz cut-off frequency determined from a residual analysis. In Chapter 4, evoked force during each NMES contraction was also recorded.

The test–retest (intra-session) reliability of MVIC measurements in the strength chair was evaluated across three sessions spaced 7-days apart with 20 adult participants. The CV was 3.8% and the MDC was 9.6 Nm.

3.7 Isokinetic Dynamometry

Isokinetic dynamometers are devices that resist applied forces and control exercise speed at a predetermined rate. These dynamometers provide a comprehensive record of applied force during various muscle actions, including isometric, concentric and eccentric actions[199]. In Chapter 5, maximal isometric, eccentric and concentric strength of the quadriceps muscles were measured using an isokinetic dynamometer (Cybex, Humac Norm, Ronkonkoma, USA) to assess peak force measurements. The dynamometer, equipped with a digital strain gauge, displayed force measurements to the nearest 0.1 N. Prior to each measurement, the instrument was calibrated following the manufacturer's instructions and specifications. Participants were comfortably seated with the backrest angled at 80˚and without shoes or orthotic devices.

MVIC for the quadriceps was tested at 30º, 45º, 60º, 90º and 105º of knee flexion to determine peak torque angle adaptations resulting from interventions at 90º knee flexion[199,201,202]. A shin pad positioned 2 cm above the lateral malleolus of the fibula was attached to a load cell[199]. Straps were used to secure the chest and hips in place. The setup for each participant was standardised and recorded during the familiarisation session and repeated for subsequent sessions. Participants performed three warm-up trials at 25%, 50% and 75% of their perceived maximal effort[201,202]. They were instructed to push as hard as possible against an immovable strain gauge pad attached to a force transducer for 5 s. Three contractions were performed, and the highest isometric torque

achieved was recorded for data analysis. Participants had 30 s rest periods between attempts[199,201].

Concentric and eccentric peak torque were measured at an angular velocity of 60° per s between 0° and 90° of knee flexion[199]. The lateral femoral condyle was aligned with the dynamometer's axis of rotation. Gravity compensation and limb weighing were performed according to the manufacturer's instructions. Participants performed a standardised warm-up and familiarisation set of ten repetitions at approximately 50% of their maximum effort for five repetitions[201]. After 1 minute of rest, they performed five maximal concentric and eccentric knee extensor contractions at 60°/s and the highest peak torque used for data analysis.

The test–retest (intra-session) reliability of isometric measurements was evaluated across three sessions spaced 7 days apart with 10 adult participants. The CV was 3.4% and an MDC of 8.3 Nm MDC. For isotonic contractions performed on the Cybex, the CV was 4.8% with an MDC of 10.2 Nm.

3.8 Quadriceps muscle thickness

Muscle cell swelling has been proposed as a acute observation after BFR interventions, suggesting that it can inhibit and promote anabolism by altering the protein balance[203]. Previous BFR studies have assessed changes in muscle cell swelling by measuring muscle thickness[203,204]. In Chapter 4, quadriceps muscle thickness (MTH) was measured using B-mode ultrasonography (Echoblaster 128 EXT-1Z, Telemed, Lithuania; 60 mm linear scanning probe, 7 MHz transducer scanner). To determine the measurement sites for the vastus medialis (VM) and VL muscles, an anthropometric tape measure was placed along the length of the thigh from the superior tip of the patella to the anterior superior iliac spine. Using the superior pole of the patella as the reference point, the thickness of VM was

measured at 20% of this distance and VL at 50% of this distance. The VM measurements were taken from 12.5% of thigh circumference in the medial direction from the midpoint of the thigh, and the VL measurements were taken from 10% of thigh circumference in the lateral direction. These locations represent the location of the maximum cross-sectional area of the VM and VL muscles[8]. The ultrasound probe was placed separately over the VM and VL musculature for three trials. Before each scan, participants rested in a supine position for 5 minutes. The measurement sites were marked using indelible ink. With the leg fully extended, the deep and superficial aponeurosis of each muscle was identified, and the distance between the two interfaces was calculated as MTH. The mean MTH from three measurements from the centre of each image was used for data analysis[46].

The test–retest (intra-session) reliability of VM and VL MTH measurements were evaluated across three sessions spaced more than 7-days apart with 20 adult participants. The CV for VM MTH was 3.2% with an MDC of 0.6 mm, while the CV for VL MTH was 5.2% with an MDC of 0.6 mm.

3.9 Quadriceps cross-sectional area

Muscle CSA is commonly used as an indicator of changes in skeletal muscle size, such as hypertrophy and atrophy[205,206]. Recently, mode-B ultrasonography (US) has emerged as a cost-effective alternative to MRI for acquiring high-quality muscle CSA images[206]. Ultrasonography allows for accurate differentiation between skeletal muscle, connective tissue and intra- and extra-muscular fat. Lixandrao et al. [206] demonstrated good validity of US-acquired CSA measurements compared to MRI measurements (CV = 1.75%). For Chapter 5, the muscle volume of the VL was measured using B-mode ultrasound scanner (Echoblaster 128 EXT-1Z, Telemed, Lithuania; 60mm linear scanning probe, 7 MHz transducer scanner), following the protocol described by Lixandrao et al. [206]. Participants initially assumed a supine position on a plinth, and the location of the lateral epicondyle of the femur was identified. A tape measure was placed from the lateral epicondyle to the

greater trochanter of the femur, with marks made at 50% and 75% of this length[206]. Semi-permanent ink was used to mark these points as references. Sequential transverse marks were made on the skin every 2 cm from the reference points, extending towards the medial and lateral aspects of the thigh, to guide the displacement of the US probe. The B-mode linear array probe was aligned with the transverse ink markers on the thigh. Sequential still images were obtained by aligning the superior edge of the probe with each mark on the skin, moving in a middle-to-lateral direction. The power and gain settings were adjusted to optimise image quality and images were recorded for each participant. The scanning head was coated with a water-soluble transmission gel to ensure acoustic contact without depressing the skin surface. Following data collection, the images of the VL muscle were reconstructed using PowerPoint (Microsoft, Redmond, WA, USA) according to the procedures described by Reeves et al. [205] and Lixandrao et al. [206]. Each image was manually rotated until the entire fascia of the VL muscle was reconstructed. The muscle CSA was measured using computerised planimetry. This involved contouring the VL muscle CSA along the muscle fascia using an 800-dpi mouse (Madena 3.2.5; EyePhysics, Los Alamitos, CA, USA) and Image J software (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA). The interfaces between the subcutaneous adipose tissue-muscle and the muscle-bone were identified from the US images and used for data analysis[206]. Image J was calibrated using fixed distance scales displayed in the US images. Each data point for statistical analysis was represented by the average of two pictures.

The test–retest (intra-session) reliability of VL CSA measurements was evaluated across three sessions spaced more than 7 days apart with 10 adult participants. The CV for VL CSA was 5.7% with an MDC of 1.2 cm^2.

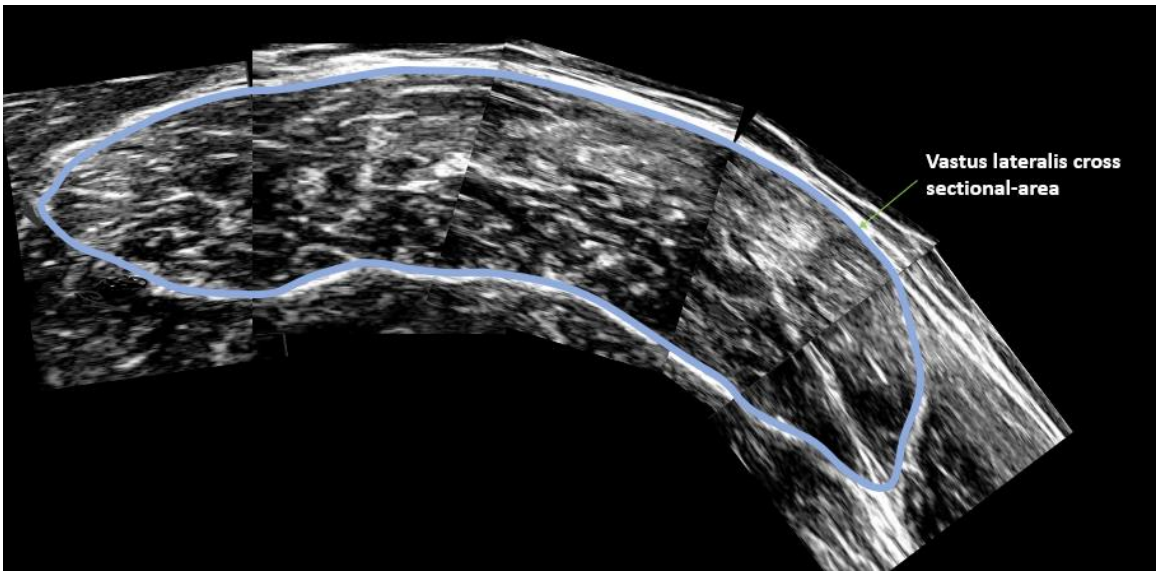

Fig 3.1. Example of vastus lateralis cross-sectional area measurement.

3.10 Quadriceps muscle architecture

Muscle fascicle length and pennation angle play crucial roles in determining muscle force production[207]. Skeletal muscle architecture was evaluated using US imaging (Echoblaster 128 EXT-1Z, Telemed, Lithuania; 60mm linear scanning probe, 7 MHz transducer scanner). Measurements of fascicle length and pennation angle were obtained between the greater trochanter and lateral epicondyle of the femur in the sagittal plane. To ensure consistent anatomical location for subsequent measurements, the US probe was positioned in the sagittal plane, perpendicular to the mediolateral axis, and indelible ink was used as reference points. Multiple images were taken and aligned to visualise the whole fascicle. In each resting US image, the fascicular path was identified as the space between echoes originating from the perimysial tissue surrounding the fascicle[208]. Fascicle length and pennation angle were measured using Image J software (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA). with the participant lying supine and the knee joint fully extended. The average of two measurements for fascicle length and pennation angle was used for data analysis[208].

The test–retest (intra-session) reliability of VL fascicle length and pennation angle measurements were assessed across three sessions spread more than 7 days apart with 10 adult participants. The CV for VL fascicle length was 2.9%, with an MDC of 0.04 cm. For VL pennation angle, the CV was 4.8%, with an MDC of 1.2º.

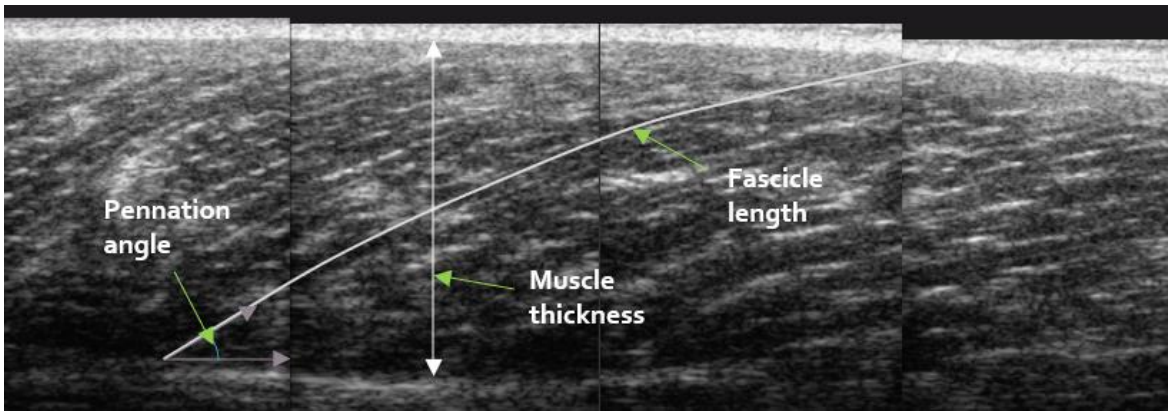

Fig 3.2. Example of vastus lateralis muscle thickness, fascicle length and pennation angle.

3.11 Blood pressure

Blood pressure serves as an important indicator of cardiovascular health[209], and in Chapter 4 and 8, it was measured both before and after each intervention to evaluate the response under various conditions and different populations. SBP and diastolic blood pressure (DBP) were measured using an automatic blood pressure monitor (Omron M3-IT, Omron Healthcare UK Ltd, Milton Keynes, UK). Participants were seated in a chair with a backrest and their feet on the floor, in a relaxed state without speaking, for at least 5 minutes. The participant's right arm was positioned on an armrest, supported at the level of the heart, while ensuring no tight clothing constricted the arm. The cuff was placed 2 cm above the brachial artery, aligned with the 'artery mark'. The bladder of the cuff encircled at least 80% of the arm but not more than 100%[210]. The appropriate cuff size was determined following the manufacturer's recommendations. The measurement process involved automatic inflation and deflation of the cuff to determine systolic and diastolic blood pressure readings. Two measurements were taken and assessed, with a third

measurement taken if variability exceeded 5 mmHg, and the mean value was recorded[210].

The test–retest (intra-session) reliability of SBP and DBP was evaluated across three sessions spaced 7 days apart, involving 20 adult participants. The CV for SBP was 3.3% CV, with an MDC of 2.5 beats/minute. For DBP, the CV was 5.1%, with an MDC of 3.2 beats/minute.

3.12 Heart rate

Measuring heart rate (HR) responses provides valuable insights into heart function[211]. In Chapter 4 HR was measured before, during and after every condition to evaluate safety[211]. In Chapter 7, HR was measured before and immediately post every intervention to assess the cardiovascular effects in knee OA patients. HR was assessed using a heart rate monitor with a coded transmitter and chest strap placed underneath each participant's xyphoid process (Polar TY1, Polar, Kempele, Finland). Participants were instructed to maintain calm breathing and avoid looking at the polar monitor. HR was recorded as the average of 5 beats and measured after 5 minutes of seated rest, both before and after the experimental conditions, as well as after each set (10 repetitions) of the NMES protocol in Chapter 4.

The test–retest (intra-session) reliability of HR was evaluated across three sessions spaced 7 days apart, involving 20 adult participants at rest. The CV for HR was 5.2%, with an MDC of 3 beats/minute.

3.13 Wellness

Prior to each training session in Chapter 5, a psychometric questionnaire was utilised to assess participant wellness (Appendix 5.4)[212]. This assessment aimed to determine if participant wellness had any influence on the tolerable currents achieved during each training session. The questionnaire consisted of five questions related to perceived fatigue, sleep quality, general muscle soreness, stress levels and mood. Participants rated each question scored on a 5-point scale, with 1 representing poor wellness and 5 representing very good wellness. The overall wellness score was calculated by summing the scores of the five questions[212]. Reliability of this questionnaire has been previously established as intraclass correlation coefficient (ICC) = 0.78[213]. The ICC is a statistic that quantifies the degree of agreement or consistency among multiple measurements, with values ranging from 0 (no agreement) to 1 (perfect agreement), interpreted as poor (ICC < 0.5), moderate (0.5 ≤ ICC < 0.75), good (0.75 ≤ ICC < 0.9), or excellent (ICC ≥ 0.9) reliability or agreement[214].

3.14 Rating of perceived exertion

The RPE scale, developed by Gunnar Borg [215], was used to assess exertion and breathlessness during activities (Appendix 5.1). Previous NMES+BFR research reported RPE scores to be higher compared to NMES alone[46]. In the context of NMES combined with varying degrees of BFR, perceptual responses were evaluated during Chapter 4, 5, 7 and 8. Participants were asked to rate their exertion on the scale, considering physical stress and fatigue rather than factors like leg pain or breathlessness. The chosen number on the scale corresponded to the intensity of the activity. familiarisation with the scale was conducted prior to data collection, and the standard Borg 6-20 scale was used[215]. RPE intra-session reliability has been previously established as ICC = 0.75-0.82[216].

3.15 Pain

The Numeric Pain Rating Scale (NPRS) is a unidimensional measure used to assess pain intensity in adults[217]. Previous research on NMES+BFR reported higher session pain scores compared to NMES alone[46]. Therefore, the perceptual responses of NMES combined with varying degrees of BFR were evaluated in Chapter 4, 5, 7 and 8. The NPRS is a segmented numeric version of the visual analogue scale, where respondents select a number from 0-10 to indicate the intensity of their pain. A rating of "0" represents no pain, while "10" represents the worst pain imaginable[218]. Participants confirmed their understanding of how to rate pain before testing. NPRS reliability has been previously established as ICC = 0.91[219].

3.16 End-test torque and impulse above end-test torque

The concept of critical power measures the highest sustainable power output that an individual can maintain without reaching exhaustion[220]. In clinical populations, such as those recovering from OA or lower limb surgery, endurance capacity is often compromised, making it challenging to engage in prolonged exercise[20,221,222]. Understanding an individual's critical power can assist healthcare professionals in designing tailored exercise programs to gradually increase endurance capacity over time[220,223]. The measurement and validation of critical power have been recently performed on the quadriceps during a 5 minute all-out test and referred to as end-test torque[224]. The test to determine end-test torque and impulse above end-test torque of the knee extensors involved 60 MVIC's of 3-5 s each, with 2 s of rest between contractions[224,225]. During the test, the muscle stimulator was triggered on the 1^{st}, 2^{nd}, 59^{th} and 60^{th} contraction to the femoral nerve to calculate voluntary activation (Section 3.17). Stimuli were delivered at the visually plateaued peak torque for each individual, and 30 s prior to the first contraction. The first and final two contractions lasted 5 s, while the remaining 56 contractions lasted 3 s, based on pilot testing that demonstrated higher reliability in voluntary activation measurements using 5 s MVIC's

compared to 3 s MVIC's. Participants were provided with feedback on their previous MVICs and were instructed to exert maximal effort during each contraction, despite the expected decline in torque by more than 50% during the test. Participants were strongly encouraged to maximise torque without being informed of the elapsed time or remaining contractions. A green light displayed on a computer screen (iPad, generation 6, Apple Inc, 1 Apple Park, Cupertino, California, USA) signalled the subjects to contract, while a red light signalled them to stop. Verbal instructions to "push" and "stop" were also provided due to participant's tendency to focus on the exercising leg rather than the screen. The test concluded upon completion of the 60th contraction cycle. The mean torque value of each contraction was used to calculate both critical torque and impulse above end-test torque. End-test torque corresponded to the average mean torque values of the final six contractions, and impulse above end-test torque was calculated using the area below the mean torque values x time curve and above the end-test torque value for each trial[224,225] (Fig 3.3). The reliability of the 5 minute all-out test has been previously established as CV 4.2-8.2%[226].

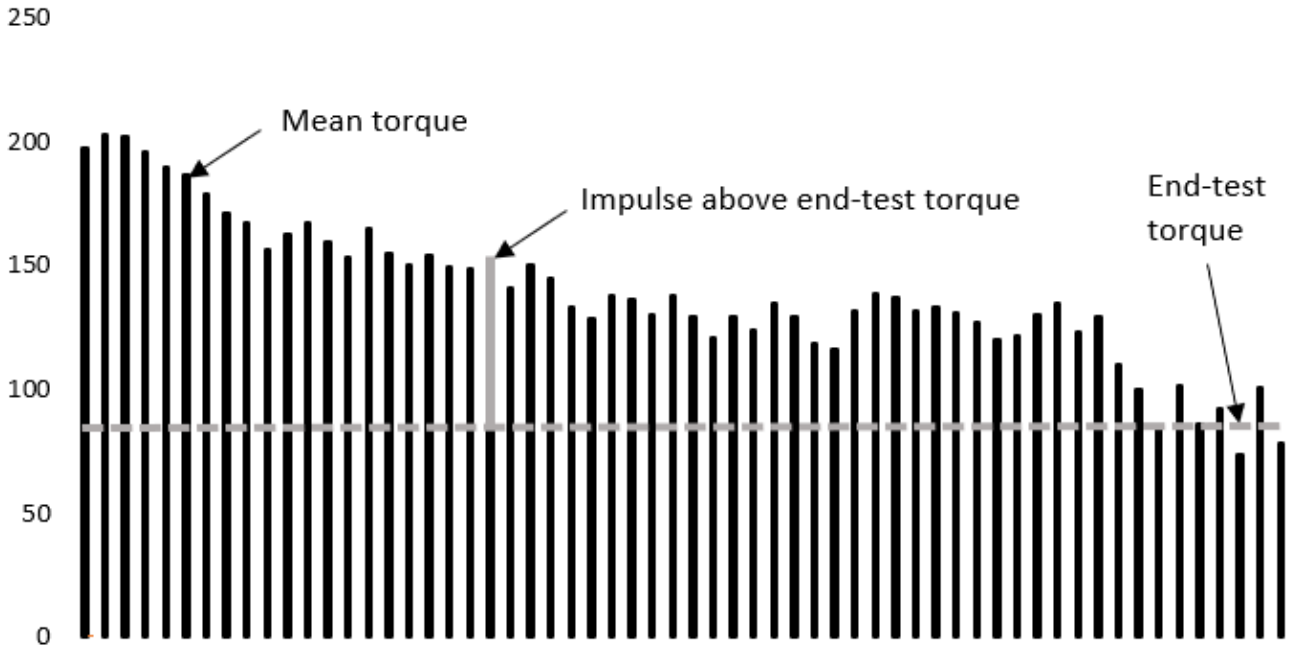

Fig 3.3. Example of impulse above end-test torque and end-test torque.

3.17 Voluntary activation

Measuring voluntary activation provides insights into the neural drive, inhibition, and central fatigue, which directly influence an individual's ability to generate force[227,228]. The percentage of voluntary activation was estimated using the twitch interpolation protocol[229]. Singlet stimuli were applied to the femoral nerve at approximately 200–300 ms intervals during the 1st, 2nd, 59th and 60th contractions of the 5 minute all-out test, as well as 5 s before the first contraction. Rectangular pulses of 200 µs duration were delivered using a high-voltage constant-current stimulator (Digitimer DS7AH, Hertfordshire, UK) with a maximum voltage of 400 V[229]. The cathode electrode (20 mm diameter, Durastick Supreme, Chattanooga Group, Hicton, TN) was positioned over the femoral nerve in the lateral portion of the femoral triangle, while the anode electrode (20 mm, Durastick Supreme, Chattanooga Group, Hicton, TN) was placed over the gluteus medius muscle, medial to the greater trochanter of the femur[225]. To determine the optimal probe location, a series of single stimuli (30 mA) were delivered, and the probe position resulting in the greatest M-wave response was identified and marked. The intensity of supramaximal stimulations was determined by progressively increasing the current until maximal twitch was observed, indicating Mmax, the point at which further increases in stimulus intensity did not yield a higher twitch amplitude. An additional 50% was added to the highest current to ensure a supramaximal stimulus[229]. MVIC was defined as the highest torque achieved prior to the superimposed single stimulation[230]. Superimposed torque referred to the increment in torque induced by the stimulation during voluntary contraction. The resting twitch was determined as the peak torque value induced by the single stimulation before the first voluntary contraction of the 5 minute all-out test. VA% was calculated using the following formula: VA% = [1 − (superimposed twitch amplitude／resting twitch amplitude)] × 100 (Fig 3.4). The reliability of this measure has been previously established as CV = 2.5%, ICC = 0.86[231,232].

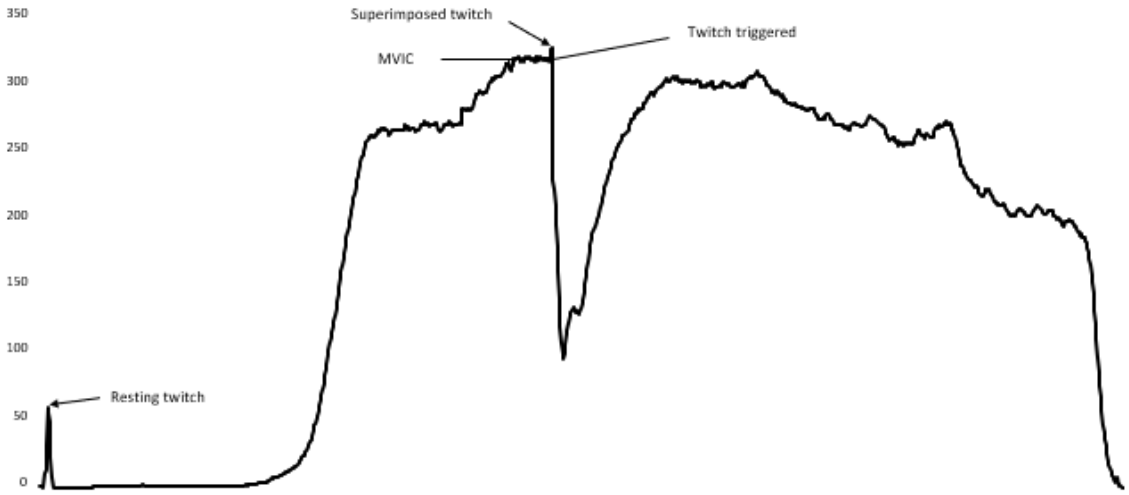

Fig 3.4. Example of voluntary activation trace.

3.18 Near-infrared spectroscopy

Measuring muscle oxygenation using near-infrared spectroscopy (NIRS) provides valuable insight into the metabolic and physiological responses of muscle tissue[233]. Previous studies have demonstrated that BFR alone can enhance muscle oxygenation after a 7-day intervention[234]. By assessing changes in oxygen utilisation and delivery in the working muscles, NIRS can help evaluate the effectiveness of NMES+BFR[233]. Prior to the 5 minute all-out test, a NIRS optode (Oxymon, Artinis Medical Systems, Zetten, The Netherlands) emitting light at 780 and 850 nm, was regularly calibrated by Artinis Medical Systems. The NIRS optode was placed on the vastus lateralis and secured with an elastic bandage (Tiger Tear, Hampshire, United Kingdom). NIRS has increasingly been used to measure real-time levels of oxygenated haemoglobin/myoglobin in small vessels and skeletal muscle, as well as muscle oxygen consumption (mVO2)[234,235]. The optode was held within a black rubber housing that maintained a constant optode spacing of 4 cm. To minimise light loss and outside light interference, the opaque housing was taped to the skin and covered with black material. The designated area was carefully shaven and cleaned with alcohol wipes prior to the experiment. Pen marks were used to indicate the correct position of the probe holder for subsequent data collections, and additional marks were made on the skin to check for any movement of the rubber housing during testing. No

movement was observed at the end of the measurements in any participant. The emitting and detecting optodes of the NIRS unit were placed distal and lateral to the greater trochanter, approximately 75% along the length of the femur. This placement on the VL was determined as the area where the greatest muscle oxygenation occurs during isometric contractions, compared to proximal locations[236,237]. The measured NIRS data analysed focused on deoxyhemoglobin (HHb) signal due to its insensitivity to changes in blood flow[238] (expressed in micromoles per litre per centimetre), as well as the derived changes in tHb (expressed in micromoles per litre per centimetre)[239,240]. The rate of change in HHb during each MVIC above 50% intensity is recognised as representative of mVO2[241,242]. With work intensity above 50% MVIC, sufficient intramuscular pressure is generated to occlude local muscle blood flow, enabling calculation of mVO2 without arterial occlusion[241,242]. The 5 minute all-out test consisted of 60, 100% MVIC's. mVO2 was determined by analysing the rate of change (slope regression) in [HHb] during the middle two seconds of each contraction period in the 5 minute all-out test when tHb was relatively constant. Specifically, the SLOPE function in excel was utilised to calculate the regression slope during the middle two seconds of every three second contraction which was manually identified. HHb resting values were obtained during the last 5 minutes of a 10 minutes rest period, during which individuals remained still and as relaxed as possible before the start of the exercise[243]. Values were determined as the difference between the HHb values obtained during the concentric phase of each repetition (as described above) and the mean HHb value during the 5 minutes resting period[244], and were used for further analysis. To minimise differences in initial values between trials, all measured parameters were normalised to the resting values acquired before each experiment, and reported as delta (Δ) values. The analysis provided a quantitative assessment of the relationships within the NIRS dataset, with a focus on changes in HHb [ΔHHb] values from baseline, during the middle 2 seconds of contraction 3-58 of the 5 minute all-out test. NIRS data were collected at a frequency of 10 Hz. Adipose tissue thickness at the site of measurement was assessed using B-mode US and within manufacturers guidelines. The first two contractions and final two contractions were not used for data analysis during the 5 minute all-out test, due to

them being 5 s in length and also being influenced by muscle stimulation[239]. The reliability of HHb signal of the VL during exercise has been previously established as CV = 2.8-3.5%, ICC = 0.40-0.81[245].

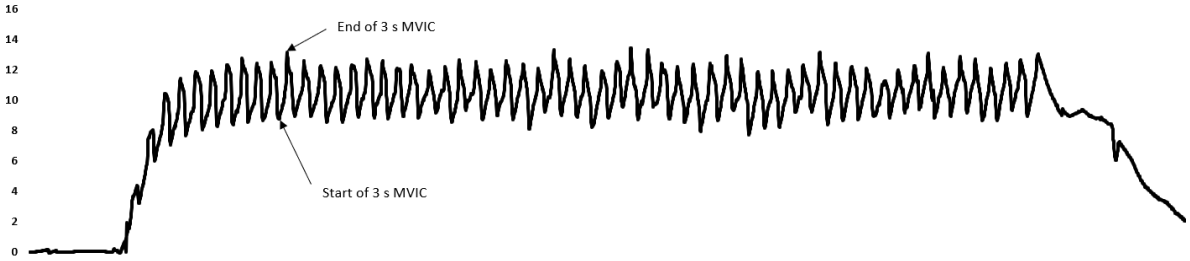

Fig 3.5. Example of deoxyhemoglobin signal during each contraction of the 5 minute all-out test.

3.19 Pressure pain thresholds

Pressure pain thresholds (PPT) quantified the pressure required to elicit a painful sensation in a specific body area[246]. PPT measurements serve to evaluate pain sensitivity and assess the effectiveness of pain treatments[246,247]. Recent studies indicate that combining BFR with resistance and aerobic exercise, as well as NMES alone, positively affects PPT[135,171,178,248]. However, the impact of NMES+BFR on PPT remains unexplored.

PPT measurements were conducted with the participants seated, with both arms resting on their thighs[43,175]. A handheld pressure algometer (J Tech Medical, United States) with a 1cm^2 stimulation area and an approximate pressure increase rate of 1 kgf/s was used for assessment[174]. Our research group has previously validated the use of a handheld algometer for PPT assessment[43]. The PPT assessment sites were marked at the midpoint of the dominant and non-dominant quadriceps muscles (20 cm proximal to the base of the patella)[43,175]. Participants were instructed to verbally indicate “now” when they first perceived the applied pressure as painful, and the PPT was recorded as kgf/cm^2 at that

point. Two PPT assessments were performed at each site, with a 20 s interval between assessments, and the average value was used for analysis[43,175].

Test–retest (intra-session) reliability for PPT was assessed in 10 adults across three sessions spaced more than 7 days apart. The CV for PPT was 3.3% and the MDC was 0.4 kgf/cm^2.

3.20 Cold pain thresholds

Cold pain thresholds (CPT) are commonly used to evaluate an individual's perceived sensitivity to cold-induced pain[249]. Studies have reported increased CPT in knee OA patients[250], but the effects of NMES+BFR on CPT have not been assessed.

All CPT measurements were performed with participants seated, with both arms resting on their thighs[43,175]. A plastic syringe filled with frozen water and ice was used, with the ice pointing out of the syringe's end. The ice was applied to the participant's skin for 30 s, and they were asked to rate their sensation of cold pain from 0-10, with 0 representing no cold pain and 10 representing severe cold pain[249]. CPT assessment sites were marked on the inferior pole of the patella on the patellar tendons of the dominant and non-dominant quadriceps[249] Two CPT assessments were conducted at each site, with a 20 s interval between assessments, and the average value was used for analysis[43,175,249].

Test–retest (intra-session) reliability for CPT was evaluated in 10 adults across three sessions spaced more than 7 days apart. The CV for CPT was 9.8%, and the MDC was 0.4.

3.21 Temporal summation of pain

Temporal summation of pain (TSP) is a measure that assesses the intensity of pain experienced by an individual over time[247]. It is commonly used in the literature to evaluate the sensitivity of the central nervous system to pain stimuli and has shown promise in diagnosing chronic pain conditions such as knee OA[251]. However, the effects of NMES+BFR on TSP have not been investigated. TSP assessment involves applying a series of identical painful stimuli to the same area of the body while recording the intensity of the pain experienced[247]. Both TSP and PPT measurements are recommended in the broader pain literature[247,251,252]. As a measure of TSP, the pin-prick evoked wind-up ratio was utilised. The wind-up ratio is calculated by dividing the pain intensity evoked by 10 pin-prick stimuli by the pain intensity evoked by a single pin-prick stimulus[247].

In the assessment, a single pin-prick stimulus was initially applied (Single PinPrick stimulator 256 mN: MRC Systems GmbH, Heidelberg, Germany), and the participant rated its severity. Subsequently, a series of 10 pin-pricks were administered at a frequency of 1 Hz within a 1 cm^2 area of skin, and the participant rated the severity of the final stimulus. Ratings were provided using the same NPRS scale used for the pain measures described previously[253]. TSP for each participant and testing site was calculated by subtracting the rating of the first pin-prick stimulus from the rating of the final pin-prick stimulus[247,252]and used for analysis.

Test–retest (intra-session) reliability for TSP was assessed in 10 adults across three sessions spaced more than 7 days apart. The CV for TSP was 6.8%, and the MDC was 0.8.

3.22 5 Sit-to-stands

Measuring sit-to-stand performance is recommended for patients with knee OA as it provides valuable insights into their lower extremity function[254]. Sit-to-stand is a common

task of daily living that involves the activation of multiple lower extremity muscles, including the quadriceps[254]. Assessing sit-to-stand ability helps healthcare professionals evaluate the functional capacity of knee OA patients and monitor their progress, as lower sit-to-stand ability is associated with an increased risk of falls[255]. The reliability of the 5-repetition sit-to-stand test has been previously examined, and it was reported to be adequate, with ICC's ranging from 0.67 to 0.90[256]. The validity of the sit-to-stand test is supported by its correlation with knee extension strength and gait performance[256,257]. During the sit-to-stand test, participants were instructed to perform the task five times, standing up from and sitting down on a slightly padded, armless chair with a height of 43 cm, as quickly as possible. They were instructed to fold their arms across their chests and ensure full standing and firm contact when sitting. The timing began with the command "go" and stopped when the participants completed the fifth sit-down. Time taken to complete the five repetitions and any knee pain experienced, rated on a 0-10 visual analogue scale, were recorded for further analysis[258].

3.23 Knee bend in 30 seconds

In individuals with knee OA, the ability to flex the knee can be restricted due to joint space narrowing, leading to an impact on physical function[259]. This test aims to assess the maximum number of knee bending's performed on one leg within a 30 s duration, as well as the ability to rapidly transition between eccentric and concentric muscle contractions around the knee joint[260]. Participants were instructed to bend and straighten their knee through their pre- intervention range of motion, assessed by goniometer, as many times as possible. The number of repetitions conducted was used for analysis and previous research demonstrated high intra-rater reliability (ICC = 0.92) using the same method[261].

3.24 Knee Injury and Osteoarthritis Outcome Score questionnaire

The Knee Injury and Osteoarthritis Outcome Score (KOOS) is a knee-specific instrument developed to assess individuals' perception of their knee and associated functional issues (Appendix 5.5)[262]. The KOOS physical function subscale provides a standardised and validated measure of physical function[262] It evaluates both short-term and long-term consequences of knee injury and consists of 42 items divided into five subscales; Pain, other Symptoms, Function activities in daily living, Function in Sport and Recreation, and knee-related Quality of Life. The KOOS meets the fundamental criteria for outcome measures and can be used to evaluate the progress of knee injury and treatment outcomes[261]. The questionnaire was administered before and immediately after the different conditions in Chapter 7. A previous meta-analysis has demonstrated reliability ICC = 0.85-0.90 of this questionnaire[263].

Chapter 4

Optimisation of effective parameters of NMES combined with BFR: The effect of restriction pressure

Journal Sport Rehabilitation. 2020 July 31;30(3): 375-383.

4.1 Preface

The main components of this chapter were published under the details previously stated. The introduction, results, discussion, and conclusion have been edited to elaborate on the publication and fit in with the rest of this thesis and assist with linking this Chapter to the following sections.

4.2 Abstract

Context: NMES+BFR has been shown to improve muscular strength and size better than NMES alone. However, previous studies used varied methodologies not recommended by previous NMES or BFR research. **Objective:** The present study investigated the acute effects of NMES combined with varying degrees of BFR using research-recommended procedures to enhance understanding and the clinical applicability of this combination. **Design:** Randomised crossover. Setting: Physiology laboratory. Participants: A total of 20 healthy adults (age: 27.1 [4.0] years; height: 177.0 [7.9] cm; body mass: 77.1 [13.2] kg). Interventions: Six sessions separated by at least 7 days. The first 2 visits served as familiarisation, with the experimental conditions performed in the final 4 sessions: NMES alone, NMES 40% BFR, NMES 60% BFR, and NMES 80% BFR. Main Outcome Measures: Maximal voluntary isometric contraction, muscle thickness, blood pressure, heart rate, rating of perceived exertion, and pain were all recorded before and after each condition. **Results:** The NMES 80% BFR caused greater maximal voluntary isometric contraction decline than any other condition (−38.9 [22.3] Nm, $p < 0.01$). VM and VL muscle thickness acutely increased after all experimental conditions ($p < 0.05$). Pain and RPE were higher after NMES 80% BFR compared with all other experimental conditions ($p < 0.05$). No cardiovascular effects were observed between conditions. **Conclusion:** The NMES combined with 80% BFR caused greater acute force decrement than the other conditions. However, greater perceptual ratings of pain and ratings of perceived exertion were observed with NMES 80% BFR. These acute observations must be investigated during

chronic interventions to corroborate any relationship to changes in muscle strength and size in clinical populations.

4.3 Introduction

NMES has been demonstrated to prevent disuse muscle atrophy[191], however, inconsistent evidence exists regarding its efficacy in enhancing muscle adaptations[37]. Prior to the commencement of this study in 2017, trials examining NMES+BFR in humans yielded varied results. Two studies reported increased muscle strength and hypertrophy compared to NMES and BFR alone in healthy and spinal cord injured adults[45,46], while others found no added benefit to muscle strength and size[47,58].

The literature review conducted in Chapter 2 revealed methodological inconsistencies in the existing NMES+BFR studies. These inconsistencies may have contributed to conflicting findings and hindered the understanding of the underlying physiological mechanisms that induce changes in muscle strength and hypertrophy. The NMES protocols utilised thus far have exhibited considerable variability, encompassing frequencies ranging from 20 to 100 Hz, with unclear reporting of other parameters such as stimulation intensities[38,45,46,58,264]. To optimise quadriceps strength after NMES, it is recommended to employ a frequency of 50 Hz, maximal tolerable intensities and proper electrode placement over muscle motor points[147]. However, these parameters have not been utilised in previous NMES+BFR studies focusing on the quadriceps[46,58]. Moreover, a majority of studies have employed arbitrary restrictive pressures to implement BFR[38,47,58,116,124] or based their occlusion pressure on SBP[45]. Neither of these approaches are effective for controlling the magnitude of BFR, with current recommendations suggesting that pressure should be prescribed via AOP[61]. Furthermore, AOP's ranging from 40% to 80% are recommended in the wider BFR literature[57], with neither AOP or these recommended pressures being previously investigated in NMES+BFR research.

The mechanisms by which NMES combined with BFR increases muscle strength and induces hypertrophy remain unknown. A recent study observed greater evoked force decline in a rat model following NMES+BFR, which correlated with increased muscle size compared to NMES alone[38]. Furthermore, resistance training with and without BFR, leading to higher levels of fatigue as evidenced by reduced force production, results in greater improvements in muscle strength and size[265,266]. These findings suggest that acute post-exercise decrements in force production could serve a surrogate marker for optimising training programmes. However, a direct comparison of the acute muscle responses to NMES in combination with varying levels of BFR has not yet been investigated.

The present study aimed to answer question 1 of this thesis and aims to standardise methodologies to gain a better understanding of how NMES alone and in combination with varying levels of BFR (40-80% AOP) acutely affect muscular, cardiovascular and perceptual variables. Established protocols for both interventions were utilised.

It is hypothesised that NMES combined with BFR would result in higher levels of muscular fatigue, muscle swelling and perceptual variables (e.g., pain and exertion) compared to NMES alone.

4.4 Method

4.4.1 Participants

Twenty recreationally active (3.1 [1.4] h/week), healthy males (*n* = 15) and females (*n* = 5) (age: 27.1 [4.0] years; height: 177.0 [7.9] cm; body mass: 77.1 [13.2] kg and body mass index: 25.1 [3.0] kg/m^2) volunteered to participate in this study. The sample size was calculated using G*Power software and the effect sizes of previous research assessing the same outcomes[267]. Inclusion criteria were: (a) absence of lower-limb injury, (b) negative

answers in the physical activity and readiness questionnaire (PAR-Q) questionnaire (Appendix 5.3), (c) no personal history of cardiovascular or metabolic disease, (d) non-smokers, (e) resting SBP < 140 mmHg and (f) normal range on the ABI test (0.9-1.4)[194]. Participants were instructed to maintain their usual level of physical activity throughout the study. All participants provided written informed consent and the study was approved by the University ethics sub-committee (Appendix 1: SMEC_2016-17_104) and conducted in accordance with the Declaration of Helsinki (2013).

4.4.2 Study design

The study followed a randomised crossover design, generated via online software (http://www.randomization.com). All testing was undertaken at the University's temperature-controlled laboratory (21-22°C). Participants were required to visit the laboratory on six occasions, separated by at least 7 days and at the same time of day (±1 h), to minimise the circadian effect. All participants were tested at least 2 h postprandial and were instructed to avoid caffeine and exercise prior to testing. The first two visits served as familiarisation sessions, with the experimental conditions performed in the final four sessions. During the first visit, height, weight, ABI, knee extension MVIC, VM and VL MTH, AOP and NMES maximal tolerable intensity were measured. During the second visit, MVIC, MTH, AOP and NMES maximal tolerable intensity were repeated. After the familiarisation sessions, participants were randomly allocated to perform the experimental conditions, with the same trained researcher performing all outcome measurements (Fig 4.1):

1) NMES and cuff not inflated (NMES alone)
2) NMES combined with 40% BFR (NMES+40% BFR)
3) NMES combined with 60% BFR (NMES+60% BFR)
4) NMES combined with 80% BFR (NMES+80% BFR)

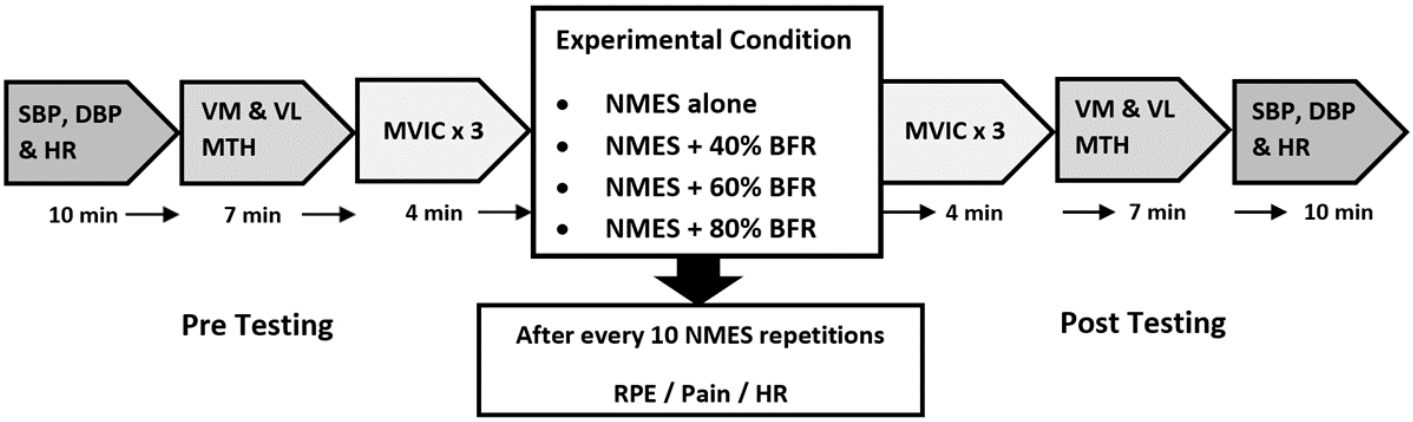

Fig 4.1. Experimental protocol. All participants performed the same neuromuscular electrical stimulation (NMES) protocol under four different blood flow restriction (BFR) pressures (0, 40, 60 and 80%) Outcome measures; systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate (HR); vastus medialis (VM) and vastus lateralis (VL) muscle thickness (MTH); knee extension maximal voluntary isometric contraction (MVIC) was assessed before (pre) and after (post) each experimental condition. Outcome measures assessed after every 10 NMES repetitions included; rating of perceived exertion (RPE), pain and HR. See abbreviations throughout.

4.5 Procedures

4.5.1 ABI

See section 3.3.

4.5.2 NMES

See section 3.4. VM and VL maximal tolerable intensities equalled 67.1 ± 44.1 mA and 70.7 ± 44.7 mA, respectively. Evoked force (% MVIC), is force generated by a muscle in response to NMES[38] and was recorded during every contraction and used for analysis.

Table 4.1. NMES protocol

Current	Bi-phasic rectangular pulse
Frequency (Hz)	50
Pulse width (μ)	400
Duty cycle (s)	5 / 5
Ramp up / ramp down (s)	1.5 / 0.5
Repetitions	40
Intensity (mA)	Max tolerable

4.5.3 Determination of BFR pressure

See section 3.5.

4.5.4 MVIC

See section 3.6.

4.5.5 MTH

See section 3.8.

4.5.6 Blood pressure

See section 3.11.

4.5.7 HR

See section 3.12.

4.5.8 RPE

See section 3.14.

4.5.9 Pain

See section 3.15.

4.6 Statistical Analysis

A two-way repeated-measures analysis of variance (ANOVA) was used to determine the effects of condition (0%, 40%, 60% and 80% BFR) and time; MVIC, MTH, SBP, DBP, HR across two time points (pre and post), HR, RPE, Pain across four time points (set 1, set 2,

set 3, set 4). If the assumptions of ANOVA were violated, the Greenhouse–Geisser correction factor was applied.

Interactions and main effects were followed with appropriate *post-hoc* analyses and Bonferroni adjustments. Statistical significance was set at $p < 0.05$.

Statistics were computed using SPSS Statistics software package version 24.0 (SPSS, Chicago, USA). Data are presented as means (standard deviation [SD]) ± standard error of mean (SEM) unless otherwise stated.

4.7 Results

No differences were observed between baseline values across the four experimental conditions ($p > 0.05$). No adverse events occurred.

4.7.1 MVIC

There was a main effect of time ($F_{(1,19)} = 37.2$, $p < 0.001$), no condition effect ($p > 0.05$) and a condition × time interaction ($F_{(3,57)} = 10.6$, $p < 0.001$) for MVIC decline (Fig 4.2). Post-hoc pairwise Bonferroni comparisons confirmed greater MVIC decline after NMES+80% BFR compared with NMES alone ($p < 0.001$), NMES 40% BFR ($p < 0.001$) and NMES+60% BFR ($p = 0.001$) (Fig 4.2). All differences were above the 9.9 Nm MDC, error of measurement.

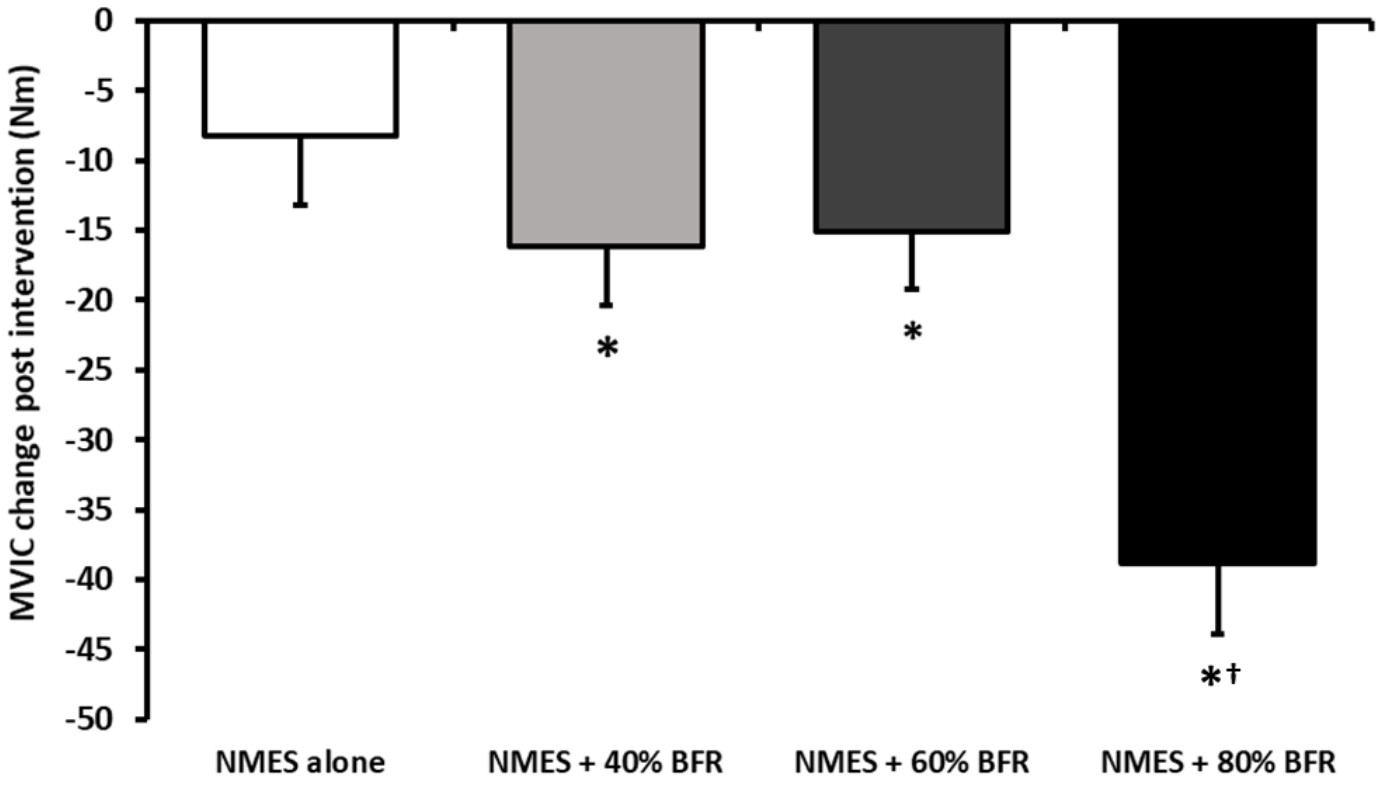

Fig 4.2. Knee extension MVIC pre-test to post-test change ∆; values as mean ± SEM. Significant difference was set at $p < 0.05$; * = significant difference between pre-test and post-test; † = significantly greater change compared to all other experimental conditions.

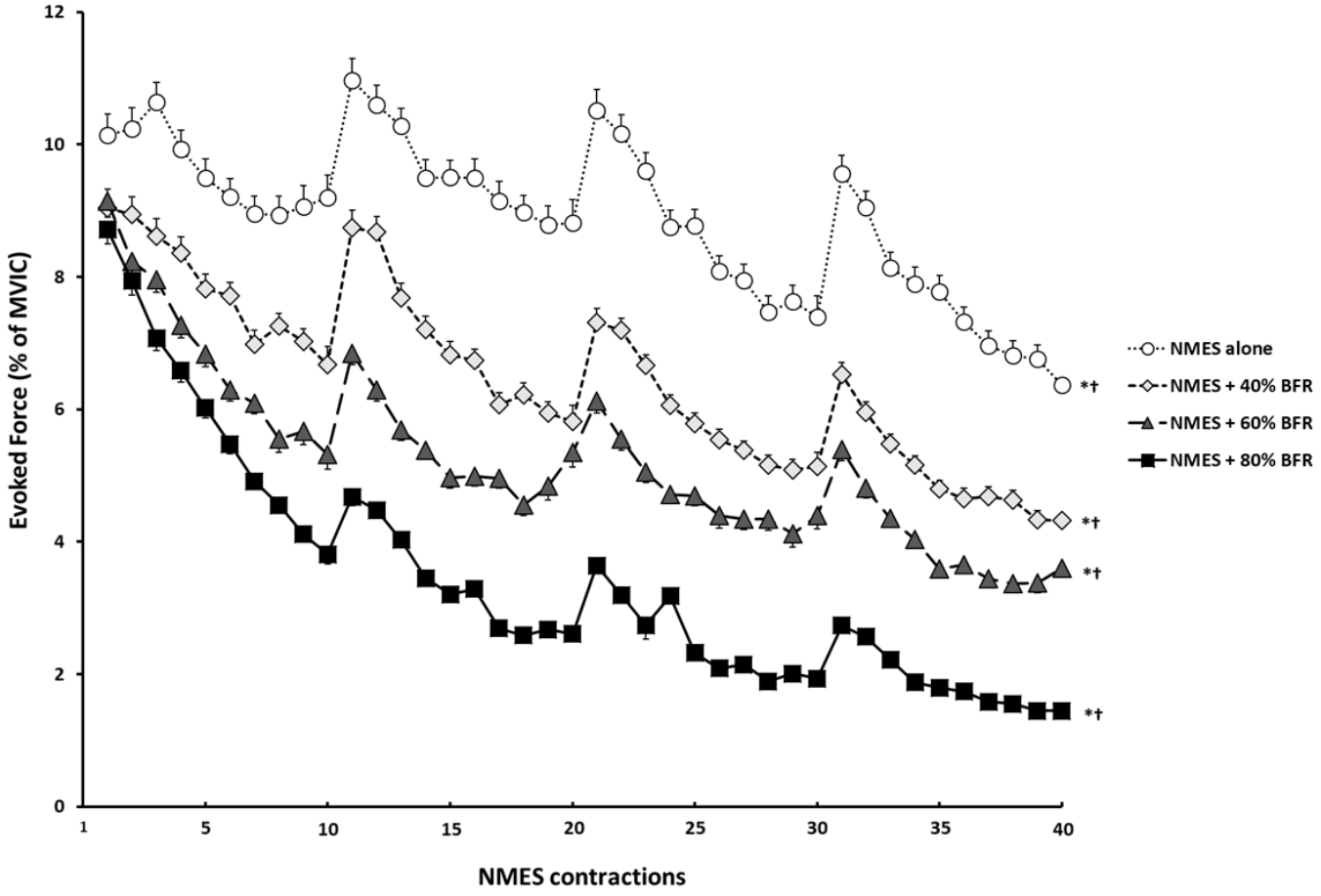

Fig 4.3. Evoked NMES force (% MVIC) during every contraction; values as mean ± SEM; * = significant decline from first repetition to the last: † = significantly different to all other conditions $p < 0.05$.

4.7.2 MTH

There was a main effect of time ($F_{(1,19)} = 43.1$, $p < 0.001$; $F_{(1,19)} = 92.1$, $p < 0.001$) for VM MTH and VL MTH increase, respectively (Table 4.2). However, there was no condition effect or condition × time interaction observed ($p > 0.05$).

4.7.3 Blood pressure

A main effect of time ($F_{(1,19)} = 12.1$, $p = 0.002$) was observed for SBP. There was no condition effect or condition × time interaction ($p > 0.05$) shown for SBP. There were no effects observed on DBP ($p > 0.05$) (Table 4.2).

Table 4.2. Knee extension MVIC, muscle thickness and cardiovascular pre-test and post-test measurement values; mean (SD) [95% CI].

	NMES alone			NMES +40% BFR			NMES + 60% BFR			NMES + 80% BFR		
	Pre	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**
MVIC (Nm)	239.8 (51.3)	231.5 (57.1)	-8.3 [-18.5; 1.9]	240.3 (48.3)	224.1 (46.8)*	-16.2 [-25.0; -7.3]	240.4 (52.3)	225.4 (55.7)*	-15.1 [-23.8; -6.4]	242.6 (55.1)	203.8 (52.1)*†	-38.9 (-49.3; -28.3]
VM MTH (mm)	25.0 (2.7)	25.6 (2.6)*	0.6 [0.3; 0.9]	25.2 (2.9)	26.0 (2.8)*	0.8 [0.3; 1.2]	25.0 (2.9)	25.8 (2.9)*	0.8 [0.4; 1.3]	24.7 (2.7)	25.9 (2.9)*	1.2 [0.8; 1.5]
VL MTH (mm)	17.2 (2.8)	17.9 (2.8)*	0.7 [0.5; 1.0]	16.6 (2.4)	17.7 (2.9)*	1.0 [0.6; 1.5]	16.9 (2.5)	18.0 (3.0)*	1.1 [0.7; 1.6]	17.0 (2.9)	18.4 (3.2)*	1.4 [0.9; 1.9]
SBP (mmHg)	122.8 (8.7)	125.2 (9.2)*	2.3 [0.7; 4.0]	121.9 (8.5)	123.9 (7.8)	1.9 [-1.4; 5.2]	123.4 (9.3)	124.7 (8.1)	1.4 [-0.6; 3.3]	123.0 (8.1)	125.5 (7.8)*	2.5 [0.8; 4.1]
DBP (mmHg)	69.4 (6.7)	71.1 (5.3)	1.7 [-0.9; 4.4]	70.2 (6.2)	71.4 (7.6)	1.3 [-0.6; 3.1]	71.2 (7.1)	71.2 (6.3)	0.1 [-2.2; 2.4]	70.7 (6.0)	71.6 (6.5)	0.9 [-1.8;3.5]
HR (bpm)	61.0 (9.3)	60.7 (9.6)	-0.3 [-2.2; 1.6]	60.7 (9.3)	61.2 (8.6)	0.5 [-1.1; 2.1]	60.6 (8.8)	58.3 (9.5)*	-2.4 [-4.6; -0.2]	62.2 (9.1)	59.5 (9.7)	-2.7 [-6.5; 1.1]

Significant differences were set at $p < 0.05$; * = significant difference between pre-test and post-test; † = significantly greater change compared to all other experimental conditions. C = change from pre to post; bpm = beats/minute.

4.7.4 HR

There was a main effect of time ($F_{(1.4,26.7)} = 54.8$, $p < 0.001$), condition effect ($F_{(3,57)} = 4.1$, $p = 0.01$) and condition × time interaction ($F_{(6.6,125.2)} = 3.9$, $p = 0.001$) for HR (Table 4.2 and 4.3). Post-hoc pairwise comparisons revealed after set 1, NMES alone was lower than NMES+80% BFR ($p = 0.019$); after set 2, NMES+80% BFR was higher than NMES alone ($p = 0.019$); after set 3, NMES 60 and NMES 80 were higher than NMES alone ($p = 0.026$ and $p = 0.01$, respectively); after set 4, NMES+80% BFR was higher than NMES alone ($p = 0.019$) (Table 4.2 and 4.3). However, all differences were below the 3.2 bpm MDC, showing no meaningful change.

4.7.5 RPE

There was a main effect of time ($F_{(1.1,21.3)} = 11.9$, $p = 0.002$), condition effect ($F_{(3,57)} = 7.7$, $p < 0.001$) and condition × time interaction ($F_{(3.8,72.4)} = 3.4$, $p = 0.015$) for RPE (Table 4.3). Post-hoc pairwise comparisons confirmed RPE to be higher; after set 1 of NMES+80% BFR compared with NMES alone ($p = 0.006$), after set 2 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR ($p = 0.018$; $p = 0.027$; $p = 0.005$, respectively), after set 3 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR ($p = 0.002$; $p = 0.002$; $p = 0.038$, respectively). Finally, RPE was higher after set 4 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR ($p = 0.001$; $p = 0.001$; $p = 0.041$, respectively).

4.7.6 Pain

There was a main effect of time ($F_{(1.6,31.2)} = 13.6$, $p < 0.001$), condition effect ($F_{(3,57)} = 19.6$, $p < 0.001$) and condition × time interaction ($F_{(5.3,100.3)} = 4.8$, $p < 0.001$) for pain (Table 4.3). Post-hoc pairwise comparisons revealed ratings of pain were higher; after set 1 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR (p

= 0.006; p = 0.001; p = 0.027, respectively), after set 2 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR (p < 0.001; p < 0.001; p = 0.010, respectively), after set 3 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR (p < 0.001; p < 0.001; p = 0.001, respectively). Finally, pain ratings were higher after set 4 of NMES+80% BFR compared with NMES alone, NMES+40% BFR and NMES+60% BFR (p < 0.001; p < 0.001; p = 0.003, respectively) and lower after set 4 of NMES alone compared with set 4 of NMES+60% BFR (p = 0.039).

Table 4.3. Measurement values after every set (10 contractions) of the interventions; mean (SD).

	NMES alone				NMES+40% BFR				NMES+60% BFR				NMES+80% BFR			
	Set 1	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**
HR	71.1	71.9	71.8	72.2	74.2	74.6	75.1	74.5	73.6	76.4	77.0	76.5	77.1	79.3	79.4	78.8
(bpm)	(9.1)	(9.7)	(8.4)	(8.7)	(9.8)	(9.1)	(10.4)	(9.7)	(11.6)	(10.5)	(9.6)	(11.4)	(11.8)	(11.4)	(11.3)	(12.2)
RPE	11.0	11.0	11.1	11.1	10.5	10.8	11.3	11.3	10.6	11.1	11.9	12.1*	12.1	12.9	13.4#	13.7†
(6-20)	(3.1)	(3.0)	(2.9)	(2.7)	(2.8)	(2.8)	(3.0)	(3.0)	(2.5)	(2.6)	(3.0)	(3.1)	(3.3)	(3.5)	(3.3)	(3.5)
Pain	3.6	3.5	3.6	3.5	3.4	3.7	3.8	3.9	3.6	4.2	4.6	4.8*	5.3	6.0#	6.6†	6.7^
(0-10)	(1.9)	(1.8)	(1.8)	(1.7)	(1.7)	(1.9)	(1.9)	(2.0)	(1.9)	(2.0)	(1.9)	(1.8)	(1.5)	(1.3)	(1.3)	(1.6)

Significant differences were set at $p < 0.05$; RPE results: * = significant difference between set 1 and set 4; # = set 3 of NMES+80% BFR significantly larger than all sets of NMES alone, NMES+60% BFR and set 1 of NMES+40% BFR; † = set 4 of NMES+80% BFR significantly larger than all sets of NMES alone, NMES+60% BFR and set 1 and 2 of NMES alone; Pain results: * = significant difference between set 1 and set 4; # = set 2 of NMES+80% BFR significantly larger than all sets of NMES alone, NMES+60% BFR and set 1 of NMES+40% BFR; † = set 3 of NMES+80% BFR significantly larger than all sets of NMES alone, NMES+60% BFR and set 1 and 2 of NMES+40% BFR; ^ = set 4 of NMES+80% BFR significantly larger than all sets of NMES alone, NMES+60% BFR and set 1 of NMES+40% BFR.

4.8 Discussion

The purpose of this study was to standardise and determine if varying BFR pressures induce different acute effects when combined with NMES. The main findings were that the addition of BFR (40-80%) to NMES was required to acutely affect muscular fatigue, with NMES+80% BFR causing greater muscular fatigue (16.2%) than NMES alone (3.5%) (Fig 4.2 and 4.3). However, higher BFR pressures (60-80%) combined with NMES caused higher ratings of pain and RPE than NMES alone and NMES combined with 40% BFR (Table 4.3), with no deleterious cardiovascular effects (Table 4.2 and 4.3).

Our result that NMES combined with 80% BFR induced the greatest muscle fatigue is consistent with findings after BFR alone and combined with low-intensity voluntary isometric contractions[267–269], demonstrating that the addition of BFR acutely reduces force generating capacity and the level of force reduction is dependent on the pressure applied to the limb. For example, Pierce et al. [269] applied BFR (163 mmHg above SBP) passively for 5 x 5 minutes and produced equal knee extension fatigue (16%) to the present study. Our results are also in accordance with prior BFR investigations that found 80% actual and estimated AOP induced acute decrements in MVIC torque[267,270–272]. The acute decrement in MVIC shown here with the addition of BFR (18%) is also similar to that observed after a single bout of resistance exercise (20%), which has correlated with increased muscular strength and size of the VL after training protocols lasting 6 weeks[136,137]. If acute fatigue is desirable for muscular adaptations, our findings provide stronger support for combining NMES with 80% BFR, compared with 40% and 60% BFR (Fig 4.2).

Although mechanistic reasons for our findings were not investigated, muscle fatigue will have occurred due to a number of physiological processes. For example, increases in intramuscular inorganic phosphate concentration have been reported after BFR[273–275]

and are a known cause of peripheral fatigue[276,277]. Indeed, others have reported that a combination of submaximal exercise with arterial occlusion rapidly depletes type I and type II muscle fibres of phosphocreatine[278], leading to increases in inorganic phosphate concentration[279]. Decreases in blood flow/O2 delivery associated with BFR, exacerbate this rate of peripheral fatigue[273,277]. Muscle fatigue can be compensated for by increased motor unit activation in an effort to maintain force output[278]. Hence, during fatiguing muscle contractions there is an increased activation of motor units that innervate type II fibres, thus increasing the potential for muscle fibre hypertrophy[279]. This provides one potential reason for the reported relationships between fatiguing tasks (induced by NMES+BFR) and muscle growth[61].

The acute increases in VM and VL MTH observed (Table 4.2), were similar to previous studies that applied BFR combined with resistance exercise using pressures from 40% AOP to 150% SBP[280–283]. Our findings also support previous BFR data, showing no greater muscle swelling effect utilising higher BFR pressures > 40% AOP[282,283]. Muscle swelling has been argued to trigger the proliferation of satellite cells, thus contributing to the hypertrophic response to exercise[284]. The present study supports the use of NMES alone and combined with BFR (40-80%) to induce acute muscle swelling (Table 4.2). However, the observed increase in muscle size, ranging from 0.6 to 1.1 mm, may not be considered clinically significant. It is important to note that the MDC for this measurement was 0.6 mm (Chapter 3). Therefore, the muscle swelling observed falls within the threshold of measurement error, indicating that the changes may not be meaningful.

Pain was increased with the addition of 80% BFR to NMES compared to all of the other conditions in the present study (Table 4.3). Additionally, NMES combined with 60% BFR produced greater ratings of pain than NMES alone (Table 4.3). This indicates that the pain experienced is mostly attributable to the level of occlusive pressure (60-80%). In addition to the nociception of pressure caused by the pneumatic cuff[285], exercise-induced muscle

pain can be generated by stimulation of group III and IV muscle afferents, elicited by metabolic perturbations of the working musculature. It is generally accepted that BFR reduces metabolite clearance, thus inducing greater pain compared to non-occluded exercise[286]. Cuff inflation at higher pressures (80% AOP) has been previously characterised as moderately painful[285], which supports the lower pain ratings observed after NMES and 40% BFR (Table 4.3). The pain ratings observed may be the direct result of pressure changes or metabolic stimulation of group III and IV afferents, induced by the NMES and 80% BFR condition[287]. However, when 60-80% BFR has been applied during resistance exercise, it acutely improved pain up to 24 h after application in knee pain patients[178,179,181]. It is currently unknown if the acute analgesic effect of BFR occurs with NMES and BFR. The lower pain and RPE scores reported with the addition of 40% compared with 60% and 80% BFR to NMES in the present study, may lead to greater clinical applicability. However, NMES combined with different BFR pressures is yet to be investigated in a training study.

There were no unanticipated effects on the cardiovascular system during any of the trials (Table 4.2 and 4.3). This supports previous NMES research using maximal tolerable intensities[288,289] and BFR research using 70% BFR pressures[290,291]. In agreement with the current findings, no adverse events have occurred in healthy and spinal cord-injured adults previously[45–47]. The present findings support the use of NMES and BFR on the selected cardiovascular measures (Table 4.2 and 4.3).

The current study has some limitations, such as the sample, which was restricted to young, healthy men and women. Thus, we acknowledge that our findings may not apply to other populations. Also, the measurements were taken immediately pre and post every experimental condition. Therefore, the time-course of change in the period of time after the intervention is unknown. The investigator and participants were not blinded to experimental conditions. Blinding aims to prevent biased assessment of outcomes and ascertainment bias after randomisation[249]. Future research should, therefore, consider evaluating the

time-course responses to BFR and NMES interventions among a wider range of clinical populations who are likely to benefit from its application.

4.9 Conclusion

This is the first study to standardise the BFR pressure using a percentage of AOP when combining it with NMES. To determine which protocol would be best suited for training, we evaluated several factors, including muscle fatigue, muscle swelling, cardiovascular response and perceptual responses. These acute findings suggest that combining NMES with 80% BFR for the quadriceps muscle group may be the most beneficial intervention. However, NMES combined with 40% BFR cannot be excluded due to lower perceptual ratings than NMES+80% BFR and acutely reducing force production (Fig 4.2; Table 4.2), which may be a surrogate marker for muscle growth. We can only speculate that the increased metabolic stress associated with BFR has led to the increased fatigue, RPE and pain ratings observed with the addition of 40-80% BFR to NMES in the present study (Fig 4.2; Table 4.3).

This study showed that the combination of 40%, 60% and 80% BFR to NMES did not cause any deleterious effect on heart rate and blood pressure. No adverse events occurred, and the combined interventions were tolerable to the participants, reflected in the RPE and pain scores. The results also showed that the combination of 40% and 80% BFR to NMES caused acute fatigue, but there was no between group difference. Therefore, aim 1 of this thesis is partially achieved.

In subsequent studies, the duration of NMES intervention times will be modified based on previous research. This decision was influenced by a study conducted by Andrade et al. [47], that showed no effects on muscular strength or hypertrophy in healthy adults when using NMES stimulus durations of less than 10 minutes. Instead, the stimulation duration of

4 x 5 minutes employed by Natsume et al. [46] will be adopted. Furthermore, Natsume et al. [46] observed improvements in muscular strength and hypertrophy after 2-weeks of NMES+BFR in healthy adults. Therefore, subsequent studies will utilise this stimulation duration to assist in optimising the methodological parameters for NMES+BFR, aiming to promote muscular adaptations and pain responses.

Overall, this study provides valuable insights into the combination of NMES and BFR, indicating potential benefits in muscle training. However, further research is needed to explore long-term effects and elucidate the underlying mechanisms for optimal NMES+BFR protocols in enhancing muscular adaptations.

Chapter 5

NMES combined with 40% and 80% BFR pressures, increases muscular strength and hypertrophy without movement: a 6-week Randomised Control Trial

5.1 Abstract

Background: NMES+BFR compared to just NMES alone has shown mixed results with regards to improving muscular strength and size However, previous studies used varied protocols not recommended by previous NMES or BFR research. **Objective:** The present study investigated the effect of NMES combined with 40% and 80% of BFR using research-recommended procedures compared to NMES alone. **Design:** A 6-week randomised control trial. A total of 42 healthy adults randomised into one of three groups: NMES alone; NMES+40% BFR; NMES+80% BFR. Training was 3 times a week for 6-weeks. **Main Outcome Measures:** Before and after 6-weeks, MVIC between 30-105°, concentric and eccentric peak torque (60°/s), quadriceps CSA's were measured and session RPE, pain and NMES current tolerated were measured in every session. **Results:** NMES+40% BFR caused greater MVIC improvements than NMES alone at 30° (11.3%, $p = 0.01$), 45° (13.8%, $p = 0.01$), and greater eccentric (12.4%, $p = 0.02$) peak torque. NMES+80% BFR caused greater MVIC improvements than NMES alone at 90° (13.6%, $p = 0.02$). NMES+40% BFR and NMES+80% BFR caused greater increases in quadriceps CSA compared to NMES alone (8.6-9.0%, $p = 0.01$-0.03). VM and VL NMES currents tolerated were higher with the addition of 40% and 80% BFR to NMES compared to NMES alone (89.1-96.8%, $p < 0.001$-0.03). Pain and ratings of perceived exertion were no different between all groups. No adverse events occurred. **Conclusion:** NMES combined with 40% and 80% BFR caused greater increases in quadriceps peak torque and CSA compared to NMES alone without any differences in session RPE and pain. The NMES currents tolerated may explain the differences between groups and this passive combination of interventions could assist with increasing muscle strength and size where voluntary movement is not possible.

5.2 Introduction

As revealed by Chapter 2, the current NMES+BFR literature base shows inconsistencies regarding improving muscular adaptations, potentially being caused by varied methodologies, specifically regarding what BFR pressure should be combined with NMES. The results obtained from Chapter 4 found that the addition of 80% BFR (measured by AOP) to NMES led to greater fatigue (force decrement) compared to NMES alone. Acute reductions in force production serve as indicators of potential muscular adaptations resulting from training protocols[292]. However, no differences in fatigue were observed between the other NMES+BFR conditions (40-80% AOP). Regarding perceptual ratings of pain during the intervention, the combination of NMES with 40% BFR resulted in reduced pain compared to NMES combined with 80% BFR and 60% BFR (Chapter 4) which could promote a more comfortable intervention to undertake. The results from Chapter 4 indicated that the acute fatigue response was the same when combining 40% and 60% BFR to NMES, with lower ratings of pain and RPE experienced with the addition of 40% BFR to NMES. Therefore, the NMES combined with 60% BFR condition was excluded from this study. The stimulation duration was adjusted in this and subsequent Chapters within this thesis to replicate the beneficial effects by Natsume et al. [46]. Chapter 4 found no differences between NMES+80% BFR and NMES+40% BFR regarding the primary outcomes measure, suggesting that the effects on training adaptations cannot be disregarded. Consequently, three groups were chosen to undertake a chronic training intervention, consisting of three sessions per week for a duration of six-weeks. Importantly, both NMES+BFR groups used AOP to determine the 40% and 80% BFR pressures.

The concept of critical power measures the highest sustainable power output that an individual can maintain without reaching exhaustion[220]. Critical power can be estimated in the quadriceps using intermittent MVIC's and is known as end-test torque[224]. In clinical populations, such as those recovering from OA or lower limb surgery, endurance capacity is often compromised, making it challenging for them to engage in prolonged

exercise[20,221,222]. Understanding an individual's end-test torque can assist healthcare professionals in designing tailored exercise programs aimed at progressively enhancing endurance capacity[220,223]. End-test torque represents a hyperbolic power-time relationship that forms the foundational basis for comprehending the physiological underpinnings of fatigue development across various exercise intensity domains[293]. When exercising at intensities below the end-test torque, skeletal muscle metabolism achieves a "steady-state"[294]. However, above end-test torque, in the "severe" intensity domain, muscle metabolism does not demonstrate steady-state behaviour, resulting in finite exercise capacity[224]. Notably, the end-test torque has been postulated to signify a 'fatigue threshold'[220], which task failure coincides with complete utilisation of a finite energy pool above the end-test torque (referred to as the impulse above end-test torque). Impulse above end-test torque represents the anaerobically biased work capacity or the additional amount of work an individual can perform above the critical power / end-test torque during high-intensity efforts[220,223,295,296]. This coincides with muscle fatigue, as evidenced by decreased maximal voluntary force[223–225]. The end-test torque and impulse above end-test torque can be estimated using an all-out knee extension test for the quadriceps[223–225,297]. These two parameters accurately predict the exercise tolerance limit at severe intensities, which occur when work is done above end-test torque[224]. Moreover, previous research suggests that impulse above end-test torque is related to a large proportion of type II muscle fibres, whereas a high end-test torque is associated with a high proportion of type I fibres[294,298]. BFR exercise causes increased neuromuscular fatigue and type II muscle fibre recruitment compared to free flow exercise[44]. Following 3-weeks of isometric training, improvements in impulse above end-test torque have been observed[225], therefore, we can hypothesise changes in this measure after NMES+BFR. Consequently, acquiring insights into potential muscle fibre type adaptations following NMES and NMES+BFR could assist clinical rehabilitation settings. Muscle disuse involving loss of neural influence and mechanical loading causing a slow-to-fast shift in fibre type and MyHC isoform profile, is usually accompanied by preferential atrophy of type I muscle fibres[50,51]. Therefore, assessing the aforementioned test can offer valuable insights into potential

muscle fibre type adaptations following NMES and NMES+BFR interventions, helping tailor rehabilitation approaches more effectively.

Training session discomfort and RPE have been reported to be higher during NMES+BFR compared to NMES alone in healthy adults[46]. This observation is similar to the wider literature base stating that BFR exercise causes increased ratings of session pain and RPE compared to free flow training[299–301]. However, Natsume et al. [46] did not use AOP to determine there restrictive pressure, which may have led to exaggerated perceptual ratings of discomfort and RPE[57,115]. Both NMES and BFR have been reported to be painful interventions[126,301]. One significant drawback associated with NMES is the elevation of pain levels at higher current intensities[40,60]. Previous research has demonstrated that BFR during resistance exercise and aerobic cycling actually reduces pain sensitivity immediately after training compared to unrestricted free flow training[43,302]. Therefore, session pain and RPE were recorded during this 6-week randomised control trial.

Blood deoxyhaemoglobin concentration [HHb], as measured by NIRS, allows the estimation of muscle microvascular oxygen extraction and provides insight into the dynamic equilibrium between O_2 delivery and utilisation[238,241,243,303]. Low-intensity BFR produces greater changes in blood deoxyhaemoglobin signal [HHb] compared to traditional low-intensity resistance training[304,305], demonstrating higher peripheral oxygen extraction with BFR exercise. The high metabolic demand during low- intensity BFR is strongly associated with a muscle hypertrophy response after only 2-weeks of training (r = 0.87)[306], indicating that reduced mechanical tension can induce muscle hypertrophy following exercise of high metabolic demand. Thus, assessing [HHb] responses during exhaustive tests of maximal torque production, such as a 5 minute all-out test, provides insight into both the mechanical and muscle microvascular responses to NMES and NMES+BFR training and could help to determine the temporal correspondence with measures of muscle hypertrophy. If muscular adaptations can be observed with less reported pain and perceived exertion, this could be

advantageous for reducing the risk of symptom flare-ups in clinical populations experiencing pain.

The primary aim of the present study was to determine if NMES combined with BFR of either (40% & or 80% AOP) is more effective at enhancing muscle strength and hypertrophy than NMES alone, using standardised protocols for 6-weeks. As a secondary aim, this study sought to explore whether any observed adaptations in muscle strength or hypertrophy were accompanied by any changes in impulse above end-test torque, end-test torque and/or HHb signal changes.

It was hypothesised that the combination of 80% BFR to NMES will induce greater changes in muscle strength, hypertrophy than NMES alone and NMES combined with 40% BFR.

A secondary hypothesis was anticipated that the combination of 80% BFR to NMES would result in enhanced impulse above end-test torque and HHb signal changes compared to NMES alone and NMES combined with 40% BFR.

5.3 Method

5.3.1 Ethical approval

All participants provided written informed consent and the study was approved by the University ethics sub-committee (Appendix 2: SMEC_2016-17_104) in accordance with the Declaration of Helsinki (2013).

5.3.2 Participants

Forty-two recreationally active healthy males (*n* = 28) and females (*n* = 14) volunteered and were randomised into one of three groups: NMES alone; NMES+40% BFR and NMES+80% BFR. The sample size was calculated *a-priori* using G*Power software and was based upon the effect sizes of research assessing the same outcomes (0.64) [178,182,208]. A group sample size of 14 was determined for the primary outcomes and 12 required for the secondary outcomes. Inclusion criteria were: (a) absence of lower-limb injury, (b) negative answers in the PAR-Q questionnaire (Appendix 5.3), (c) no personal history of cardiovascular or metabolic disease, (d) non-smokers, (e) resting SBP < 140 mmHg and (f) normal range on the ABI test (0.9-1.4)[194]. Participants were instructed to avoid lower limb resistance training for the duration of the study.

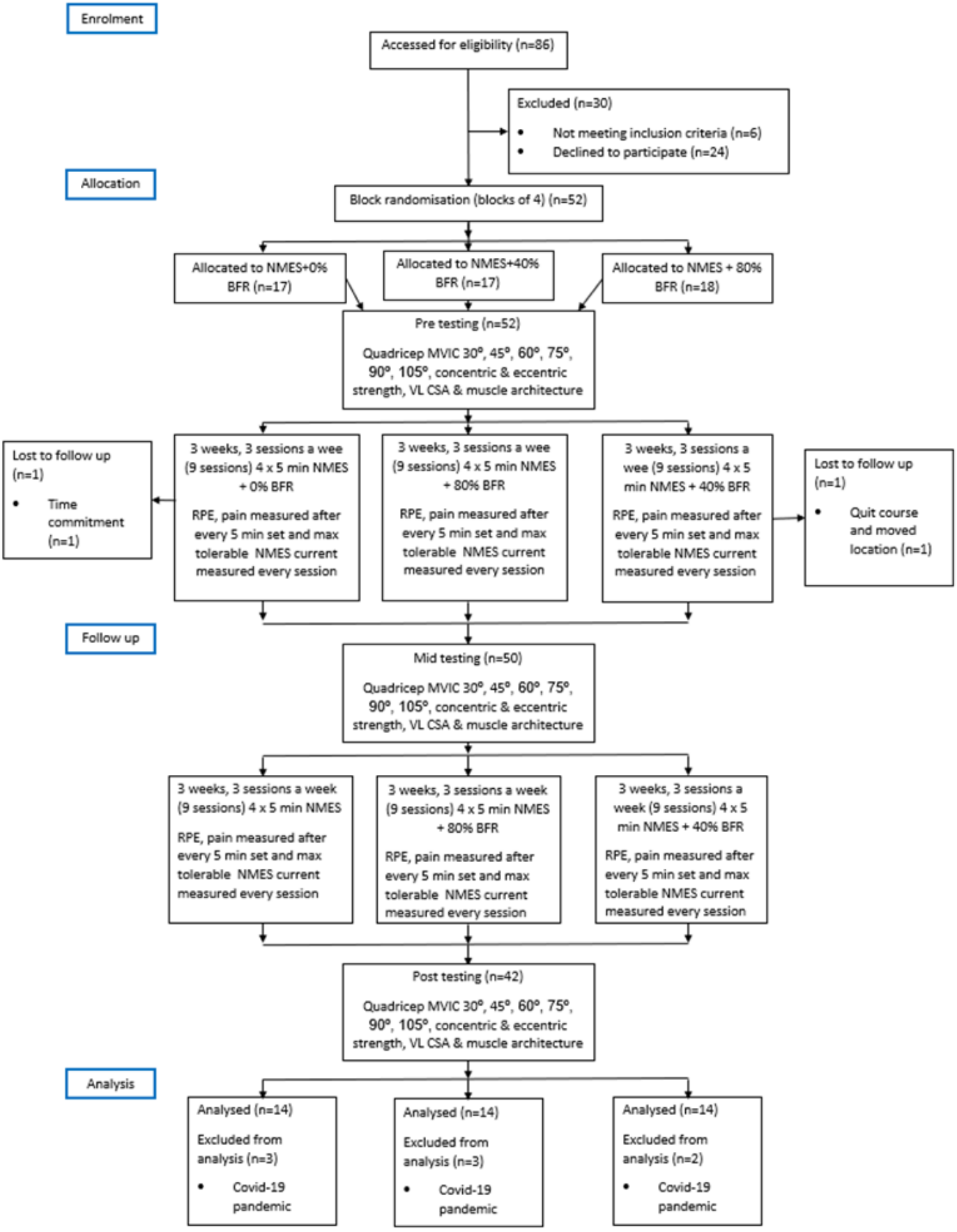

Fig 5.1. Consort flow diagram of participants through the study.

Table 5.1. Baseline characteristics of participants; mean (SD).

	NMES alone (n=14)	NMES+40% BFR (n=14)	NMES+80% BFR (n=14)	*P*
Demographics				
Male : female	9 : 5	9 : 5	10 : 4	>0.86
Age (years)	25.1 (5.6)	26.4 (6.6)	24.6 (6.4)	>0.91
Height (m)	1.75 (0.2)	1.74 (0.1)	1.74 (0.1)	>0.92
Weight (kg)	70.8 (10.8)	72.8 (10.2)	68.4 (9.5)	>0.71
BMI (kg/m^2)	22.9 (2.7)	23.9 (2.3)	22.9 (2.0)	>0.79
Muscle characteristics				
MVIC 30^0 (Nm)	102.2 (22.0)	105.2 (32.6)	103.4 (25.0)	>0.30
MVIC 45^0 (Nm)	141.3 (36.2)	133.8 (35.4)	142.7 (43.6)	>0.18
MVIC 60^0 (Nm)	181.9 (44.4)	184.7 (38.3)	180.3 (50.6)	>0.22
MVIC 75^0 (Nm)	198.3 (40.1)	210.8 (54.5)	205.4 (67.2)	>0.12
MVIC 90^0 (Nm)	195.2 (50.6)	208.7 (54.5)	202.3 (64.1)	>0.07
MVIC 105^0 (Nm)	161.5 (36.1)	168.6 (53.3)	184.2 (59.0)	>0.07
Quadriceps Concentric (Nm)	146.7 (38.7)	150.1 (55.2)	152.7 (38.9)	>0.13
Quadriceps Eccentric (Nm)	179.0 (40.5)	185.0 (50.5)	182.2 (50.8)	>0.09
Vastus lateralis CSA at 50% length (cm^2)	24.7 (6.0)	26.9 (6.0)	27.5 (10.1)	>0.07
Vastus lateralis CSA at 75% length (cm^2)	14.4 (4.6)	14.7 (4.5)	17.5 (11.3)	>0.16

5.3.3 Study design

The study followed a multi-arm parallel, randomised controlled design. Randomisation (blocks of 4) was generated via online software (http://www.randomization.com). All testing and training were undertaken in the university laboratory. The study underwent pre-trial registration https://clinicaltrials.gov/ct2/show/NCT03662555. Laboratory testing took place on 22 separate occasions, 2 familiarisations, 2 testing and 18 intervention sessions. The initial two sessions served as a familiarisation to the NMES protocol, BFR stimulus and strength testing. These took place > 7-days prior to the beginning of the intervention period and served as a standardised control period prior to the main testing. The third session (PRE) took place 1-day prior the start of the intervention period and all future sessions consisted of muscle strength and hypertrophy outcome measures. The fourth session (POST) took place 48-72 h following 6-weeks of training. All repeated measures were performed at a similar time of day (±1 h) to diminish any circadian effect. All participants were tested at least 2 h postprandial and were instructed to avoid caffeine and exercise prior to testing. After the familiarisation sessions, participants were randomly allocated by

the lead investigator to their experimental groups, with the same trained researcher performing all outcome measurements (Fig 5.2):

1) NMES alone
2) NMES+40% BFR
3) NMES+80% BFR

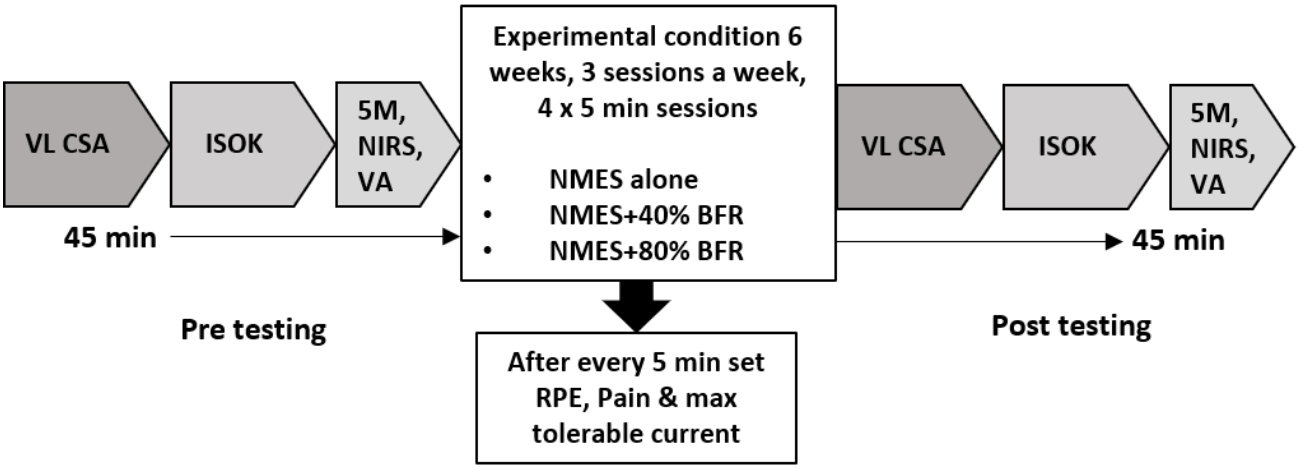

Fig 5.2. Experimental protocol. All participants performed the same NMES protocol under three different BFR pressures (0, 40 and 80%) for 6-weeks, 3 sessions a week. Outcome measures; VL CSA, isometric peak torque and concentric and eccentric peak torque (ISOK), 5 minute all-out test, NIRS, VA was assessed before and post the 6-week intervention period. Outcome measures assessed after every 5 minutes set included; RPE, pain and max tolerable current achieved.

5.4 Procedures

5.4.1 NMES

See section 3.4. Every training session involved each participant remaining seated with their knees bent to 90°, which matched the same testing position as Chapter 4 and their foot placed in front of an immovable object. The training position was standardised and

remained the same for every participant. Every training session involved a 4 x 5 minutes intervention, with maximum tolerable NMES currents verbally encouraged throughout. Each VM and VL maximal tolerable intensities were also recorded after every training session and used for analysis.

5.4.2 Determination of BFR pressure

See section 3.5. AOP was determined with each participant seated with their knees bent at 90°, matching the position that the intervention was undertaken[116].

5.5 Primary outcomes

5.5.1 Isokinetic Dynamometry

See section 3.7.

5.5.2 Quadriceps CSA

See section 3.9.

5.5.3 Quadriceps muscle architecture

See section 3.10.

5.5.4 RPE

See section 3.14.

5.5.5 Pain

See section 3.15.

5.6 Secondary outcomes

5.6.1 End-test torque and impulse above end-test torque

See section 3.16.

5.6.2 VA

See section 3.17.

5.6.3 Slope analysis on ΔHHb values

See section 3.18.

5.7 Statistical Analysis

One-way analyses of covariance (ANCOVA) were used to determine the effects of group (NMES alone; NMES+40% and NMES+80% BFR) at post-testing. The pre-test measurements were used as covariates for: MVIC30°, MVIC45°, MVIC60°, MVIC90°, MVIC105°, CON, ECC, end-test torque, impulse above end-test torque, VA%, Δ[HHb]; CSA50, CSA75, fascicle length and pennation angle.

Two-way ANCOVAs was used to determine the effects of group (NMES alone; NMES+40% and NMES+80% BFR) and time across 18 time points (intervention week 1-18) for session maximum tolerable currents for VL and VM, pain, RPE. Familiarisation 2 was used as the covariate for max tolerable current and Session 1 was used as the covariate for pain and

RPE. Interactions and main effects were followed with appropriate post-hoc analyses and Bonferroni adjustments.

Statistical significance was set at $p < 0.05$. Cohens d = 0.2, 0.5 and 0.8 are reported for pairwise comparisons and correspond to small, medium and large effects, respectively.

Statistics were computed using SPSS Statistics software package version 28.0 (SPSS, Chicago, USA). Data are presented as means (SD) unless otherwise stated.

5.8 Results

No adverse events occurred. All participants completed all training sessions (100% adherence).

Table 5.2. Knee extension peak torque, post-test measurement values; mean SD.

	NMES alone (n=14)		NMES+40% BFR (n=14)		NMES+80% BFR (n=14)	
	Post	SD	Post	SD	Post	SD
MVIC 30º (Nm)	105.9	24.2	121.0	30.5#	112.4	26.3
MVIC 45º (Nm)	149.3	37.2	159.8	46.8#	161.0	34.9
MVIC 60º (Nm)	191.7	43.3	205.8	49.9	204.3	42.2
MVIC 75º (Nm)	207.1	44.6	241.4	68.1	231.2	61.3
MVIC 90º (Nm)	202.2	53.0	226.0	65.2	237.0	71.4#
MVIC 105º (Nm)	170.9	48.1	184.4	19.5	207.9	68.9
Concentric (Nm)	151.2	37.1	171.5	51.2	174.0	51.3
Eccentric (Nm)	190.2	46.8	219.6	64.9#	207.6	52.7

Significant difference was set at $p < 0.05$; # = significantly greater group effect compared to NMES alone.

5.8 Muscle strength

5.8.1 MVIC at 30º knee angle

There was a significant main effect of group on MVIC at 30º [$F_{(2,38)} = 5.69$, $p = 0.007$, $\eta^2 = 0.23$]. Post-hoc analysis showed higher values in MVIC at 30º for NMES+40% BFR compared with NMES alone ($p = 0.005$). The mean difference between NMES+40% BFR

and NMES alone was 12.14 (SEM = 3.61, n = 14), resulting in a Cohen's d of 0.95. This indicates a large effect size in favour of NMES+40% BFR (d = 0.95, 95% CI [3.1, 21.185]). No differences were observed between NMES+40% BFR and NMES+80% BFR (Table 5.2).

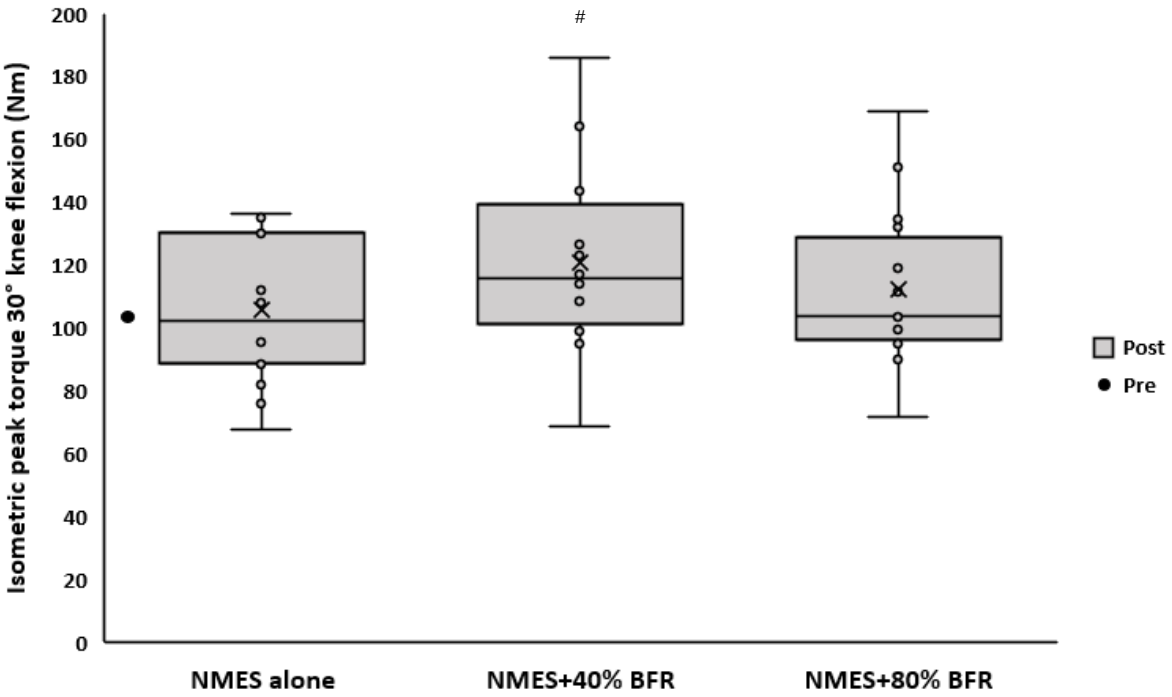

Fig 5.3. Isometric peak torque at a knee angle of 30°; values as mean ± SEM; # = significant increase compared to NMES alone.

5.8.2 MVIC at 45º knee angle

There was a significant main effect of group on MVIC at 45º [$F_{(2,38)} = 4.53$, $p = 0.017$, $\eta^2 = 0.19$]. Post-hoc analysis showed higher values in MVIC at 45º after NMES+40% BFR compared with NMES alone ($p = 0.014$, d = 0.94, 95% CI [2.89, 32.45]). No differences were observed between NMES+40% BFR and NMES+80% BFR (Table 5.2).

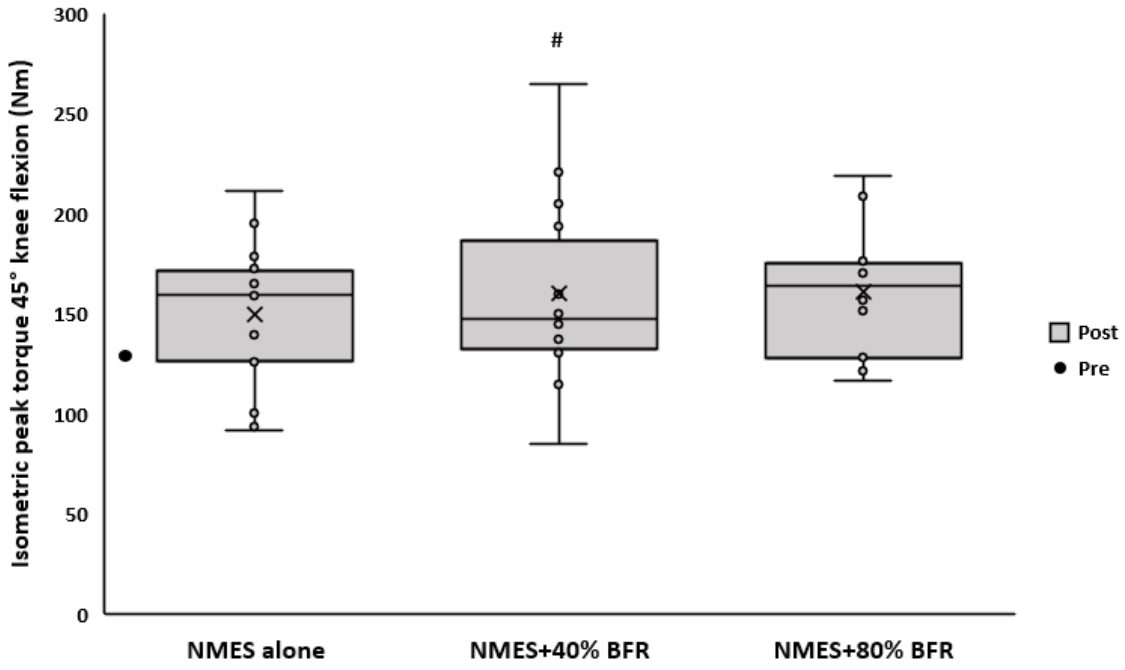

Fig 5.4. Isometric peak torque at a knee angle of 45°; values as mean ± SEM; # = significant increase compared to NMES alone.

5.8.3 MVIC at 60º knee angle

There was no significant main effect of group on MVIC at 60º [$F_{(2,38)} = 2.38$, $p = 0.106$, $\eta^2 = 0.11$] (Table 5.2).

5.8.4 MVIC at 75º knee angle

There was no significant main effect of group on MVIC at 75º [$F_{(2,38)} = 3.1$, $p = 0.057$, $\eta^2 = 0.14$] (Table 5.2).

5.8.5 MVIC at 90º knee angle

There was a significant main effect of group on MVIC at 90º [$F_{(2,38)} = 4.05$, $p = 0.025$, $\eta^2 = 0.18$]. Post-hoc analysis showed higher values in MVIC at 90º after NMES+80% BFR compared with NMES alone ($p = 0.023$, d = 0.84, 95% CI [3, 51.98]). No significant differences were observed between NMES+40% BFR and NMES+80% BFR (Table 5.2).

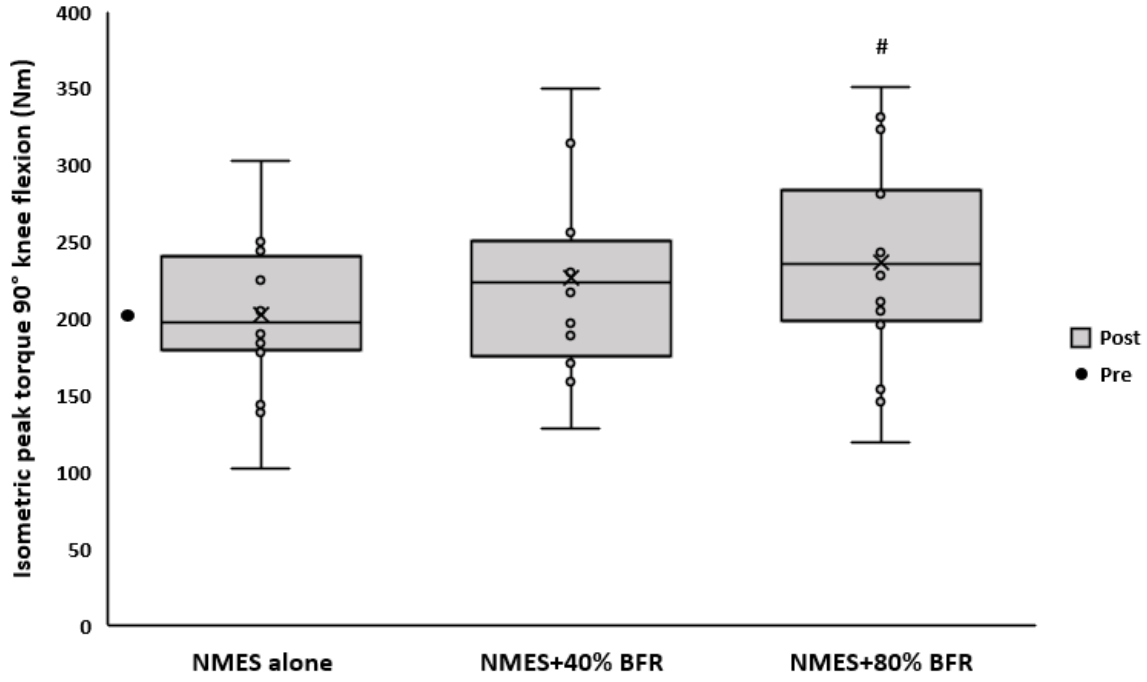

Fig 5.5. Isometric peak torque at a knee angle of 90°; values as mean ± SEM; # = significant increase compared to NMES alone.

5.8.6 MVIC at 105º knee angle

There was no significant main effect of group on MVIC at 105º [$F_{(2,38)} = 1.19$, $p = 0.32$, $\eta^2 = 0.06$] (Table 5.2).

5.8.7 Concentric strength

There was a significant main effect of group on concentric peak torque [$F_{(2,38)} = 3.5$, $p = 0.03$, $\eta^2 = 0.17$]. However, Post-hoc analysis could not identify any significant differences between groups ($p > 0.064$) (Table 5.2).

5.8.8 Eccentric strength

There was a significant main effect of group on eccentric peak torque [$F_{(2,38)} = 4.5$, $p = 0.017$, $\eta^2 = 0.19$]. Post-hoc analysis showed higher values in eccentric peak torque following NMES+40% BFR compared with NMES alone ($p = 0.015$, d = 0.80, 95% CI [3.7, 42.013]).

No significant differences were observed between NMES+40% BFR and NMES+80% BFR (Table 5.2).

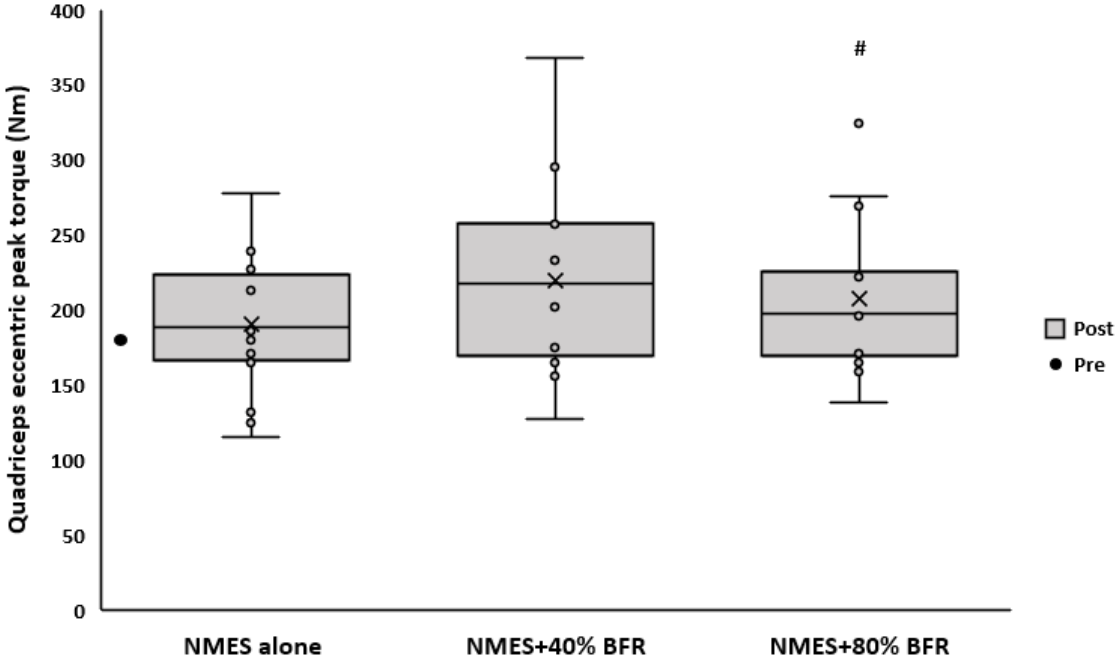

Fig 5.6. Quadriceps eccentric peak torque; values as mean ± SEM; # = significant increase compared to NMES alone.

Table 5.3. VL CSA at 50% and 75% length of femur, post-test measurement values; mean SD.

	NMES alone (n=14)		NMES+40% BFR (n=14)		NMES+80% BFR (n=14)	
	Post	SD	Post	SD	Post	SD
VL CSA at 50% length (cm^2)	25.2	6.0	29.8	5.6#	30.4	10.5#
VL CSA at 75% length (cm^2)	14.3	4.8	16.3	4.0#	19.5	11.9#
VL fascicle length (cm)	7.8	0.8	8.3	0.9	8.3	0.7
VL pennation angle (º)	19.6	2.2	21.3	3.3*	21.9	4.0

Significant difference was set at $p < 0.05$; # = significantly greater group effect compared to NMES alone; * = significantly greater group effects compared to NMES+80% BFR

5.9 Muscle size

5.9.1 VL CSA at 50% length of femur

There was a significant main effect of group on VL CSA at 50% femur length [$F_{(2,38)} = 8.8$, $p < 0.001$, $\eta^2 = 0.32$] (Table 5.3). Post-hoc analysis showed higher values in VL CSA at 50% femur length after NMES+40% BFR and NMES+80% BFR compared with NMES alone ($p = 0.002$, d = 0.97, 95% CI [0.79, 4.25] and $p = 0.002$, d = 0.98, 95% CI [0.8, 4.3], respectively). No significant differences were observed between NMES+40% BFR and NMES+80% BFR (Table 5.3).

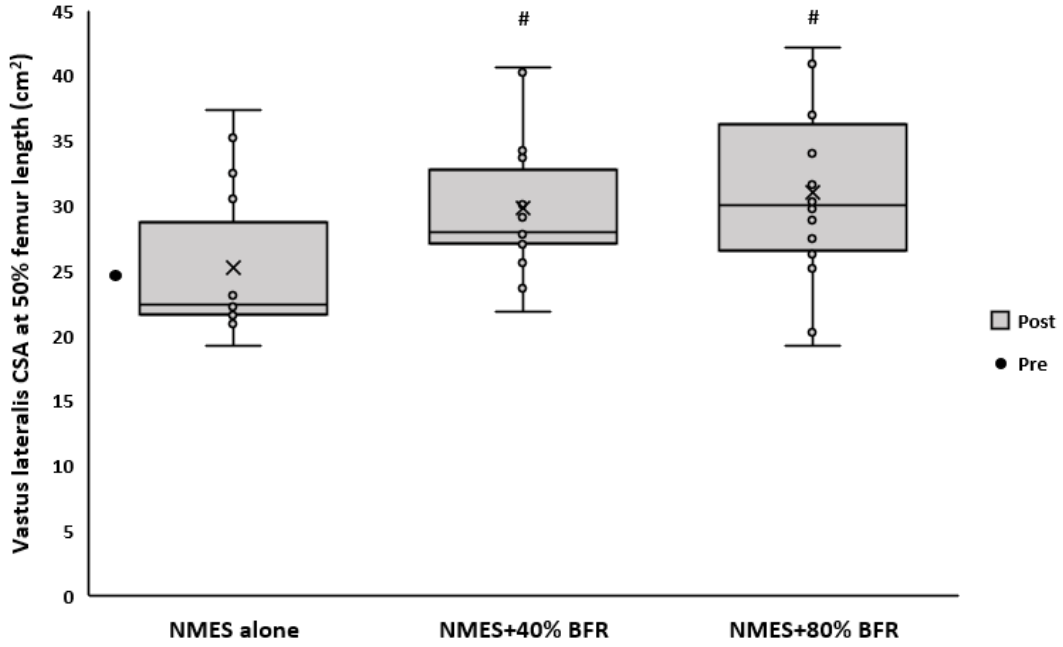

Fig 5.7. VL CSA at 50% length of femur; values as mean ± SEM; # = significant increase compared to NMES alone.

5.9.2 VL CSA at 75% length of femur

There was a significant main effect of group on VL CSA at 75% femur length [$F_{(2,38)} = 5.7$, $p = 0.007$, $\eta^2 = 0.23$]. Post-hoc analysis showed higher values in VL CSA at 75% femur length after NMES+40% BFR and NMES+80% BFR compared with NMES alone ($p = 0.028$, d = 0.73, 95% CI [0.11, 2.49] and $p = 0.011$, d = 0.82, 95% CI [0.29, 2.73], respectively).

No significant differences between NMES+40% BFR and NMES+80% BFR were observed (Table 5.3).

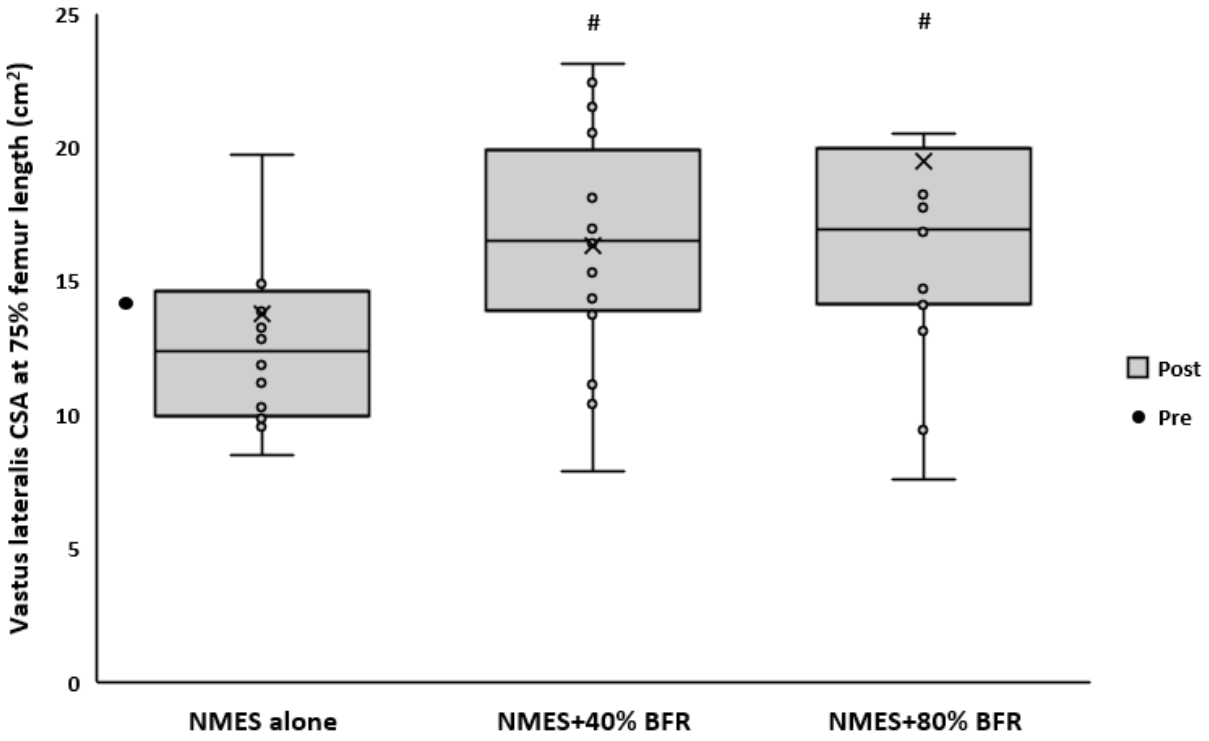

Fig 5.8. VL CSA at 75% length of femur; values as mean ± SEM; # = significant increase compared to NMES alone.

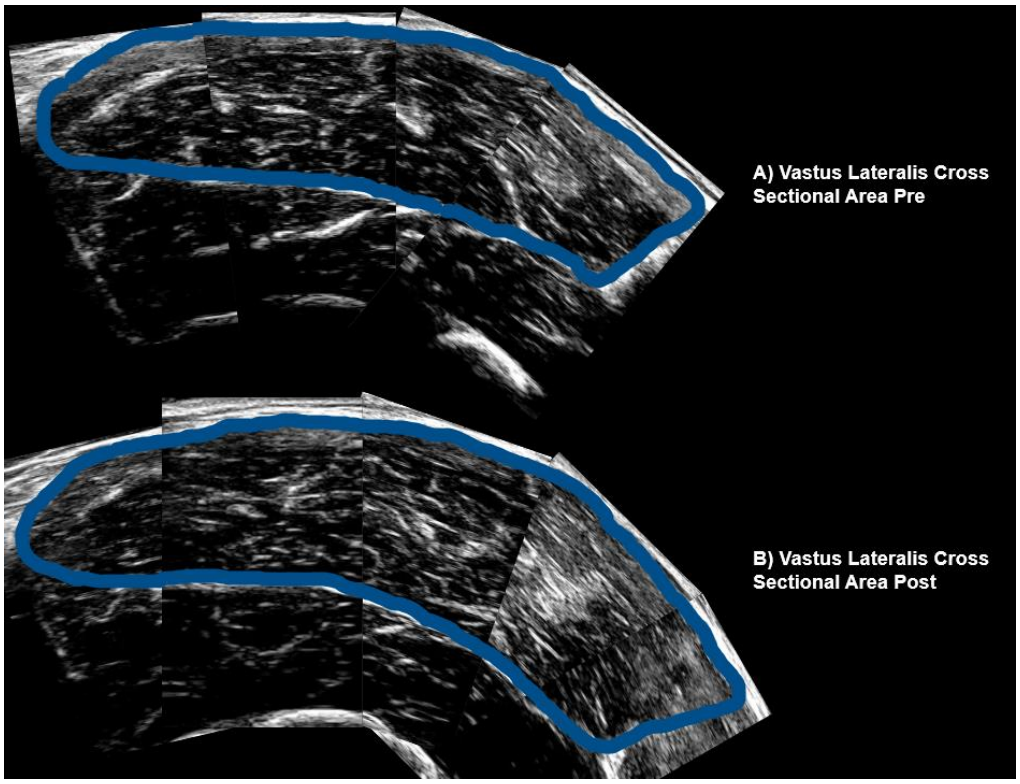

Fig 5.9. Example of VL CSA at 50% femur length; pre and post.after NMES+40% BFR.

5.9.3 Fascicle length

There was no significant main effect of group on fascicle length [$F_{(2,38)} = 2.9$, $p = 0.065$, $\eta^2 = 0.13$] (Table 5.3).

5.9.4 Pennation angle

There was a main group effect on pennation angle [$F_{(2,38)} = 3.9$, $p = 0.029$, $\eta^2 = 0.17$] (Table 5.3). Post-hoc analysis showed higher values in pennation angle after NMES+40% BFR compared with NMES+80% BFR ($p = 0.031$, d = 0.72, 95% CI [0.07, 1.84]). No other differences were observed (Table 5.3).

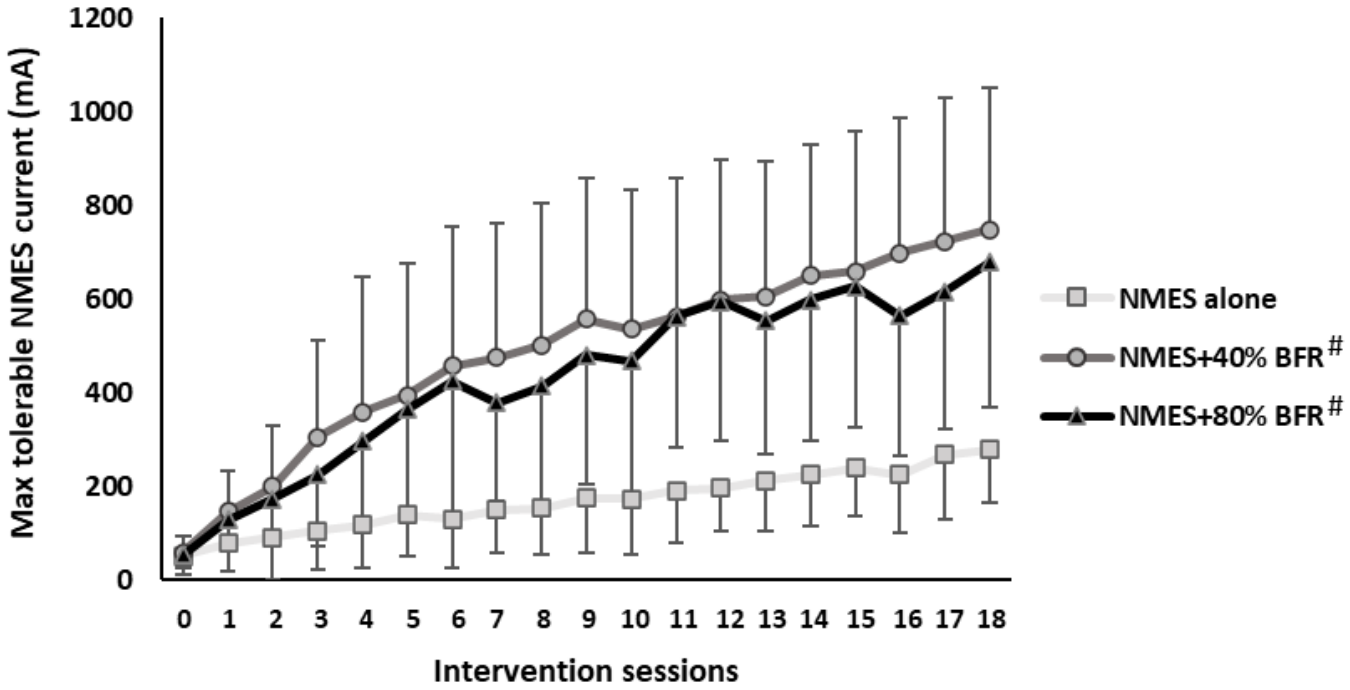

Figure 5.10. Maximal tolerable NMES current after every intervention session. Values as mean ± SEM; # = significantly greater group effect than NMES alone.

5.10 Perceptual variables

5.10.1 Maximum tolerable NMES current

There was a significant effect of time [$F_{(1,38)} = 40.3$, $p < 0.001$, $\eta^2 = 0.52$] and group × time interaction for greater vastus medialis [$F_{(2,38)} = 10$, $p < 0.001$, $\eta^2 = 0.34$] and vastus lateralis time [$F_{(1,38)} = 21.6$, $p < 0.001$, $\eta^2 = 0.36$] and group × time [$F_{(2,38)} = 10.1$, $p < 0.001$, $\eta^2 = 0.35$], respectively for max tolerable NMES current increase. Post-hoc analysis confirmed higher values in vastus medialis max tolerable NMES current following NMES+40% BFR and NMES+80% BFR compared with NMES alone ($p < 0.001$, d = 1.14, 95% CI [143.59, 546.6] and $p = 0.031$, d = 0.72, 95% CI [18.72, 497.55], respectively). Additionally, higher values in the vastus lateralis max tolerable NMES current was observed following NMES+40% BFR compared with NMES alone ($p < 0.001$, d = 1.12, 95% CI [134.67, 533.77] and NMES+80% BFR compared with NMES alone $p = 0.004$, d = 0.94, 95% CI [79.72, 477.19]. No differences between NMES+40% BFR and NMES+80% BFR was observed (Fig 5.10).

Table 5.4. Training session pain and RPE; mean (SD)

	NMES alone		NMES+40% BFR		NMES+80% BFR	
Session	**Pain**	**RPE**	**Pain**	**RPE**	**Pain**	**RPE**
1	3.3 (1.7)	11.8 (1.6)	4.5 (2.1)	12.5 (2.8)	5.0 (2.1)	13.4 (2.0)
2	3.6 (1.9)	12.2 (2.1)	4.7 (1.9)	12.9 (3.1)	4.9 (2.6)	13.2 (2.8)
3	4.0 (1.9)	12.7 (1.3)	4.8 (2.0)	12.8 (2.7)	4.6 (3.0)	12.7 (3.2)
4	3.9 (1.6)	12.4 (1.6)	4.7 (1.8)	12.9 (2.0)	4.8 (2.2)	12.8 (2.5)
5	3.6 (1.4)	12.5 (2.0)	4.1 (1.3)	12.3 (2.3)	5.0 (2.1)	12.6 (2.7)
6	4.1 (1.3)	12.1 (1.8)	4.4 (1.4)	12.6 (2.0)	5.0 (1.8)	12.4 (2.8)
7	4.4 (1.9)	12.4 (2.6)	4.0 (1.4)	11.9 (1.9)	4.5 (1.9)	12.4 (2.6)
8	4.5 (2.0)	12.0 (2.8)	4.2 (1.4)	12.4 (1.9)	4.5 (2.0)	12.1 (2.7)
9	5.4 (3.3)	12.3 (3.1)	3.9 (1.5)	11.8 (2.4)	4.8 (3.3)	3.3 (3.1)
10	4.6 (2.0)	12.5 (2.8)	4.2 (1.6)	12.5 (1.9)	4.4 (2.0)	12.5 (2.8)
11	4.9 (2.1)	13.2 (2.8)	4.3 (1.8)	12.1 (2.1)	4.9 (2.1)	12.9 (2.8)
12	5.3 (3.5)	11.8 (3.0)	4.3 (1.4)	12.2 (1.8)	4.6 (3.5)	12.3 (3.0)
13	4.2 (1.7)	12.1 (2.6)	4.1 (1.6)	12.0 (1.8)	4.2 (1.7)	12.2 (2.6)
14	4.6 (1.7)	12.1 (2.2)	4.1 (1.6)	12.4 (2.3)	4.9 (1.7)	12.5 (2.2)
15	4.7 (2.1)	12.4 (2.7)	4.0 (1.5)	12.2 (1.6)	4.5 (2.1)	12.7 (2.7)
16	4.3 (1.7)	12.2 (2.6)	3.9 (1.8)	12.1 (2.3)	4.3 (1.7)	12.2 (2.6)
17	4.1 (1.6)	11.9 (2.4)	4.3 (2.1)	12.5 (2.7)	4.6 (1.6)	12.1 (2.4)
18	4.5 (2.0)	12.3 (2.8)	4.2 (2.0)	12.4 (2.6)	4.3 (2.0)	12.6 (2.8)

Significant difference set at $p < 0.05$.

5.10.2 Pain

There was a significant effect of time [$F_{(1,38)} = 55.8$, $p < 0.001$, $\eta^2 = 0.60$], however, no group × time interaction observed for pain ratings ($F_{(2,38)} = 0.6$, $p = 0.55$, $\eta^2 = 0.031$) (Table 5.4).

5.10.3 RPE

There was a significant effect of time [$F_{(1,38)} = 28.3$, $p < 0.001$, $\eta^2 = 0.43$], however, no group × time interaction observed for RPE ratings ($F_{(2,38)} = 0.98$, $p = 0.38$, $\eta^2 = 0.049$) (Table 5.4).

Table 5.5. 5 minute all-out test: impulse above end-test torque; end-test torque; VA at the start and end of the test; Δ[HHb] signal during middle 2 s of each contraction, post-test

	NMES alone (n=12)		NMES+40% BFR (n=12)		NMES+80% BFR (n=12)	
	Post	SD	Post	SD	Post	SD
IAETT (Nms)	7137.4	2306.2	7680.7	3170.2	7729.9	3199.5
ETT (Nms)	78.3	19.2	76.6	10.7	76.1	14.1
VA start (%)	90.8	2.7	90.3	1.6	90.6	1.9
VA end (%)	75.2	6.7	75.1	5.3	75.8	5.8
Δ HHb (mmol/s)	1.29	0.8	1.44	0.6	1.39	0.6

Significant difference was set at $p < 0.05$.

5.11 Secondary Outcomes

5.11.1 Impulse above end-test torque

There was no significant main effect of group on impulse above end-test torque [$F_{(2,32)} = 0.8$, $p = 0.46$, $\eta^2 = 0.05$] (Table 5.4).

5.11.2 End-test torque

There was no significant main effect of group on end-test torque [$F_{(2,32)} = 0.3$, $p = 0.74$, $\eta^2 = 0.02$] (Table 5.4).

5.11.3 VA%

There was no significant main effect of group on VA% [$F_{(2,32)} = 0.3$, $p = 0.76$, $\eta^2 = 0.02$] (Table 5.4).

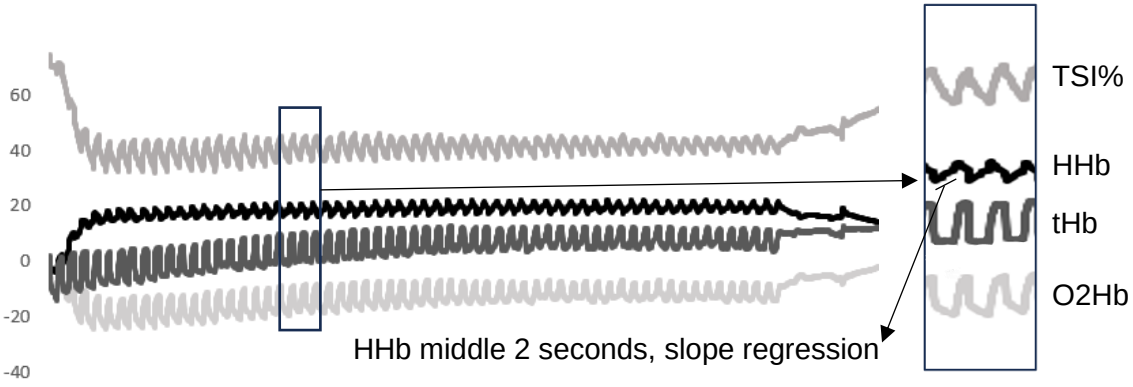

Fig 5.11. NIRS trace example zoomed into three, 3 second contractions. TSI%: tissue oxygen/saturation index, O2Hb: oxyhemoglobin, HHB: deoxyhemoglobin, tHB: total hemoglobin

5.11.4 Slope analysis on ΔHHb values

There was no significant main effect of group on slope analysis on ΔHHb values [$F_{(2,164)}$ = 2.09, p = 1.3, η^2 = 0.03] (Table 5.4).

5.12 Discussion

The main findings of the present study revealed that combining NMES with either 40% or 80% BFR (AOP) for six-weeks, resulted in greater increases in muscular strength (isometric and eccentric) and quadriceps CSA compared to NMES alone (Table 5.2 and 5.3). Secondary findings were that NMES combined with 40-80% BFR caused the participants to tolerate higher NMES currents during the training sessions compared to NMES alone (Fig 5.9). Contrary to our hypothesis, both NMES+40% BFR and NMES+80% BFR were more effective at enhancing muscle strength and hypertrophy than NMES alone. However, greater changes in pennation angle were observed after NMES+40% BFR compared to NMES+80% BFR. Despite the observed increases in muscle strength and hypertrophy after six-weeks of NMES+BFR, no effects on impulse above end-test torque, end-test torque and Δ[HHb] were observed (Table 5.5).

The present study observed differences between the NMES+BFR groups and NMES alone for increases in isometric (8.1-8.2%), concentric (10.9-11.1%) and eccentric strength (7.7-12.4%) (Table 5.2). Our findings indicate that the current tolerated by the NMES+40% BFR (749.3 [312.0] mA) and NMES+80% BFR groups (678.6 [367.3] mA) was 2.4-2.7 times greater than that tolerated by NMES alone (296.4 [129.1] mA). No differences observed between groups in current tolerated after the familiarisation sessions (36.5-40.1 mA: Fig 5.10). The greater current tolerated in the NMES+BFR groups may have contributed to the enhancement in muscle strength and hypertrophy observed in the present study compared to NMES alone. It is widely recognised that current intensity is a crucial parameter to control when using NMES to enhance isometric strength, with higher current intensities providing a greater stimulus for muscle adaptation[84]. Numerous studies have investigated the effect of NMES training on muscle strength adaptation, and based on previous findings, strength gain following NMES training depends on the magnitude of electrically evoked force during the intervention period[36–40]. Natsume et al. [84] found that using maximum tolerable currents caused greater changes in muscle strength and size after eight-weeks of training vs. using 50% maximum tolerable current. Additionally, Li et al. [81] recently evaluated the effect of BFR, NMES and NMES+BFR during low-intensity squat training on muscle adaptations and muscle activation assessed through surface EMG. They observed a greater increase in muscle activation increase in the NMES+BFR group compared with the BFR group and the control group who only performed the squatting intervention, where the increased muscle activation led to increased muscle strength and may have contributed the effects observed after NMES+BFR in the present study.

The present findings show that the greater current tolerated during NMES+BFR did not cause increased session pain and RPE during the training sessions compared to NMES alone (Table 5.4). One of the main limitations of NMES is the increase in pain with higher current levels[40,60]. Previous research has demonstrated that BFR during resistance exercise and aerobic cycling reduces pain sensitivity immediately after training compared

to unrestricted free flow training[43,302]. This reduction in pain sensitivity after BFR exercise has been partially explained by endogenous opioid and endocannabinoid system pain modulation mechanisms[43,184,302]. The endogenous opioid system utilises peptides such as endorphins, which selectively bind to mu, delta, and kappa receptors, to modulate pain perception by inhibiting pivotal neurotransmitters like substance P. Concurrently, the endocannabinoid system employs retrograde neurotransmitters, such as anandamide, creating interactions with CB1 and CB2 receptors, assisting with the regulation of pain[167,307]. Additionally, activation of a nociceptive descending inhibitory system has been proposed as an explanation of how pain perception is altered by the recruitment of high-threshold motor units[307]. Moreover, increased motor cortex activity has been observed after the recruitment of high-threshold motor units due to increased force production and this has been shown to induce analgesia in humans[308]. Therefore, increased fatigue, as reported previously after NMES+BFR[186] and the previously discussed pain inhibitory mechanisms induced by BFR exercise may explain the higher current tolerances observed in the present study and the subsequent greater muscle strength and hypertrophy after NMES+BFR. This apparent pain modulating effect of NMES+BFR compared to NMES alone needs to be explored regarding pressure, thermal and central mechanisms in both healthy and clinical populations. This will be explored in Chapter 6 and 7 of this thesis, to elucidate whether the same effects of BFR exercise on pain modulation are present after NMES+BFR.

In the present study, hypertrophy changes were 8.9% greater with the addition of BFR to NMES alone; 11% after NMES+40% BFR and NMES+80% BFR vs. 2.1% after NMES alone (Table 5.3). Increased motor unit recruitment levels during strength training, as a result of peripheral fatigue, contributes to muscle hypertrophy. When the working muscle fibres are fatigued, other muscle fibres must be activated in order to maintain the desired levels of force, increasing motor unit recruitment[309–311]. A clear positive relationship exists in the literature between baseline muscle CSA and strength, with greater CSAs correlating with

greater strength capacities[94,312–314]. Our previous study demonstrated that the combination of 40% and 80% BFR to NMES resulted in a decrease in knee extension force immediately after the session, indicating the presence of acute post-session fatigue[186]. This finding is consistent with the notion that session fatigue plays a crucial role in promoting improvements in quadriceps hypertrophy, as observed in the current study.

Regarding architectural changes, the present study observed increases in pennation angle after NMES+40% BFR, which were greater than that observed after NMES+80% BFR. Greater pennation angle allows for a larger amount of contractile tissue to attach to a given area of tendon or aponeurosis, resulting in an increased physiological CSA of the muscle[207]. This enlarged CSA optimises the length tension relationship and facilitates the recruitment of more muscle fibres, ultimately contributing to increased strength[315]. This finding helps explain the greater amount of group effects observed after NMES+40% BFR for isometric and isotonic strength compared to NMES+80% BFR in the present study. Similar observations of muscle thickness increase primarily related to an increase in pennation angle, without changes in fascicle length have been reported in previous BFR research[316]. The lack of change in fascicle length in the present study can be attributed to the specific nature of NMES+BFR contractions, which impose lower demands on the body compared to high load and high-speed isotonic contractions typically used to induce muscle fascicle lengthening[207,317]. It is possible that the lower loads and slower contraction velocities associated with NMES+BFR do not provide a sufficient stimulus for fascicle elongation.

Interestingly, despite the higher current tolerances in the NMES+BFR groups, there were no differences in session pain and RPE scores compared to NMES alone (Table 5.4). Natsume et al. [46] showed contrasting findings, with increased RPE and CR10 scores on the NMES+BFR limb compared with the NMES alone limb in their participants. The differences observed could be due to the present study utilising evidence-based protocols

to improve the comfort and efficacy of NMES and BFR, namely using AOP to prescribe the BFR restrictive pressure and placing the NMES electrodes over motor points[57,147]. Placing NMES electrodes over motor points reduces the pain experienced and the evoke torque produced compared to placing the electrodes without locating the motor points[38,98,147]. Furthermore, to maximise quadriceps strength after NMES, it is recommended to use a frequency of 50 Hz[123]. These NMES parameters were not utilised in previous NMES+BFR studies on the quadriceps[46,58,69] but proved effective in increase strength and hypertrophy in the present study when combined with 40% and 80% BFR.

A methodological factor that was addressed in this study was the standardisation of BFR pressures when combined with NMES. Previous studies have implemented BFR by prescribing an arbitrary restrictive pressure[46,47,58] or based their occlusion pressure on SBP[45]. Actual pressure exerted on the vascular system may vary wildly with cuff width, limb thickness and body composition[115]. Therefore, current recommendations are that the BFR pressure should be prescribed via AOP[57]. The present study addressed this issue by prescribing the BFR pressure via AOP, providing a more standardised and accurate approach. Our findings support the beneficial effects of NMES+BFR increasing muscle strength and size reported among healthy and spinal cord injured participants[45,46] and interestingly found no difference between the NMES+40% BFR and NMES+80% BFR conditions, with both being superior to NMES alone (Table 5.2 and 5.3). However, the NMES+40% BFR group showed greater group effects for more isometric angles and eccentric contractions (Table 5.2). This aligns with the recommended BFR pressure range of 40-80% AOP in recent methodology and application guidelines[57]. The same reasoning might therefore explain the lack of effect reported by Slysz et al. [58], where 100% occlusion was applied as part of their BFR method. The authors reported no group differences for quadriceps strength and hypertrophy after NMES+BFR compared to NMES alone. Our findings currently support future NMES+BFR studies utilising the same NMES parameters

and a restrictive pressure of 40% AOP, however, NMES+80% BFR cannot be discounted. In clinical practice, the NMES+40% BFR intervention is recommended based on observed increases in eccentric strength, isometric strength, and vastus lateralis hypertrophy without voluntary movement or exercise, as shown in Table 5.2 and 5.3 of the present study.

The secondary objective of this study was to investigate the effects of NMES and/or combined with BFR on impulse above end-test torque, end-test torque and Δ[HHb] during a 5 minute all-out knee extension MVIC test after six weeks of training. These measurements provide valuable insights into muscle and vascular responses[220,224,242,318]. In contrast to previous studies examining whole-body exercise[296,319] and isometric training[225], our findings demonstrated no effects of NMES or NMES+BFR on impulse above end-test torque, end-test torque and Δ[HHb] (Table 5.5).

Impulse above end-test torque serves as a measure of the overall force output generated by a muscle during an MVC by integrating force over-time[224]. However, in our study, despite the observed increases in muscle strength and hypertrophy following both six-week NMES+BFR interventions, no effects were observed on impulse above end-test torque and end-test torque. This discrepancy may be attributed to various factors. One potential explanation could be the involvement of neural adaptations that enhance activation and co-ordination, resulting in increased strength without a concurrent change in torque produced over time (e.g. impulse)[225]. Additionally, the observed changes in pennation angle, which result in improved recruitment patterns[315] or alterations in muscle fibre composition, may have contributed to increased strength independently of changes in force output measured by IACT[225,315]. Furthermore, increased impulse above end-test torque has been observed after 3 weeks of training. The discrepancy in results could be associated with involuntary NMES contractions compared to voluntary isometric exercises by De-Menezes et al. [225]

Muscle oxygen extraction, reflected by Δ[HHb], plays a crucial role in evaluating tissue perfusion and vascular health[233,234,242,320]. However, similar to impulse above end-test torque and end-test torque, no effect on Δ[HHb] was observed in our findings despite the aforementioned muscle adaptations following both NMES+BFR interventions. Improvements in muscle size and strength are intricately linked to enhanced oxygen extraction during exercise. This adaptation involves enhanced capillarization, promoting increased blood flow and efficient oxygen delivery to the muscle[233,321]. Due to an improvement in VL strength and size after NMES+BFR, a concurrent improvement in HHb extraction would be expected. This inconsistency may be explained by factors such as changes in capillary density, alterations in oxygen utilisation or adaptations in local blood flow regulation observed after BFR exercise that do not directly manifest as changes in Δ[HHb][154,242]. Furthermore, the reduced intensity of the NMES+BFR interventions along with the physiological and metabolic difference to involuntary NMES compared with voluntary resistance exercise could have contributed to the lack of effect observed[322–325]. These findings underscore the complex interplay between muscle adaptations, vascular responses and functional outcomes which cannot fully explain the muscle strength and hypertrophy improvements observed after NMES+BFR compared to NMES alone cannot be fully explained by changes in impulse above end-test torque, end-test torque or Δ[HHb].

Further research is needed to fully understand the underlying mechanisms and physiological responses associated with NMES+BFR interventions. Additionally, investigating the long-term effects of NMES+BFR on functional outcomes and evaluating its applicability across different clinical populations and muscle groups would contribute to a more comprehensive understanding of this intervention’s potential benefits.

5.13 Conclusion

The present findings provide evidence that the addition of 40% and 80% BFR to NMES is more effective at enhancing muscular strength and hypertrophy than NMES alone in healthy adults. This is the first training study to standardise the BFR pressure using a percentage of AOP when combining it with NMES. The perceptual ratings of RPE and pain show no clinically difference between the groups and are lower than those reported by Natsume et al. [46], suggesting that the protocols used in this study may be more effective and comfortable. Importantly, no differences were observed between the NMES+40% BFR and NMES+80% BFR groups, with both showing superiority over NMES alone following the six-week, three session a week protocol. The main factor contributing to these findings is that NMES+BFR groups tolerated higher currents during the training sessions compared to the NMES alone condition. This study assists in answering research aims 1-3 of this thesis. When voluntary exercise is not possible due to pain and pathology, or when adherence to exercise is challenging, the utilisation of NMES combined with BFR to promote muscular adaptations is a promising and possible solution. Due to the increased tolerable currents observed, subsequent studies in this thesis will explore the effects of NMES+BFR on pain modulation in healthy and clinical populations.

Chapter 6

Effect of NMES combined with BFR on pressure pain, cold pain and temporal summation of pain in healthy adults

6.1 Abstract

Context: BFR with resistance training and aerobic exercise has observed an analgesia effect that can last up to 24 h post its application. This response has not been investigated after NMES+BFR. **Objective:** The present study investigated the acute effects of NMES combined with varying degrees of BFR on PPT, CPT and TSP **Design:** Randomised crossover. Setting: Physiology laboratory. Participants: A total of 12 healthy adults (age: 28.2 [5.3] years; height: 177.4 [5.0] cm; body mass: 75.3 [8.1] kg). Interventions: five sessions separated by at least 7 days. The first 2 visits served as familiarisation, with the experimental conditions performed in the final three sessions: NMES alone, NMES+40% BFR, and NMES+80% BFR. Main Outcome Measures: PPT, CPT, TSP were recorded pre, immediately post, 20 minutes post and 45 minutes post each condition, rating of perceived exertion, and pain were recorded after every 5 minutes set of the interventions. **Results:** The NMES+80% BFR caused greater PPT increase immediately post compared to NMES alone (p = 0.036, d = 0.8, 95% CI [0.1, 3.9]). No other group differences observed. Pain and ratings of perceived exertion observed no group differences. **Conclusion:** This study showed the NMES+80% BFR acutely increased pain sensitivity as measured by PPT, but this effect had dispersed 20 minutes post the intervention. The acute effect on pain sensitivity observed helps to explain the increased current tolerated during NMES+BFR compared with NMES alone in Chapter 5.

6.2 Introduction

The findings of Chapter 4 indicated that the addition of 40% and 80% BFR to NMES was necessary to induce fatigue, as evidenced by a decrement in force production. Notably, NMES+80% demonstrated higher fatigue compared to NMES alone. The following Chapter (Chapter 5) observed greater muscle strength and size increases after 6-weeks of NMES+BFR compared to NMES alone. Notably, both NMES+BFR groups exhibited greater maximal tolerable currents achieved throughout the training period compared to NMES

alone, without increasing perceptual ratings of pain (Table 5.5; Fig 5.9). This finding is contrasting, due to the wider NMES literature observing increased pain and discomfort with increasing currents[40,126,198,326]. This observation from Chapter 5 warrants further exploration to assist with the understanding of the underlying mechanisms in action and this study will assess the effects of NMES+BFR compared to NMES alone on pain sensitivity and session perceptual ratings of pain and RPE in healthy adults.

A notable limitation of both NMES and BFR interventions is that some individuals find the pain associated with these modalities intolerable[49,126], making it challenging to administer an adequate stimulus[38]. As the intensity of the NMES stimulus is increased, excitation of sensory, motor, and pain fibers occurs[97]. While the excitation of the motor neurons is the fundamental premise behind NMES enhancing muscular strength, they are located near free nerve endings and nociceptive receptors, which results in discomfort, pain, and occasionally a burning sensation[326]. BFR combined with aerobic and resistance exercise has observed improvements in pain sensitivity lasting up to 24 hours after its application[43,178,179,181,302]. Korakakis et al. [182] reported an immediate pain reduction during single-leg squat tasks with large to very large effect sizes (d = 0.56-1.32) after BFR resistance exercise, which was sustained for 45 minutes post-intervention in anterior knee pain patients. Hughes et al. [181] reported lower knee pain in anterior cruciate ligament reconstruction (ACL-R) patients following BFR resistance exercise compared to HI-RE, that lasted 24 hours post. The acute effect of NMES+BFR on pain sensitivity is currently unknown. Pain sensitivity is the degree to which an individual is responsive or susceptible to perceiving and experiencing pain, encompassing the threshold for pain initiation as well as the intensity and duration of the pain sensation, with various factors such as genetic predispositions, psychological aspects, previous pain experiences, and underlying medical conditions contributing to its multifaceted nature[33,167,327–329]. Pain pathways involve the intricate transmission and processing of signals from nociceptors to the brain, contributing to the conscious experience of pain. The endocannabinoid system,

with its CB1 receptors in the central nervous system, plays a role in modulating these pain signals[330]. In the context of BFR exercise, studies suggest potential analgesic effects linked to the release of endogenous opioids and the modulation of endocannabinoids[43,302]. Furthermore, exercise induced hypoalgesia (EIH) is a reduction in pain sensitivity following exercise has also been proposed as a heightened response inducing reductions in pressure pain following BFR exercise similar to high intensity resistance training[331]. However, EIH responses differ from healthy to clinical populations[32,332]. In knee OA, EIH can be blunted or even reversed compared to healthy adults, with exercise increasing pain or causing symptom flare ups rather than reducing it.

The greater NMES currents tolerated in Chapter 5 compared to NMES alone (Fig 5.9) suggests that the BFR stimulus has a dampening effect on the pain induced by NMES. The blunting of NMES induced pain could be via reducing sensitivity to pressure pain, cold pain or the central processing of pain[126,333]. Local pain modulation mechanisms encompass processes such as peripheral sensitisation, where injury or inflammation heightens pain sensitivity, as well as peripheral inhibition, where the release of endogenous opioids and activation of specific nerve fibres dampen pain signals[176,307,329]. Central mechanisms include descending pain modulation pathways, where brain regions like the periaqueductal gray and rostral ventromedial medulla release neurotransmitters to inhibit pain transmission[127,334]. Additionally, the gate control theory suggests that non-painful sensory input can block pain signals at the spinal cord level. The assessment of pressure, thermal and central mechanisms will lead to a greater understanding of the NMES+BFR methodologies used and whether they will be appropriate for knee OA patients, who suffer from higher central and peripheral mechanisms of pain than healthy controls[20,25,34,176]. Improved PPT and CPT have been observed after BFR exercise[331,335]. However, no BFR study to date has assessed TSP, which will help elucidate any central processing analgesic effect after NMES+BFR in the present study.

Therefore, the present study aimed to investigate whether NMES+BFR has any impact on pain sensitivity in healthy adults, prior to being tested in knee OA patients. Specifically, the study aims to investigate the acute impact of NMES+BFR on PPT, CPT and TSP compared to NMES alone. To explore whether the observed effects of NMES+BFR on pain sensitivity are consistent with the previously reported pain-reducing effects of BFR when combined with aerobic and resistance exercise. To assist with answering research aims 1 and 4 of this thesis.

Drawing upon the observations from Chapters 4 and 5 and the existing literature, it was hypothesised that NMES+BFR (40% and 80% AOP) would reduce TSP and increase PPT and CPT pain thresholds acutely after NMES+BFR (40% and 80% AOP) compared with NMES alone.

6.3 Method

6.3.1 Participants

Twelve healthy males (*n* = 6) and females (*n* = 6) (age: 27.7 [4.5] years; height: 177.0 [5.0] cm; body mass: 74.6 [7.5] kg, and body mass index: 23.9 [1.9] kg/m²) volunteered to participate in this study. The sample size was calculated a priori using G*Power software and the effect sizes of previous research assessing the same outcomes[178,182,208]. Inclusion criteria will be: (a) absence of lower-limb injury, (b) negative answers in the PAR-Q questionnaire (Appendix 5.3), (c) no personal history of cardiovascular or metabolic disease, (d) non-smokers, (e) resting SBP < 140 mmHg and (f) normal range on the ABI test (0.9-1.4)[194]. The study was approved by the University ethics sub-committee at Level 2 (Appendix 3) and in accordance with the Declaration of Helsinki (2013).

6.3.2 Study design

This study followed a randomised crossover design, generated via online software (http://www.randomization.com). All testing was undertaken at the University. Participants were required to visit the University on five occasions, separated by at least 7 days and at the same time of day (±1 h), to minimise the circadian effect. After the two familiarisation sessions, participants were randomly allocated to perform the experimental conditions, with the same trained researcher performing all outcome measurements:

1) NMES alone
2) NMES+40% BFR
3) NMES+80% BFR

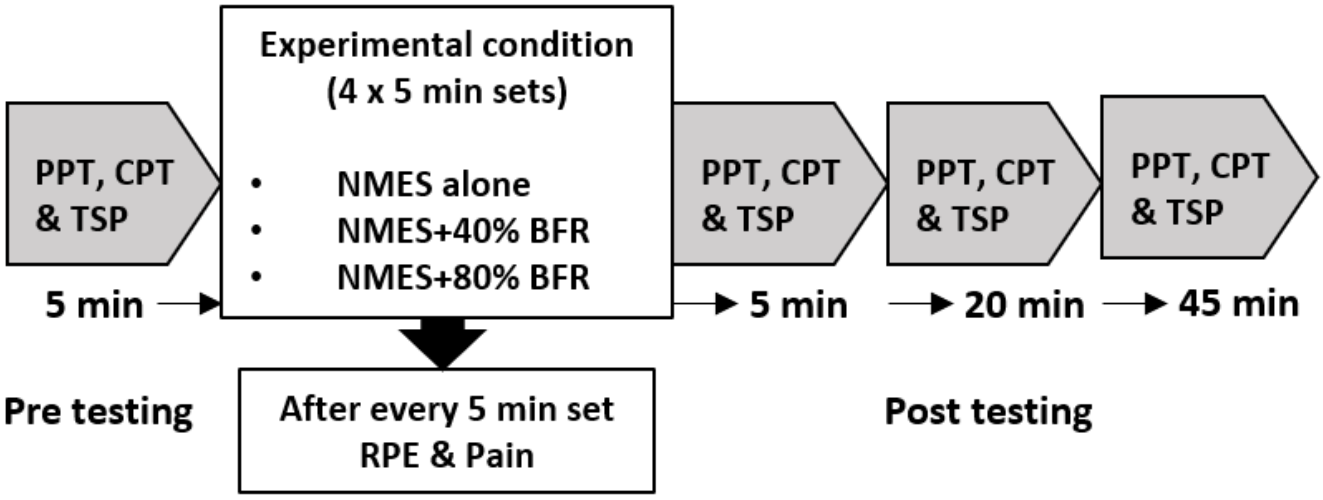

Fig 6.1. Experimental protocol. All participants performed the same NMES protocol under three different BFR pressures (0%, 40% and 80%). Outcome measures; PPT, CPT and TSP were assessed before (pre) and immediately post, 20 minutes and 45 minutes post each experimental condition. Outcome measures assessed after every 5 minutes included; RPE and pain.

6.4 Procedures

6.4.1 ABI

See section 3.3.

6.4.2 NMES

See section 3.4.

6.4.3 Determination of BFR pressure

See section 3.5.

6.4.4 PPT

See section 3.19.

6.4.5 CPT

See section 3.20.

6.4.6 TSP

See section 3.21.

6.4.7 RPE

See section 3.14.

6.4.7 Pain

See section 3.15.

6.5 Statistical Analysis

A two-way repeated-measures ANOVA was used to determine the effects of condition (0%, 40% and 80% BFR) and time; PPT, CPT, TSP across four time points (pre, immediately post, 20 minutes post and 45 minutes post), RPE, Pain across four time points (set 1, set 2, set 3, set 4). If the assumptions of ANOVA were violated, the Greenhouse–Geisser

correction factor was applied. Interactions and main effects were followed with appropriate *post-hoc* analyses and Bonferroni adjustments.

Statistical significance was set at $p < 0.05$. Cohens d = 0.2, 0.5 and 0.8 are reported for pairwise comparisons and correspond to small, medium and large effects, respectively.

Statistics were computed using SPSS Statistics software package version 28.0 (SPSS, Chicago, USA). Data are presented as means (SD) unless otherwise stated.

6.6 Results

No adverse events occurred.

6.6.1 PPT

There was a main effect of time ($F_{(2.3,75.6)} = 26.3$, $p < 0.001$) and group x time interaction for PPT on the dominant leg [$F_{(4.6,75.6)} = 6.5$, $p < 0.001$, $\eta^2 = 0.28$]. Post-hoc tests showed greater PPT increase immediately post NMES+80% BFR compared with NMES alone ($p = 0.036$, d = 0.8, 95% CI [0.1, 3.9]). No effect on the non-dominant leg was observed and non-dominant leg [$F_{(4.7,77.6)} = 0.5$, $p = 0.61$, $\eta^2 = 0.05$] (Table 6.1).

Table 6.1. PPT (kgf/cm^2), pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Dominant	Non dominant	Dominant	Non dominant	Dominant	Non dominant
Pre	5.6 (1.7)	5.5 (1.6)	5.7 (1.6)	5.5 (1.6)	5.6 (1.5)	5.4 (1.3)
Post	5.7 (1.8)	5.6 (1.5)	6.7 (1.9)*	5.4 (1.5)	7.7 (1.8)*#	5.2 (1.4)
Post 20 minutes	5.5 (1.7)	5.3 (1.6)	5.9 (1.8)~	5.2 (1.4)	6.0 (2.2)~	5.1 (1.3)
Post 45 minutes	5.4 (1.8)	5.5 (1.8)	5.7 (1.6)~	5.2 (1.4)	5.8 (2.0)~	5.0 (1.4)

Significant difference was set at $p < 0.05$; * = significant increase compared to pre time point; # = significantly greater group effect compared to NMES alone; ~ = significant decrease compared to post time point.

6.6.2 CPT

There was no main group x time interaction for CPT on the dominant [$F_{(5,82.4)} = 0.6$, $p = 0.67$, $\eta^2 = 0.04$] and non-dominant leg [$F_{(4.7,77.6)} = 0.6$, $p = 0.80$, $\eta^2 = 0.03$] (Table 6.2).

Table 6.2. CPT, pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Dominant	Non dominant	Dominant	Non dominant	Dominant	Non dominant
Pre	1 (0.9)	0.9 (0.9)	1.1 (1.1)	1.1 (1.2)	1.1 (1.1)	1.1 (1.3)
Post	0.8 (0.9)	0.8 (0.9)	0.8 (0.8)	0.9 (1)	0.7 (0.8)	1 (1.1)
Post 20 minutes	0.8 (0.9)	0.8 (0.9)	0.8 (0.9)	0.8 (0.9)	0.9 (0.9)	0.9 (1.1)
Post 45 minutes	0.9 (0.9)	0.8 (0.9)	0.8 (0.9)	0.8 (1)	0.9 (1.1)	1 (1.2)

Significant difference was set at $p < 0.05$.

6.6.3 TSP

There was no main group x time interaction for TSP [$F_{(3.2,52)} = 0.3$, $p = 0.83$, $\eta^2 = 0.02$] (Table 6.3).

Table 6.3. TSP, pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Mean	SD	Mean	SD	Mean	SD
Pre	1.67	(0.98)	1.79	(0.99)	1.83	(1.53)
Post	1.67	(1.83)	1.58	(1.24)	1.42	(1.31)
Post 20 minutes	1.50	(1.57)	1.42	(1.24)	1.50	(1.51)
Post 45 minutes	1.46	(1.59)	1.46	(1.34)	1.50	(1.62)

Significant difference was set at $p < 0.05$.

6.6.4 Pain

There was no main group x time interaction for session Pain [$F_{(3.7,60.4)} = 0.1$, $p = 0.97$, $\eta^2 = 0.06$] (Table 6.4).

6.6.5 RPE

There was no main group x time interaction for session RPE [$F_{(3.2,52.2)} = 0.2$, $p = 0.88$, $\eta^2 = 0.01$] (Table 6.4).

Table 6.4. Pain and RPE values after set 1, 2, 3 and 4 mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Pain	RPE	Pain	RPE	Pain	RPE
Set 1	4.3 (1.3)	12.6 (2)	4.1 (0.8)	13.1 (1.7)	5.5 (1.3)	14.5 (1.1)
Set 2	4.2 (0.9)	12.4 (2.1)	4.2 (1)	13.0 (1.8)	5.5 (1.2)	14.6 (1.6)
Set 3	4.0 (0.9)	12.2 (2.2)	3.9 (0.8)	12.8 (2)	5.4 (0.8)	14.5 (1.5)
Set 4	3.5 (0.5)	11.8 (2.2)	3.6 (0.9)	12.5 (1.8)	5.0 (1.1)	13.7 (1.8)

Significant difference was set at $p < 0.05$.

6.7 Discussion

The main finding of this study is that adding 80% BFR to NMES results in an immediate increase in PPT compared to NMES alone (Table 6.1; Fig 6.2). However, it does not affect CPT and TSP. The PPT effect was not observed at the 20 minutes post-intervention time point (Table 6.1). No effect on the contralateral thigh was observed after all conditions or measures (Table 6.1-6.3).

Our findings align with previous research demonstrating that BFR during resistance exercise and aerobic cycling reduces pain sensitivity (i.e., increased PPT) immediately after training compared to unrestricted free flow training[59,297]. However, in contrast to the 24 h duration observed after BFR aerobic exercise[302], BFR resistance exercise in ACL-R patients[181] and 45 minutes in anterior knee pain patients[182], the observed effect in the present study did not last up to 20 minutes post-intervention. Additionally, no effect on PPT was observed in the contralateral quadricep, suggesting that spinal level inhibitory mechanisms were not involved in the present findings[167]. The observed absence of an effect on TSP further aligns with the discernment that NMES coupled with BFR yields no discernible systemic or central nervous system pain-modulating effects in the context of healthy adults. These findings strongly suggest that NMES combined with an 80% BFR stimulus imparts a localised and transient impact on PPT within the specifically targeted

quadricep muscle. However, the apparent lack of influence on spinal level inhibitory mechanisms prompts considerations regarding its potential clinical efficacy, particularly in populations afflicted with knee OA, characterised by heightened central sensitisation relative to pain-free controls[25]. Further exploration of these implications is undertaken in Chapter 7. Previous BFR research assessing PPT locally and distally has found a distal effect and reductions in PPT in the contralateral limb[336]. Moreover, previous research in healthy adults has shown that isometric exercise produces the largest effect on reducing pain sensitivity compared to dynamic movements and the effect sizes reported are much greater than those observed in our study[173]. One key difference is the use of involuntary isometric contractions elicited by NMES in the present study, compared to voluntary actions. This disparity in contraction modes may contribute to the different findings observed in our study compared to both BFR exercise and isometric contractions on PPT. NMES actions produce involuntary contractions that bypass the central nervous system and activate muscles directly through the recruitment of both type I and type II muscle fibres with minimal current[80]. On the other hand, voluntary actions recruit muscles according to the size principle, with type I fibres recruited at low intensities and type II fibres recruited as the intensity of exercise increases[76]. Previous NMES research has also observed metabolic differences between voluntary and NMES contractions[326], suggesting that differences in contraction physiology could explain the contrasting effects observed in the present study, which warrant further exploration.

NMES applied using moderate and maximum tolerable currents in healthy adult's has observed acute increases in PPT[337]. They utilised 10 s contractions compared to the 5 s contractions used in this study, and the electrode placement was different, with electrodes placed just below the inguinal crease compared to 13.4 cm lower as in the present study due to the width of the BFR cuff. These methodological differences could have contributed to the contrasting findings[337].

Pain inhibition is complex and multifactorial, involving mechanisms including; EIH, long term depression of nociceptive synaptic connections and descending inhibition via mechanisms involved in diffuse noxious inhibitory control and conditioned pain modulation[332,334,337]. The magnitude of EIH depends on various factors, including exercise parameters such as type, dose, duration and intensity, with EIH responses increasing as the exercise intensity increases[33,332].

Regarding muscle pain during each set of the interventions, the NMES+80% BFR condition was more painful by 1.3-1.5 on a scale of 0-10 during each set compared to NMES alone and NMES+40% BFR (Table 6.4) and lower than those observed in Chapter 4. Similar findings were observed by Natsume et al. [46] who utilised a similar protocol as this study and reported higher pain and RPE values compared to NMES alone. However, 5 on the 0-10 NPRS represents moderate pain only[219], with no participants withdrawing from this study due to the discomfort experienced, indicating this observation warrants little concern in healthy adults. This finding is also similar to previous BFR research, which reported higher muscle discomfort after cycling with 80% BFR compared to 40% BFR[302]. RPE values for resistance exercise combined with 40% BFR ranged from 15 to 17, while for 80% BFR it was 18 to 19[43]. The results observed in the present study are similar or lower, which may also contribute to explaining the reduced effect observed. The phenomenon known as diffuse noxious inhibitory control, or the “pain inhibits pain” effect, is part of the descending endogenous analgesia system. Experimental studies in healthy adults have demonstrated a decrease in pain intensity after a noxious conditioning stimulus when stimulated at adjacent and remote sites[178,337]. The decreased perceptual ratings in the present study may help explain the reduced and shorter-lasting findings compared to previous BFR research.

Furthermore, previous research has suggested that exercise intensity and duration affect the magnitude of EIH response[173]. During heavy load lifting or when the muscle is fatigued, high-threshold motor units are typically recruited in addition to low-threshold motor

units to prevent force failure based on the size principle[76,338]. Both high-load resistance exercise and low-load resistance exercise performed to volitional failure result in similar magnitudes of hypoalgesia, which are greater compared to low-load resistance exercise performed to failure[187,302]. This suggests that the recruitment of high-threshold motor units may be one of the underlying mechanisms of EIH. The addition of 40-80% BFR to NMES causes greater neuromuscular fatigue than NMES alone (Chapter 4) and promotes increased muscle strength and size (Chapter 5), indicating recruitment of high threshold motor units. Activation of a nociceptive descending inhibitory system has been proposed as an explanation of how pain perception is altered by the recruitment of high-threshold motor units[303]. Furthermore, increased motor cortex activity has been observed after the recruitment of high-threshold motor units due to increased force production, and this has been shown to induce analgesia in humans[304]. The increased fatigue seen in Chapter 4, could have led to the increased PPT immediately after NMES+80% BFR observed in these findings. However, future research should elucidate other physiological mechanism that could have contributed to the observed results.

The previously observed hypoalgesia responses after BFR exercise have been partially explained by endogenous opioid and endocannabinoid system pain modulation mechanisms[59,297,298]. The endogenous opioid system consists of naturally occurring peptides that bind to opioid receptors in the central nervous system[299]. These receptors are involved in pain perception and activation of the opioid system can lead to a reduction in pain the sensation[167]. The endocannabinoid system consists of endogenous cannabinoids such as anandamide and 2-arachidonoylglycerol, along with their receptors CB1 and CB2. The endocannabinoid and endogenous opioid systems can interact with each other and with other neurotransmitter systems in the body to modulate pain perception[299]. The acute improvement in PPT after NMES+80% BFR is comparable to, but lower in magnitude and shorter in duration than, the improvements seen following BFR resistance and aerobic exercise[43,183,302]. These findings suggest that while NMES+80% BFR can enhance PPT, the effects are less pronounced and do not persist as

long as those achieved through BFR exercise modalities. One plausible explanation for the observed improvements in PPT with NMES+80% BFR is the activation of the endogenous opioid and endocannabinoid systems, which are known to play significant roles in pain modulation and have been implicated in the analgesic effects associated with various forms of exercise[307]. The endogenous opioid system involves the release of opioid peptides, such as endorphins, enkephalins, and dynorphins, which bind to opioid receptors in the nervous system to produce analgesic effects. Similarly, the endocannabinoid system, which includes endocannabinoids like anandamide and 2-arachidonoylglycerol (2-AG), interacts with cannabinoid receptors to modulate pain and inflammation[307]. BFR exercise has been shown to elevate PPT and levels of these endocannabinoids after aerobic and resistance exercise[302,336]. However, since blood tests were not conducted in the present study, it is not possible to conclusively determine the involvement of these endogenous systems in the pain modulation observed with NMES+80% BFR. Future research should include biochemical analyses to measure levels of endogenous opioids and endocannabinoids pre- and post-intervention to clarify their role in mediating the analgesic effects of NMES+80% BFR.

No effect on TSP was observed in the present study. Factors that have been found to affect TSP include: stimulus intensity, frequency and duration, psychological factors such as fear, anxiety and attentional focus, and previous pain experiences, such as individuals suffering from chronic pain, can heighten TSP[251,252]. To the authors knowledge, this study is the first to assess TSP after a BFR intervention. TSP involves central nervous system mechanisms including, peripheral sensitisation, enhanced synaptic transmission, wind-up phenomenon and central sensitisation[334]. The present study involved healthy subjects, which may explain the lack of effect on TSP observed, as the TSP ratings were relatively low compared to clinical populations[219,305].

Furthermore, no effect was observed on cold pain thresholds. A similar finding was reported after an ischemic pre-conditioning stimulus (100% BFR), which found no effect on cold pain

sensitivity[306]. This result could also be attributed to the relatively low CPT scores observed during pre-testing in the present study[307] (Table 6.2).

6.8 Conclusion

In conclusion, the present study demonstrated that the addition of 80% BFR to NMES resulted in a greater acute increase in PPT immediately after the intervention compared with NMES alone (Table 6.1). However, this effect was not sustained at the 20 minutes post-intervention assessment and did not affect the contralateral limb. No group differences were observed in CPT and TSP. These findings partially align with previous research demonstrating that BFR during resistance exercise and aerobic cycling reduces pain sensitivity immediately after training compared to free flow training[43,302]. Nevertheless, the reduced and shorter-lasting effect observed in the present study could be attributed to the use of involuntary NMES contractions instead of voluntary actions and methodological variations to previous NMES research. The underlying mechanisms responsible for the observed analgesic effect remain unclear, however, the greater PPT in the present study may be attributed to increased neuromuscular fatigue and subsequent recruitment of high threshold motor units to prevent force failure[188,190,255,297,298,301]. Notably, no differences in session RPE and pain were observed, and there was no effect on TSP or CPT. The perceptual ratings in this study were lower than Chapter 4 and previous BFR research, which may have contributed to the reduced effect on PPT. Overall, our findings suggest that the addition of BFR to NMES may have potential as an acute pain management strategy and help to explain the greater NMES currents tolerated during NMES+BFR in Chapter 5. Additionally, the lack of effects of TSP and CPT may be attributed to the relatively low baseline scores and the healthy population studied.

The final experimental Chapter investigated if NMES+BFR promotes an analgesic effect in knee OA patients.

Chapter 7

Effect of NMES combined with BFR on pressure pain, cold pain, temporal summation of pain and function in knee OA patients

7.1. Abstract

Context: Acute reductions in pain sensitivity have been observed after BFR exercise and NMES+BFR in Chapter 6 of this thesis. However, no effect lasted 20 minutes and it was conducted using healthy adults. Knee OA patients report increased pressure, thermal and conditioned pain responses to healthy adults. **Objective:** The present study investigated the acute effects of NMES combined with varying degrees of BFR on PPT, CPT, TSP, 5-repetition sit-to-stand, and knee bends in 30 seconds in knee OA patients. **Design:** Randomised crossover. Setting: Private physiotherapy clinic, London. Participants: A total of 12 knee OA patients (age: 68.8 [5.8] years; height: 174.0 [8.1] cm; body mass: 80.2 [9.4] kg, and body mass index: 26.6 [3.4] kg/m²). Interventions: five sessions separated by at least 7-days. The first 2 visits served as familiarisation, with the experimental conditions performed in the final three sessions: NMES alone, NMES+40% BFR, and NMES+80% BFR. **Main Outcome Measures:** PPT, CPT, TSP, 5-repetition sit-to-stand and knee bends in 30 seconds were recorded pre, immediately post, 20 minutes post and 45 minutes post each condition, RPE, and pain were recorded after every 5 minutes set of the interventions. **Results:** The NMES+80% BFR caused greater 5-repetition sit-to-stand improvement at 45 minutes post compared to NMES alone (p = 0.04, d = 0.76, 95% CI [0.09, 4.71]). NMES+80% BFR also caused acute improvements in pain during the 5STS, PPT and TSP ($p < 0.05$). However, no between group differences were observed. Session pain and RPE were higher after NMES+80% BFR compared to both other groups ($p > 0.05$). **Conclusion:** This study showed that NMES+80% BFR acutely improved 5-repetition sit-to-stand function 45 minutes post its application, with reduced pain and also improved PPT and TSP. The findings present an acute pain modulating effect after NMES+80% BFR in knee OA patients.

7.2 Introduction

Chapter 6 within this thesis demonstrated that the addition of 80% BFR to NMES led to a transient reduction in pain sensitivity, as measured by PPT compared to NMES alone in healthy adults (Chapter 6). However, this effect did not last 20 minutes post the intervention and no effects on CPT or TSP were observed (Chapter 6). It is important to note that this previous study was conducted on healthy adults without any injury or pathology. This current study aims to extend these findings to a clinical population experiencing pain. Knee OA is a degenerative joint disease that affects the articular cartilage, subchondral bone and synovium of the knee joint. It is characterised by inflammation, pain, stiffness and a loss of joint function[308]. The early signs and symptoms of knee OA may include mild pain and stiffness, particularly after periods of inactivity or overuse. As the condition progresses, the pain can increase in severity and become more persistent, and may be accompanied by swelling and crepitus and associated loss of knee range of motion[308]. Because OA is considered to be a mechanically driven disease, altered joint loads are likely a requirement for its development and progression. Functional limitations associated with knee OA include muscle weakness and atrophy, particularly of the quadriceps, which play a key role in stabilising the knee joint[23,308]. These limitations can lead to individuals being unable to perform activities that require bending, lifting and supporting their body weight. However, due to the clinical signs and symptoms traditional resistance exercise to help restore these functional limitations can commonly be too painful to perform and exacerbating this condition[308]. Therefore, interventions that promote muscular adaptations with reduced mechanical stress i.e., NMES and BFR have been investigated in knee OA patients[309–311].

In knee OA, alterations in pain sensitivity compared to healthy controls are intricately linked to physiological mechanisms[20,25,32]. The degeneration of joint tissues induces peripheral sensitisation, marked by the release of inflammatory mediators and heightened responsiveness of nociceptors. Knee OA also involves central sensitisation, where the

central nervous system undergoes changes amplifying pain perception. Dysfunctions in temporal summation and diffuse noxious inhibitory control contribute to an increased sensitivity to pain stimuli[20,25,32]. Moreover, pain in knee OA influences quadriceps function, creating a reciprocal relationship. Quadriceps dysfunction, often attributed to pain-related inhibition and altered neuromuscular control, can exacerbate the pain experience and contribute to the functional limitations observed in knee OA[15].

Exercise-induced hypoalgesia (EIH), the reduction of pain following exercise, manifests differently in individuals with knee OA compared to healthy controls, largely influenced by distinct physiological mechanisms[32,348]. Knee OA is characterised by heightened baseline pain sensitivity as detailed above. In contrast to healthy individuals, the magnitude of the EIH response in knee OA may cause no reduction in pain sensitivity or increased pain perception and symptom flare ups, potentially attributable to the persistent nociceptive input and altered pain processing associated with the condition[32]. The mechanisms underlying EIH in healthy controls, involving the activation of endogenous pain-inhibitory systems such as the release of endorphins and engagement of descending inhibitory pathways, may be compromised or less efficient in knee OA[32,307]. Moreover, the chronic nature of knee OA pain, rooted in structural joint changes and ongoing inflammation, may limit the duration of EIH effects in individuals with the condition. Therefore, testing NMES+BFR on pain modulation in knee OA patients may produce different results than observed in Chapter 6 on healthy adults and warrants investigation.

The current literature suggests that there may be a hypoalgesia effect after BFR exercise[178–181]. Blood flow restriction training has been shown to reduce pain across a range of training programs and in a variety of clinical conditions including knee pain and OA[178–181]. Interestingly, the reduction in pain with low load BFR appears to be greater than high load resistance exercise (HL-RE). In contrast, a network meta-analysis on electrical stimulation therapies concluded that interferential

current compared with NMES, is the most promising pain relief treatment for individuals with knee OA. However, the findings suggest that among electrical stimulation therapies, interferential current stands out as the most promising option for alleviating pain in knee OA[309]. In contrast to NMES, interferential electrical stimulation does not enhance muscular strength. Therefore, its effectiveness on assisting knee OA with their functional limitations is limited[148,349]. Due to the previously observed acute improvements in PPT in healthy controls along with enhanced muscular strength and size seen after NMES+80% BFR (Chapter 5 and 6), which could assist with the previously mentioned functional declines in knee OA, this study investigated the effect of NMES+BFR on pain and function in knee OA patients.

Due to Chapter 6 observing greater reductions in pain sensitivity with the addition of 80% BFR to NMES in a healthy population, the aim of the present study is to assess if these findings also extend to a clinical population experiencing pain. An acute pre to post change in PPT was also observed after NMES+40% BFR and therefore was not discounted from further investigation. Sit-to-stand and knee bending functional activities are reduced and reported to be painful in knee OA patients[20]. Korakakis et al. [178] observed reduced pain and improved performance during a single leg squatting task after a single session of BFR exercise in anterior knee patients. Therefore, functional measures were added to the previously used pain outcome measures in Chapter 6 to be investigated in knee OA patients. The purpose of this study is to determine if NMES+BFR has any acute effect on TSP, PPT, CPT and function in knee OA patients compared to NMES alone. Additionally, the study aims to investigate if these acute improvements in pain thresholds, if observed, translate to enhanced physical function acutely following NMES+BFR compared to NMES alone to assist in answering research aim 4 and 5.

The aim of the present study was to determine if NMES+BFR has any acute lasting up to 45 minutes post its application on PPT, CPT, TSP in knee OA patients. Secondary aims include exploring whether any observed acute improvements in pain thresholds translate into enhanced physical function immediately following NMES+BFR (40% and 80% AOP) compared to NMES alone, addressing research aims 4 and 5 of this thesis. Additionally, assessing the safety of NMES+BFR in knee OA patients, a population associated with older age and potential cardiovascular comorbidities[29,34], by re-evaluating cardiovascular measures previously examined in Chapter 4.

It was hypothesised that knee OA patients subjected to NMES+BFR (40% and 80% AOP) will exhibit acutely increased PPT compared to NMES alone. Physical function assessed immediately after NMES+BFR will improve compared to NMES alone. No significant changes in CPT or TSP are expected between NMES+BFR and NMES alone. Finally, the acute cardiovascular measures assessed in Chapter 4, will demonstrate the safety of NMES+BFR in knee OA patients.

7.3 Method

7.3.1 Participants

Twelve knee OA patients were recruited and volunteered for this study; males (*n* = 8) and females (*n* = 4) (age: 68.8 [5.8] years; height: 174.1 [8.1] cm; body mass: 80.2 [9.4] kg, and body mass index: 26.6 [3.4] kg/m^2). The sample size was calculated using G*Power software and the effect sizes of previous research assessing the same outcomes[178,182,208]. Inclusion criteria were: (a) diagnosis of knee osteoarthritis from GP / consultant following the NICE guidelines and X ray imaging[350], (b) one sided symptomatic knee OA, (c) no personal history of cardiovascular or metabolic disease, (d) non-smokers, (e) resting SBP < 140 mmHg and (f) normal range on the ABI test (0.9-

1.4)[194]. The study was approved by the University ethics subcommittee (Appendix 4: SMU_ETHICS_2021-22-329) in accordance with the Declaration of Helsinki (2013).

7.3.2 Study design

The study followed a randomised crossover design, generated via online software (http://www.randomization.com). All testing was undertaken at a private Physiotherapy Clinic in London. Participants were required to visit the clinic location on five occasions, separated by at least 7 days and at the same time of day (±1 h), to minimise the circadian effect. After two familiarisation sessions, participants were randomly allocated to perform the experimental conditions, with the same trained researcher performing all outcome measurements:

1) NMES alone
2) NMES+40% BFR
3) NMES+80% BFR

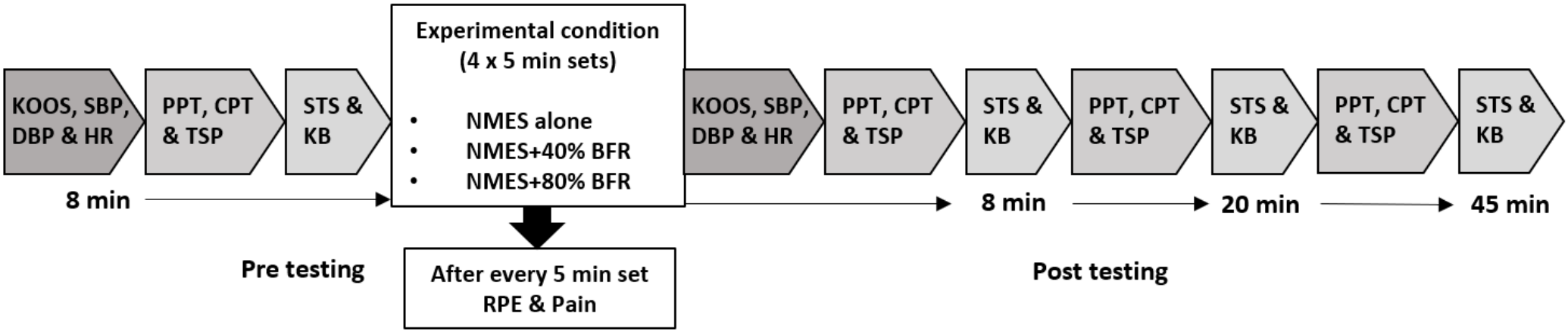

Fig 7.1. Experimental protocol. All participants performed the same NMES protocol under three different BFR pressures (0%, 40% and 80%) Outcome measures; KOOS, SBP, DBP, HR, PPT, CPT, TSP, 5-repetition sit-to-stand, knee bends in 30 seconds were assessed before (pre) and immediately post, 20 minutes and 45 minutes post each experimental condition. Outcome measures assessed after every 5 minutes included; RPE and pain.

7.4 Procedures

7.4.1 ABI

See section 3.3.

7.4.2 NMES

See section 3.4.

7.4.3 Determination of BFR pressure

See section 3.5.

7.4.4 PPT

See section 3.16.

7.4.5 CPT

See section 3.17.

7.4.6 TSP

See section 3.18.

7.4.7 5-repetition sit-to-stand

See section 3.19.

7.4.8 Knee bends in 30 seconds

See section 3.20.

7.4.9 KOOS questionnaire

See section 3.21.

7.5 Statistical Analysis

A two-way repeated-measures ANOVA was used to determine the effects of condition (0%, 40% and 80% BFR) and time; PPT, CPT, TSP, 5-repetition sit-to-stand, and knee bends in 30 s across four time points (pre, immediately post, 20 minutes post and 45 minutes post), Session pain and RPE across four time points (set 1, 2, 3 and 4) and KOOS, SBP, DBP, HR across two time points (pre, immediately post). If the assumptions of ANOVA were violated, the Greenhouse–Geisser correction factor was applied. Interactions and main effects were followed with appropriate *post-hoc* analyses and Bonferroni adjustments.

Statistical significance was set at $p < 0.05$. Cohens d = 0.2, 0.5 and 0.8 are reported for pairwise comparisons and correspond to small, medium and large effects, respectively.

Statistics were computed using SPSS Statistics software package version 28.0 (SPSS, Chicago, USA). Data are presented as means (SD) unless otherwise stated.

7.6 Results

No differences were observed between baseline values across the three experimental conditions ($p > 0.05$) and no adverse events occurred.

7.6.1 PPT

There was a main group x time interaction for PPT on the symptomatic leg [$F_{(5,78.5)} = 5.0$, $p < 0.001$, $\eta^2 = 0.23$]. However, Post-hoc analysis could not identify any significant differences between groups at any time point ($p > 0.14$). No effect was observed on the non-symptomatic quadricep ($p < 0.05$) (Table 8.1).

There was no main group x time interaction for PPT on the non-symptomatic leg [$F_{(6,99)} = 0.8$, $p = 0.54$, $\eta^2 = 0.05$] (Table 8.1).

Table 7.1. PPT (kgf/cm^2), pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	OA	Non-OA	OA	Non-OA	OA	Non-OA
Pre	4.4 (2.0)	4.3 (2.0)	4.3 (1.7)	4.3 (2.0)	4.3 (2.0)	4.4 (2.1)
Post	4.4 (1.9)	4.4 (2.0)	5.1 (2.3)*	4.3 (2.0)	5.4 (2.3)*	4.2 (2.0)
Post 20 minutes	4.5 (1.7)	4.7 (2.1)~	5.0 (2.3)*	4.4 (2.0)	5.8 (1.5)*	4.7 (1.4)
Post 45 minutes	4.3 (2.0)	4.8 (2.4)	4.7 (2.0)	4.4 (2.0)	5.9 (1.5)*	4.7 (1.5)

Significant difference was set at $p < 0.05$; * = significant increase compared to pre time point; ~ = significant increase compared to post time point.

7.6.2 CPT

There was no main group x time interaction for CPT on the symptomatic leg [$F_{(4.5,74.2)} = 1.7$, $p = 0.16$, $\eta^2 = 0.09$] and non-symptomatic leg [$F_{(4.6,68.0)} = 0.9$, $p = 0.49$, $\eta^2 = 0.03$] (Table 8.2).

Table 7.2. CPT, pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	OA	Non-OA	OA	Non-OA	OA	Non-OA
Pre	2.6 (1.1)	2.3 (1.0)	2.2 (1.1)	1.9 (1.1)	2.1 (1.4)	2.0 (1.2)
Post	2.5 (1.3)	2.3 (1.2)	2.1 (1.4)	2.0 (1.3)	2.0 (1.4)	2.0 (1.2)
Post 20 minutes	2.2 (1.4)	1.9 (1.3)	2.2 (1.5)	1.9 (1.3)	1.8 (1.6)	1.5 (1.5)
Post 45 minutes	2.5 (1.2)	2.4 (1.0)	2.1 (1.1)	2.0 (1.2)	1.8 (1.4)	1.6 (1.4)

Significant difference was set at $p < 0.05$.

7.6.3 TSP

There was no main group x time interaction for TSP [$F_{(4.4,72.7)}$= 0.5, p = 0.75, η^2 = 0.03] (Table 8.3).

Table 7.3. TSP, pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values on the OA limb, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Mean	SD	Mean	SD	Mean	SD
Pre	4.00	(1.48)	3.50	(1.45)	3.75	(1.14)
Post	3.58	(1.98)	3.42	(1.51)	3.17	(1.47)
Post 20 minutes	2.75	(1.96)	2.17	(1.11)	2.42	(0.79)
Post 45 minutes	3.04	(1.76)	2.04	(1.84)	1.88	(1.17)*

Significant difference was set at $p < 0.05$; * = significant decrease compared to pre time point.

7.6.4 5-repetition sit-to-stand

There was a main group x time interaction for 5-repetition sit-to-stand time [$F_{(4.2,69.4)}$ = 4.2, p = 0.03, η^2 = 0.20]. Post-hoc analysis showed reduced time to perform 5-repetition sit-to-stand following NMES+80% BFR compared to NMES alone at the 45 minutes post time-point (p = 0.04, d = 0.76, 95% CI [0.09, 4.71]). No significant differences were observed between NMES+40% BFR and NMES+80% BFR.

There was a main group x time interaction for pain experienced during the 5-repetition sit-to-stand [$F_{(3.1,50.7)}$ = 4.0, p = 0.01, η^2 = 0.19] (Table 8.4). However, Post-hoc analysis could not identify any significant differences between groups at any time point (p > 0.29) (Table 7.4).

Table 7.4. 5 sit-to-stand time (s) and pain (0-10), pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		NMES+40% BFR (n=12)		NMES+80% BFR (n=12)	
	Time	Pain	Time	Pain	Time	Pain
Pre	14.5 (2.2)	4.5 (1.8)	14.1 (2.6)	4.6 (1.9)	14.1 (2.6)	4.8 (1.9)
Post	14.0 (2.4)	4.3 (2.1)	12.6 (2.4)*	3.8 (2.0)*	12.7 (2.4)*	3.8 (2.1)*
Post 20 minutes	14.1 (2.1)	4.5 (1.5)	12.3 (2.4)*	4.0 (2.1)	12.0 (1.9)*	3.3 (2.0)*
Post 45 minutes	14.0 (2.3)	4.4 (1.5)	12.2 (2.4)*	3.8 (2.0)	11.6 (2.1)*~#	3.2 (1.9)*

Significant difference was set at $p < 0.05$. * = significant decrease compared to pre time point; ~ = significant decrease compared to pre time point. # = significant group effect compared to NMES alone.

7.6.5 Knee bends in 30 seconds

There was no main group x time interaction for knee bends performed in 30 s [$F_{(3.3,54.2)} = 1.8$, $p = 0.15$, $\eta^2 = 0.10$]. Additionally, there was no main group x time interaction for pain experienced during knee bends performed in 30 s [$F_{(2.5,41.3)} = 1.6$, $p = 0.21$, $\eta^2 = 0.09$] (Table 8.5).

Table 7.5. Knee bends on OA limb; amount and pain (0-10), pre-test, post-test, 20 minutes post-test and 45 minutes post-test measurement values, mean (SD).

	NMES alone (n=12)		NMES+40% BFR (n=12)		NMES+80% BFR (n=12)	
	Amount	Pain	Amount	Pain	Amount	Pain
Pre	18.6 (4.2)	3.7 (2.0)	19.6 (4.9)	3.6 (2.1)	19.3 (5.0)	3.7 (2.0)
Post	19.2 (4.4)	3.5 (2.1)	20.8 (4.7)	3.2 (1.8)	20.5 (5.3)	3.3 (2.0)
Post 20 minutes	19.3 (4.6)	3.6 (1.9)	21.3 (4.8)	2.2 (1.6)	21.4 (4.7)*	2.3 (1.5)
Post 45 minutes	18.9 (4.5)	3.6 (1.6)	22.2 (4.8)*	2.3 (1.6)	22.0 (4.9)*	2.3 (1.6)

Significant difference was set at $p < 0.05$. * = significant increase compared to pre time point.

7.6.6 KOOS

There was no main group x time interaction for session Pain [$F_{(2,33)} = 0.1$, $p = 0.88$, $\eta^2 = 0.08$].

7.6.7 SBP

There was no main group x time interaction for session RPE [$F_{(2,33)} = 1.4$, $p = 0.26$, $\eta^2 = 0.08$].

7.6.8 DBP

There was no main group x time interaction for session DBP [$F_{(2,33)} = 0.6$, $p = 0.58$, $\eta^2 = 0.03$].

7.6.9 Pain

There was no main group x time interaction for session Pain [$F_{(2.7,44.4)} = 0.3$, $p = 0.82$, $\eta^2 = 0.02$]. (Table 7.6).

7.6.10 RPE

There was no main group x time interaction for session RPE [$F_{(3.5,58.5)} = 1.7$, $p = 0.16$, $\eta^2 = 0.10$] (Table 7.6).

Table 7.6. Pain and RPE values after set 1, 2, 3 and 4, mean (SD).

	NMES alone (n=12)		**NMES+40% BFR (n=12)**		**NMES+80% BFR (n=12)**	
	Pain	RPE	Pain	RPE	Pain	RPE
Set 1	4.9 (0.8)	13.0 (1.1)	5.2 (1.5)	13.6 (1.7)	6.3 (1.0)~#	14.2 (1.6)
Set 2	4.9 (0.8)	12.9 (1.2)	5.0 (1.0)	13.5 (1.8)	6.3 (0.9)~#	14.5 (1.7)
Set 3	4.8 (0.7)	12.8 (1.1)	5.0 (1.0)	13.5 (1.8)	6.3 (0.9)~#	14.7 (1.6)#
Set 4	4.8 (0.9)	12.3 (1.5)	4.8 (1.0)	13.3 (1.4)	6.3 (0.9)~#	14.6 (1.4)#

Significant difference was set at $p < 0.05$. ~ = significant group effect compared to NMES+40% BFR. # = significant group effect compared to NMES alone.

7.7 Discussion

The purpose of this study was to investigate the acute effects of different BFR pressures combined with NMES and NMES alone on pain thresholds and function in knee OA patients. The main findings demonstrated that 4 x 5 minutes sets of NMES+80% BFR improved time taken to perform 5 sit-to-stand repetitions by 17.7% compared to NMES alone (3.4%) at the 45 minutes post-intervention assessment (Table 7.4). Additionally, the addition of 80% BFR to NMES was necessary to acutely affect pain sensitivity, as indicated by an improvement

in PPT (Table 7.1) and reduction in TSP ($p < 0.05$) (Table 7.3). Both the 40% and 80% NMES+BFR groups acutely improved sit-to-stand and knee bend abilities, but only NMES+80% BFR was greater than NMES alone for 5-repetition sit-to-stand performance ($p < 0.05$), without observed effects on CPT or self-reported KOOS (Table 7.2). However, it is important to note that NMES+80% BFR resulted in higher ratings of session pain and RPE than NMES alone and NMES+40% BFR (Table 7.6), without any detrimental effects on cardiovascular parameters.

The findings of this study can be explained through several mechanisms. Firstly, the combination of NMES+BFR may have acutely improved muscle activation[46,81], leading to enhanced functional performance in the 5-repetition sit-to-stand test, compared to NMES alone. The addition of 80% BFR to NMES likely induced increased metabolic stress, an ischemic environment and muscle recruitment, which could promote local muscle adaptations and growth factors, ultimately acutely enhancing muscle function in knee OA patients[46,81]. Moreover, NMES+80% BFR may have reduced inhibitory signals (AMI), allowing for increased quadriceps force production[56]. AMI is a reflex inhibition of quadriceps activation resulting from abnormal afferents from a damaged joint, leading to decreased motor drive and limited force generation by the muscles[19]. Pain and quadriceps weakness are closely related in knee OA, with AMI being associated with pain rather than structural damage[19,25,56]. Previous research has shown that reducing discomfort, can decrease AMI and improve muscle function in knee OA patients[19,56]. Furthermore, reducing pain has shown to enhance muscle activation and MVC in knee OA patients[351]. The reduction in pain experienced during the 5-repetition sit-to-stand after NMES+80% BFR likely contributed to the reduced time taken to perform the 5 repetitions at the 45 minutes post-intervention assessment time point(Table 7.4). The reduction in pain and performance enhancement observed in the present study could be attributed to reductions in AMI[351].

BFR may also improve AMI through various mechanisms, including the promotion of reactive hyperaemia and increased microvascular filtration capacity induced by ischemic reperfusion[234,304,352,353]. These physiological responses may contribute to enhanced vascular reactivity, such as FMD responses as seen in previous NMES+BFR research[45]. Reactive hyperaemia refers to the increased blood flow that occurs following a period of reduced blood flow or ischemia[354]. BFR restricts blood flow to the working muscles, creating ischemia. Once the BFR is released, there is a rapid increase in blood flow to the muscles, resulting in reactive hyperaemia[44]. Furthermore, BFR has been shown to increase the production of nitric oxide within the muscle[44]. Nitric oxide has various physiological functions, including promoting blood vessel dilation and improving blood flow, thus enhancing the delivery of oxygen, nutrients and other essential factors to the muscles[19,44,56]. This may help mitigate the inhibitory effects of AMI in knee OA[56]. Additionally, ischemic reperfusion, which refers to the restoration of blood flow after a period of ischemia, can lead to improved microvascular filtration capacity[234]. Ischemia followed by reperfusion triggers a cascade of physiological responses that can enhance vascular function, including improved endothelial function, increased vasodilation, the release of hypoxia-inducible factors which play a role in tissue repair and angiogenesis potentially improving muscle function and contributing to the results observed in the present study[234,304,352,353].

NMES+BFR in Chapter 4 induced higher levels of fatigue than NMES alone. This fatigue leads to increased recruitment of muscle fibres, including fast-twitch fibres responsible for generating high force[279] which may have helped overcome the inhibitory effects of AMI and promote increased function in the present study and the previously observed chronic strength and hypertrophy gains observed in Chapter 5.

In addition to the physiological mechanisms mentioned above, the acute reduction in TSP observed from pre to post, in conjunction with improved 5-repetition sit-to-stand performance and reduced pain during this task after NMES+80% BFR, may be attributed to

the activation of large diameter sensory fibres through the gate control theory, inhibiting pain signals transmitted through small diameter fibres due to OA, potentially alleviating pain during functional movements observed[167,176]. Moreover, BFR-induced release of endogenous opioids and neuro-modulatory effects may have further modulated pain perception, resulting in improved performance of functional movements[43,331].

TSP refers to an increased perception of pain in response to a repeated stimulus due to amplification of pain processing mechanisms in the central nervous system[355]. In the present study, the absence of an effect observed after NMES alone suggests that the NMES+80% BFR may have modulated the central nervous system's response to pain in knee OA, leading to reduce pain perception during the 5-repetition sit-to-stand test[355]. This finding warrants further exploration to better understand the underlying mechanisms.

Higher ratings of session pain and RPE reported during the NMES+80% BFR sessions compared to NMES alone or NMES+40% BFR suggest that this combination imposes a greater subjective discomfort in knee OA patients. However, still only moderate scores on the NPRS were reported and with no drop outs due to the discomfort experienced, or flare ups in their pain or OA symptoms reported, this warrants little concern. The findings in the present study are supported by previous BFR research, which reported higher muscle discomfort and RPE after cycling and resistance exercise with 80% BFR compared to 40% BFR[302]. The phenomenon known as diffuse noxious inhibitory control, or the "pain inhibits pain" effect, is part of the descending endogenous analgesia system[356]. Experimental studies in healthy adults have demonstrated a decrease in pain intensity after a noxious conditioning stimulus[178,337]. The increased perceptual ratings of pain and RPE in the present study may help explain the acute effects of PPT and TSP observed. The difference in the results observed in the present study compared to Chapter 7 of this thesis is primarily due to the reduced PPT and increased TSP ratings observed in the OA patients at baseline compared to healthy adults, which aligns with previous findings in knee OA populations[25]. Nonetheless, it is reassuring that no adverse events occurred, or any deleterious

cardiovascular responses were observed, suggesting the safety of the interventions used in knee OA patients.

In line with the findings of Chapter 6 and 7 of this thesis, previous research did not find any effect on cold pain thresholds in healthy adults or knee OA patients following NMES alone or in combination with 40% and 80% BFR (Chapter 6 and 7). Similar results were observed in BFR research that utilised 100% AOP during cold water immersion[357]. Cold pain, or the sensation of pain in response to cold stimuli, is typically transmitted through the cold thermoreceptors in the skin and travels via the spinothalamic pathway to the central nervous system[250,328]. Whereas, pressure pain travels through the spinothalamic pathway via nociceptors (pain receptors)[358]. The findings that NMES+BFR affects pressure pan but not cold pain, although speculative, suggests that it likely targets the nociceptors or pain signalling pathways specific to pressure pain rather than impacting thermal signals[328,359] and warrants further investigation.

7.8 Conclusion

In conclusion, the findings of this study demonstrated that the addition of 80% BFR to NMES resulted in an improvement in the time taken to perform 5 sit-to-stand repetitions and reduced pain during the task, indicating enhanced functional ability. Moreover, this coincided with acute improvements in PPT and TSP after NMES+80% BFR. However, both NMES+BFR groups demonstrated acute improvements in sit-to-stand and knee bending abilities from pre to post intervention assessments.

Although speculative, the observed benefits of NMES+80% BFR can be attributed to several mechanisms, including reductions in AMI, increased muscle activation, ischemic environment as well as potential modulation of pain perception, improved vascular reactivity and reduced inflammation. However, it is important to note that the NMES+80% BFR intervention was associated with higher levels of session pain and RPE. Despite this

discomfort, no adverse events or cardiovascular issues were reported, emphasising the safety of the interventions in knee OA patients.

These findings highlight the potential of NMES combined with BFR as a safe adjunctive therapy for managing knee OA. Further research is needed to explore the long-term effects, optimal pressures and individualised approaches to maximise the benefits of this intervention, due to disparities in acute effects observed in the present study compared to Chapter 6 in healthy adults in this thesis. Considering patient comfort and subjective experiences will be crucial in implementing these interventions in clinical practice. Ultimately, the integration of NMES+BFR holds promise for improving muscle function, reducing pain sensitivity and potentially leading to enhanced long-term outcomes in knee OA patients.

Chapter 8

General discussion

8.1 Main findings

This thesis set out to investigate the synergistic benefits of combining NMES with BFR to enhance muscular adaptations and pain modulation compared to NMES alone. The research encompassed acute and chronic training studies on healthy adults and those suffering from knee OA. The initial phase focused on methodological considerations, assessing safety and efficacy of NMES+BFR at different pressures. Chapter 4 led to the exclusion of NMES+60% BFR. A subsequent 6-week study confirmed NMES+BFR's safety and efficacy in enhancing muscle strength and size without movement and no differences between NMES+40% BFR and NMES+80% BFR. The final chapters evaluated NMES+BFR's impact on pain and function, revealing reduced pain sensitivity and improved physical function, particularly in knee OA patients. No adverse events were reported throughout the thesis. The collective findings warrant a comprehensive discussion on muscle adaptation, cardiovascular safety, and pain modulation.

8.2 Muscle size

Chapter 5 observed, 8.6-8.8% greater quadriceps hypertrophy at 50% length of the femur were 8.3-8.8% greater with the addition of BFR to NMES alone; 10.8% total after NMES+40% BFR (d = 0.97) and 10.6% after NMES+80% BFR (d = 0.98) compared to 2% after NMES alone. Additionally, 9.0-9.1% greater quadriceps CSA was observed at 75% length of the femur with the addition of BFR to NMES alone; 11.2% after NMES+40% BFR (d = 0.73) and NMES+80% BFR 11.3% after NMES+80% BFR (d = 0.82) compared to 2.2% after NMES alone.

Results observed during Chapter 5 observed lower hypertrophy than Gorgey et al. [45] who reported extensor carpi radialis longus CSA increased in the NMES+BFR forearm of their participants by 17%, d = 1.1, using a similar CSA US measurement for hypertrophy as used in Chapter 5, but for the forearm rather than the quadriceps. Additionally, Skiba et al. [69]

observed a slightly higher percentage increase of 11.7% from Chapter 5, but a lower effect size d = 0.6 from pre to post training ($p < 0.05$) on twenty-one men with complete spinal cord injury (SCI). They used shorter sessions lasting only 12 minutes (3 x 4 minutes sets), rather than 20 minutes (4 x 5 minutes sets) used throughout this thesis and they used unrecommended parameters of 20 Hz frequency, minimal current to elicit a contraction, rather than max tolerable and not placing the electrodes over the quadriceps motor points which could explain the lower results observed using similar restrictive pressure of 40% used in Chapter 5. It is hypothesised that the spinal cord injured muscles used in both of these may be more responsive to training than the healthy adults used in Chapter 5 [90,166,360,361].

The other NMES+BFR studies using healthy adults, rather than clinical populations all observed lower effect sizes for improvements in muscle size than Chapter 5 of this thesis. Natsume et al. [46] observed muscle thickness of the quadriceps increased after 2-weeks of training (+3.9%, d = 0.18) and decreased after 2-weeks of detraining (-3.0%), whereas no notable change was observed under NMES alone. Although they used muscle thickness, the biggest difference is their intervention only lasted 2-weeks. Previous BFR reviews suggest that training durations of 6-weeks and over are optimal for enhancing muscular adaptations[48,49]. This is supported by the NMES+BFR research showing greater hypertrophy than NMES alone when performing 6-weeks training interventions[45,69] and Chapter 5.

Slysz et al. [58] only observed a 1% increase (d = 0.04) in the quadriceps size after their 6-week intervention. Bergamasco et al. [112] observed increased CSA of the quadricep, using the same CSA measurement as used in Chapter 5, NMES+BFR = 23.0 (2.7) cm^2, 4.6%, d = 0.4. 20 training sessions were performed for both protocols over 6-weeks. Both used 100% BFR and found the lowest effect on muscle hypertrophy, using 6-week interventions, Natsume et al. [46] was slightly lower using only a 2-week intervention. The high 100% BFR

pressure is not recommend in the wider BFR literature[57] and could have contributed to the reduced results observed compared to Chapter 5. Furthermore, a partial occlusion method (40-80%) is also supported by animal model data observing muscle growth after NMES+BFR[64,362] and by this thesis.

8.3 Muscle strength

Chapter 5 observed isometric strength increases from 30-105° were on average 8.1-8.2% greater with the addition of BFR to NMES alone; 13% total after NMES+40% BFR (d = 0.43) and NMES+80% BFR 12.8% (d = 0.44) compared to 4.8%, d = 0.2 after NMES alone for isometric contractions. Isokinetic strength, concentric and eccentric strength increases 9.2-11.7% greater with the addition of BFR to NMES alone; 16.5% total after NMES+40% BFR (d = 0.5) and NMES+80% BFR 14% (d = 0.48) compared to 4.8%, d = 0.17 after NMES alone for isokinetic contractions. These results observed are similar, but also more or less pronounced to previous NMES+BFR studies conducted prior to Chapter 5 of this thesis[45–47,58] and two studies published after it began[69,112].

Slysz et al. [58] observed a moderate effect size d = 0.69, 23.6%, for increased isometric knee extension using max tolerable currents. Natsume et al. [46] reported NMES-BFR isometric strength improved greater than Chapter 5 (+14.2%), but lower isokinetic improvements (+7.0% at 90°/s and +8.3% at 180°/s) conditions after their 2-weeks of training, (d = 0.64 isometrically, 0.31 at 90°/s and 0.35 at 180°/s). The reduced training duration and BFR stimulus could have equated to the reduced isokinetic strength observed. Furthermore, greater pennation angle increases were observed after NMES+40% BFR in Chapter 5 of this thesis, which could have contributed to the greater increases isokinetic strength observed. Increasing the pennation angle has differing effects on isokinetic and isometric strength. This observed effect on pennation in Chapter 5 may also help to explain the greater isokinetic strength increases.

Regarding strength increases after NMES, the current tolerated is the main factor[38,126,363]. The greatest improvement in isometric observed previously used max tolerable currents[58], then Chapter 5 results using max tolerable currents, followed by Natsume et al. [46] using a current intensity that produced a 5%–10% MVIC and the smallest effect observed by Andrade et al. [47] using a current amplitude to achieve a 20% MVIC. The wider NMES literature, the NMES+BFR research to date along with this thesis, supports that max tolerable currents, rather than aiming for low level MVIC's, is recommended for optimising both isometric and isokinetic strength using NMES combined with BFR.

Other parameters of note are the NMES frequency, with the most effective for enhancing strength for the quadriceps is 50 Hz[58] observed in Chapter 5. However, the improvements observed by Natsume et al. [46] using only 30 Hz need to be explored in a longer duration study to determine its efficacy compared to 50 Hz. The work-to-rest ratio of 1:1 for NMES contractions also seems optimal regarding previous NMES+BFR research, which differs from the wider NMES literature recommending large rest periods to limiting fatigue[38] and leads to enhancement of muscle strength and hypertrophy after NMES+BFR (40-80%).

8.4 Cardiovascular safety

The findings of Chapter 4 revealed no meaningful differences in HR responses between the different groups; NMES alone, NMES+40% BFR, NMES+60% BFR and NMES+80% BFR. For blood pressure, no interaction effect was observed. The BP responses after training for SBP ranged from 1.4-2.5 mmHg and DBP 0.1-1.7 mmHg, with no between group differences observed. The findings from Chapter 6, using NMES alone, NMES+40% BFR and NMES+80% BFR, observed no effects on HR, SBP, or DBP in knee OA patients. No effect of NMES+BFR on blood pressure was also observed in spinal cord injured patients also reporting no adverse events[45]. The lack of deleterious cardiovascular effect observed

in Chapter 4 and 6 of this thesis, using different population groups is promising for determining the safety of this approach along with no adverse events occurring throughout all of the studies in this thesis.

8.5 Pain modulation

The findings of Chapter 4, showed higher session ratings of pain and RPE during there NMES alone and NMES combined with 40, 60% and 80% BFR interventions compared to Chapter 5 onwards. The main difference in the parameters used was the set durations being 1.4 minutes in Chapter 4 and 5 minutes from Chapter 5 onwards. The longer set duration of 5 minutes from Chapter 5 onwards resulted in in no interaction and lower pain and RPE scores. These results are supported by research on conditioned pain modulation, which suggests that session duration and frequency play a role in reducing pain modulation experienced. The findings suggest that the 5 minutes set duration used from Chapter 5 onwards is optimal for perceptual comfort.

During Chapter 5, pain values, RPE, and maximum tolerable currents were reported. The pain experienced in each session decreased from the first session to the last for both NMES+BFR groups, while the NMES alone group experienced an increase in pain. This coincided with higher NMES currents used and tolerated under both NMES+BFR conditions compared to NMES alone. The subjects in the NMES alone group did exhibit conditioning to the stimulus, as evidenced by the greater currents tolerated from the first to the last session. However, the conditioning effect was greater with the addition of BFR. This finding aligns with the observations made by Natsume et al. [46] who reported greater reductions in session discomfort with NMES+BFR compared to NMES alone and the acute reductions in PPT observed after NMES+BFR in Chapter 6. After NMES+40% BFR, the effect size at the immediate post time point was moderate (d = 0.6) and after NMES+80% BFR was large (d = 0.8). However, the effect did not last for 20 minutes, which differs from previous BFR

research showing that the PPT increase lasts up to 24 hours[331]. In Chapter 7, the effect size observed for pain reduction during the 5-repetition sit-to-stand task at the 45 minutes post-intervention time point was d = 0.5 compared to NMES alone. We observed similar improvements in pain during functional squatting tasks as reported by Korakakis et al. [182]. This improvement in pain and 5-repetition sit-to-stand performance also coincided with a reduction in TSP after NMES+80% BFR and lasted 45 minutes as observed previously[182]. Although speculative, the reduced pain sensitivity and TSP could have led to improvements in AMI, leading to enhanced muscle activation[81] and therefore 5-repetition sit-to-stand performance in knee OA patients in Chapter 7[355].

8.6 Clinical practice guidelines

The results of this thesis suggest the optimal NMES+BFR protocol for enhancing muscular strength (isometric and eccentric) and VL size, particularly in situations where exercise is contraindicated or challenging due to pain, is NMES combined with 40% BFR for 4 sets of 5 minutes, three times a week for six weeks.

In the context of knee OA patients, the use of NMES combined with 80% BFR is recommended to contribute to short-term improvements in pain modulation, characterized by more than a 19% reduction in the time taken to perform 5 sit-to-stand movements with decreased pain after a single session.

In line with results from this thesis, clinicians should expect to observe >10% improvements in muscle size and >12% improvements in muscle strength following a 6 week, 3 session a week protocol. The NMES electrodes should be placed over muscle motor points and AOP should be determined in the position that the treatment is undertaken. The NMES parameters reported in Chapter 5 onwards should be utilised to enhance muscular strength,

size and assist in modulating pain when combined with 40% and 80% BFR. Max tolerable currents should be encouraged throughout all treatment sessions.

8.7 Limitations

Throughout this thesis, only the quadriceps muscle group has been tested due to its importance to physical function and to help provide an optimal methodology. The same protocol may have different results on varying muscles due to their physiological characteristics and their functional demands.

The cohort used for three experimental studies of this thesis was based around university students and staff members. Therefore, a further limitation will be the results are limited to the age groups tested and also socioeconomic population associated with the students and staff at St Marys University, Twickenham.

8.8 Future Research Considerations

One consistent theme throughout the thesis is the need for standardised protocols and methodologies for NMES and BFR interventions. Further research should focus on establishing consensus guidelines for the application of NMES+BFR, including parameters such as stimulation frequency, intensity, duration and BFR pressure.

While this thesis investigated three different BFR pressures (40%, 60% and 80%), there is still a need to explore a wider range of pressures. Future research should examine the acute and long-term effects of NMES combined with lower and higher BFR pressures to determine the optimal pressure for specific populations and outcomes.

Further research is warranted to elicit to exact mechanisms behind the muscle adaptations and acute improvements in pain sensitivity and function observed throughout this thesis. Further research should explore the neural, vascular, and molecular mechanisms involved in the NMES+BFR response. NMES+BFR potentially reducing inhibition and enhancing muscle function at an accelerated rate is really promising and holds a lot of value for the rehabilitation of knee OA patients.

8.9 Conclusion

In conclusion, this thesis investigated the effects of combining NMES with BFR on muscle strength, size, function and pain in both healthy and knee OA patients. Overall, the findings of this thesis support the use of NMES+40% BFR and NMES+80% BFR in increasing muscle strength, size and pain modulation compared to NMES alone in both healthy adults and knee OA patients. In knee OA, the greater reductions in pain sensitivity and functional improvements after NMES+80% BFR currently make it a preferable choice over NMES+40% BFR. However, further controlled trials are needed to determine the long-term effectiveness of NMES+80% BFR compared to NMES+40% BFR.

When voluntary exercise is not possible or contraindicated due to pain or pathology, NMES+BFR provides a safe and efficacious intervention to enhance muscular adaptations and improve pain modulation, which can assist with the rehabilitation of clinical populations.

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Appendix 1

St Mary's University Twickenham London

25 May 2017 SMEC_2016-17_104

Paul Head (SHAS): 'The acute effect of neuromuscular electrical stimulation combined with varying degrees of blood flow restriction on physiological and haemodynamic variables'.

Dear Paul

University Ethics Sub-Committee

Thank you for submitting your ethics application for consideration.

I can confirm that your application has been considered by the Ethics Sub-Committee and that ethical approval is granted.

Yours sincerely

Prof Conor Gissane
Chair of the Ethics Sub-Committee

Cc Stephen Patterson, Mark Waldron, Nicola Theis

Appendix 2

St Mary's University Twickenham London

11 September 2018 SMEC_2017-18_143

Paul Head (SHAS): 'Effect of neuromuscular electrical stimulation combined with varying degrees of blood flow restriction on muscular and cardiovascular function'.

Dear Paul

University Ethics Sub-Committee

Thank you for re-submitting your ethics application for consideration.

I can confirm that all required amendments have been made and that you therefore have ethical approval to undertake your research.

Yours sincerely

Appendix 3

St Mary's University Twickenham London

Approval Sheet

Name of proposer(s)	Paul Head
Name of supervisor	Dr Stephen Patterson, Dr Mark Waldron & Dr Nicola Theis
Programme of study	PhD
Title of project	Effect of neuromuscular electrical stimulation combined with varying degrees of blood flow restriction on pressure pain

Supervisors, please complete section 1. If approved at level 1, please forward a copy of this Approval Sheet to the School Ethics Representative for their records.

SECTION 1: To be completed by supervisor.			
☐ Approved at Level 1. ☒ Refer to School Ethics Representative for consideration at Level 2 or Level 3.			
Signature of Supervisor (for student research projects):		Date:	20.10.22

SECTION 2: To be completed by School Ethics Representative

Appendix 4

St Mary's University Twickenham London

10 November 2022 SMU_ETHICS_2021-22-329

Paul Head (SAHPS): 'Effect of neuromuscular electrical stimulation combined with varying degrees of blood flow restriction on pressure and cold pain with knee pathology'

Dear Paul,

University Ethics Sub-Committee

Thank you for re-submitting your ethics application for consideration.

I can confirm that all required amendments have been made and that you therefore have ethical approval to undertake your research.

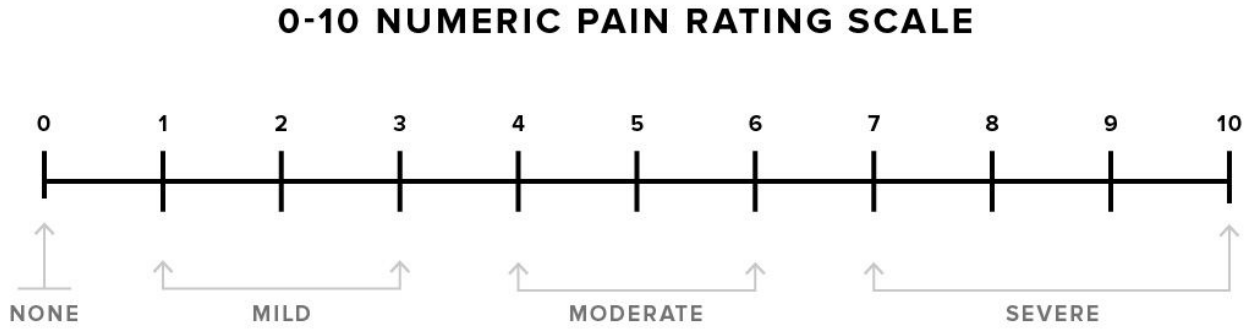
Yours sincerely

Appendix 5

Appendix 5.1 Borg RPE scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix 5.2 Numeric pain rating scale



Appendix 5.3 Physical activity and readiness questionnaire

CONFIDENTIAL MEDICAL HISTORY / PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q) FORM

This screening form must be used in conjunction with an agreed Consent Form.

Full Name: _____ Date of Birth: _____

Height (cm): _____ Weight (kg): _____

Have you ever suffered from any of the following medical conditions? If yes please give details:

	Yes	No	Details
Heart Disease or attack	☐	☐	_____
High or low blood pressure	☐	☐	_____
Stroke	☐	☐	_____
Cancer	☐	☐	_____
Diabetes	☐	☐	_____
Asthma	☐	☐	_____
High cholesterol	☐	☐	_____
Epilepsy	☐	☐	_____
Allergies	☐	☐	_____
Other, please give details	☐	☐	_____

Do you suffer from any blood borne diseases? If yes please give details;

Please give details of any **medication** you are currently taking or have taken regularly within the last year:

Please give details of any **musculoskeletal injuries** you have had in the **past 6 months** which have affected your capacity to exercise or caused you to take time off work or seek medical advice:

Other Important Information

During a typical week approximately how many hours would you spend exercising?

If you **smoke** please indicate how many per day:

If you drink **alcohol** please indicate how many units per week:

Are you currently taking any **supplements or medication?** Please give details:

Is there any reason not prompted above that would prevent you from participating within the relevant activity?

By signing this document I agree to inform the relevant individual(s) of any change(s) to my circumstances that would prevent me from participating in specific activities.

Signature (Participant): _____ **Date:** _____

Signature (Test Coordinator*): _____ **Date:** _____

Appendix 5.4 Wellness questionnaire

	5	4	3	2	1	Record Score
FATIGUE	Very fresh	Fresh	Normal	More tired than normal	Always tired	
SLEEP QUALITY	Very restful	Good	Difficulty falling asleep	Restless sleep	Insomnia	
GENERAL MUSCLE SORENESS	Feeling great	Feeling good	Normal	Increase in soreness/tightness	Very sore	
STRESS LEVELS	Very relaxed	Relaxed	Normal	Feeling stressed	Highly stressed	
MOOD	Very positive mood	A generally good mood	Less interested in others &/or activities than usual	Snappiness at team-mates, family and co-workers	Highly annoyed/ irritable/down	

Appendix 5.4 Knee injury and osteoarthritis outcome score questionnaire

KOOS KNEE SURVEY

Today's date: _____/_____/_____ Date of birth: _____/_____/_____

Name: _____

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities.
Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms

These questions should be answered thinking of your knee symptoms during the **last week**.

S1. Do you have swelling in your knee?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S3. Does your knee catch or hang up when moving?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

S5. Can you bend your knee fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

Stiffness

The following questions concern the amount of joint stiffness you have experienced during the **last week** in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

S7. How severe is your knee stiffness after sitting, lying or resting **later in the day**?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐