Remote Ischaemic Conditioning for Fatigue after Stroke (RICFAST), a pilot, single-blind, randomised, placebo-controlled trial.

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ABSTRACT

Post-stroke fatigue (PSF) affects up to 75% of stoke survivors at some stage after stroke. The precise mechanisms that underlie PSF are still being unravelled but it may develop due to changes in cortical excitability, inflammation, and impaired cellular energetics. It negatively impacts neurological recovery, return to work and quality of life (QoL). There are currently no well evidenced treatments.

Remote ischaemic conditioning (RIC) is a novel treatment whereby brief and reversible episodes of ischaemia and reperfusion are applied to a limb. It may lead to alterations in inflammation, tissue perfusion and mitochondrial function, that theoretically counteract factors contributing to PSF.

The primary aim of this thesis was to investigate the safety, acceptability, and feasibility of RIC to treat PSF by undertaking a single-centre, single-blind, pilot, randomised, placebo-controlled trial.

Participants with PSF were randomised to RIC or sham control for 6 weeks and measures of fatigue, physical function, mood, and QoL were assessed at baseline, 6-weeks, 3-months, and 6-months. Mechanistic evaluation using cardiopulmonary exercise testing and ^{31}Phosphorus-Magnetic Resonance Spectroscopy (^{31}P-MRS) of peripheral muscles was also undertaken.

RIC was safe, acceptable, and feasible for people with PSF. RIC appears to result in clinically meaningful improvements in fatigue that persisted beyond treatment cessation, independent to mood or functional state and was associated with increased walking distances. These changes may be associated with improvements in thresholds for efficient energy production (ventilatory anaerobic threshold). ^{31}P-MRS suggested this may be driven by improvements in the energy producing capabilities of mitochondria (adenosine triphosphate content of muscle).

We have demonstrated that RIC is a promising treatment strategy for patients with PSF that is low cost and widely implementable if found to be effective. It warrants further definitive investigation in larger studies.

PUBLICATIONS ASSOCIATED WITH THIS THESIS

Remote ischaemic conditioning for stroke: unanswered questions and future directions. ***Stroke and Vascular Neurology.*** Baig S, ***Moyle B***, Nair K P S, Redgrave J, Majid A, Ali A. https://pubmed.ncbi.nlm.nih.gov/33903181/

Post-stroke fatigue - an evidence update. *Neurorehabiliation.* (*in press*). ***Moyle B***, Baig S, Redgrave J, Majid A, Ali A.

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DECLARATION

I, the author of this thesis, confirm that the work presented is my own. I am aware of and understand the University's Guidance on plagiarism and the use of unfair means (www.sheffield.ac.uk/ssid/unfair-means) and I confirm any information that has been obtained from other sources has been clearly stated in my thesis. This work has not been previously submitted to the University for an award.

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LIST OF ABBREVIATIONS

31P-MRS: Phosphorus magnetic resonance spectroscopy

6MWT: Six-minute walk test

Ach: Acetylcholine

ADL: Activities of daily living

ADP: Adenosine diphosphate

AE: Adverse event

ALS: Amyotrophic lateral sclerosis

ANS: Autonomic nervous system

AP: Action potential

ATP: Adenosine triphosphate

BAIPC: Bilateral arm ischemic preconditioning

BOLD: Blood oxygen level dependant

BP: Blood pressure

$Ca2^{+}$: Calcium ion

CABG : Coronary artery bypass surgery

CAF: Central activation failure

CAO: Coronary artery occlusion

CBF: Cerebral blood flow

CBT: Cognitive behavioural therapy

CCA: Common carotid artery

CFS: Chronic fatigue syndrome

Cho: Choline

CIS-f: Checklist individual strength - subscale fatigue

CO: Cognitive therapy

COGRAT: Cognitive therapy and graded activity training

CPAP: Continuous positive airway pressure

CPET: Cardiopulmonary exercise testing

CPP: Cerebral perfusion Pressure

Cr: Creatine

CRF: Cardiorespiratory fitness

CRIC: Chronic remote ischemic conditioning

CRP: C-reactive protein

CSVD: Cerebral small vessel disease

CT: Computerised tomography

ECT: Electron transport chain

EMG Electromyography

EMG: Electromyography

eNOS: Endothelial nitric oxide synthase

EPO: Erythropoietin

ESR: Erythrocyte sedimentation rate

FAO: Femoral artery occlusion

FAS: Fatigue assessment scale

FIS: Fatigue impact scale

FMD: Flow-mediated dilation

FSS: Fatigue severity scale

HIF: Hypoxia inducible factor

HR: Heart rate

HSP70: Heat shock protein

I/R: Ischemia/reperfusion

IAS: Intracranial arterial stenosis

ICA: Internal carotid artery

ICP: Intracranial pressure

IL-1: Interleukin-1

IL-6: Interleukin-6

IPC: Ischemic preconditioning

ITT: Interpolation twitch technique

LLEP: Lower limb extensor power

LMN: Lower motor neurone

MAO: Mesenteric artery occlusion

MCA: Middle cerebral artery

MCID: Minimal clinically important difference

MFCV: Muscle fibre conduction velocity

MFI-20: Multidimensional Fatigue inventory

MRI: Magnetic resonance imaging

MRS: Magnetic resonance spectroscopy

MS: Multiple sclerosis

MVC: Maximal voluntary contraction

MVIC: Maximal voluntary isometric contraction

NAA: N-acetyl aspartate

NMJ: Neuromuscular junction

NMR: Nuclear magnetic resonance

NO: Nitric oxide

OCR: Oxygen consumption rates

OSA: Obstructive sleep apnoea

PCI: Percutaneous coronary intervention

PCr: Phosphocreatine

PCr: Phosphocreatine

PDE: Phosphodiesters

PET: Positron emission tomography

Pi: Inorganic phosphate

PMC: Primary motor cortex

PME: Phosphomonoesters

Ppm: Parts per million

PROMS: Patient reported outcome measures

PSD: Post-stroke depression

PSF: Post-stroke fatigue

RCT: Randomised controlled trial

RIC: Remote ischemic conditioning

ROS: Reactive oxygen species

SAE: Serious adverse event

SAH: Subarachnoid haemorrhage

SAH: Subarachnoid haemorrhage

SBP: Systolic blood pressure

SD: Standard deviation

SDF-1: Stromal cell-derived factor 1

SIAS: Symptomatic intracranial arterial stenosis

SLE: Systemic lupus erythematosus

SPECT: Single-photon emission computed tomography

TBI: Traumatic brain injury

TCD: Transcranial Doppler sonography

tDCS: Transcranial direct current stimulation

TIA: Transient ischemic attack

TMS: Transcranial magnetic stimulation

TNF- α: Tumor necrosis factor-alpha

TRAIL: TNF-related apoptosis-inducing ligand

TTI: Time to systolic inflexion

VA: Voluntary activation

VAS: Visual analogue scale

VCAM-1: Vascular cell adhesion molecule-1

VCI: Vascular cognitive impairment

VO_2 max: Maximal oxygen consumption

VO_2peak: Peak oxygen consumption

CHAPTER 1. INTRODUCTION

1.1 Stroke

Stroke is the second biggest cause of mortality worldwide and a leading cause of adult disability (Johnson et al., 2019, Gorelick, 2019). In 2015 the estimated cost of stroke in Europe alone was €45 billion in direct (diagnostic testing, treatment, care) and indirect (informal care costs, lost productivity) healthcare costs (European Heart Network, 2017). It is estimated that between 2015-2035 the number of strokes in the UK each year will increase by 60% and the number of stroke survivors will more than double (King et al., 2020). Increasing incidence (Feigin et al., 2014) and lower mortality rates after stroke (Lackland et al., 2014) mean there is an increasing population who are at risk of developing longer term complications after stroke, such as fatigue. Post-stroke fatigue (PSF) has recently received scientific attention as one of the most prevalent complications stroke survivors suffer, without well-established treatment pathways. It has been highlighted as a research priority by patients, carers and healthcare professionals (Rudberg et al., 2021, James Lind Alliance, 2021).

1.2 Post-stroke fatigue

Post-stroke fatigue (PSF) is a complex, multidimensional motor-perceptive, emotional and cognitive experience characterised by a subjective feeling of exhaustion, physical tiredness, lack of energy and weariness which persists even after rest (Aaronson et al., 1999, Staub and Bogousslavsky, 2001, De Groot et al., 2003). Fatigue can be classified as either physical (referring to motor activities) or mental (refers to the cognitive or perceptual functions) (Gruet et al., 2013). However, the psychophysical and multidimensional nature of PSF makes it difficult to define for purposes of quantification and comparison across individuals (De Doncker et al., 2018). Definitions of PSF are largely based on felt experience of stroke survivors, however, more mechanistic definitions have been proposed such as a 'feeling arising from difficulty in initiation of or sustaining voluntary effort' (Chaudhuri and Behan, 2004), or an 'amplified sense of normal (physiological) fatigue induced by changes in one or more variables regulating work output' (De Doncker et al., 2018, Kuppuswamy et al., 2015). Researchers have postulated differing entities and definitions of PSF based on whether it develops early (predominantly motor characteristics) or late (predominantly mental characteristics), however, the development of both phenotypes are not mutually exclusive (Annoni et al., 2008, Tseng et al., 2010). Although the concept of early (up to 3 months post-

stroke) and late (over three months post-stroke) onset fatigue has been described (Wu et al., 2015b, Kirchberger et al., 2022) there is little evidence to support the notion that these are two distinct entities. In longitudinal studies of PSF, early fatigue is often associated with late fatigue, thus the idea of early and late fatigue could be viewed as an evolving process. Although, it is plausible that different factors may contribute to the development of fatigue at different timepoints. For example, one study that investigated associations of fatigue at different timepoints after stroke found that infratentorial stroke was associated with fatigue at 2-months post-stroke but not at 18-months; however baseline anxiety and depression were associated with PSF at both timepoints (Snaphaan et al., 2011). In another study, the three strongest correlates of PSF at 6-months were initial stroke severity, depression and disability, whereas the strongest correlates at 1-year were anxiety, depression and language impairments (Radman et al., 2012). These findings suggest that the type of stroke someone has suffered may be a more important determinant of early onset fatigue, while psychological factors are important for both early and late fatigue. To explore whether early and late fatigue are distinct entities longitudinal studies of stroke patients with fatigue and biomarkers (e.g., inflammation, immune system activation, mitochondrial bioenergetics) (Klinedinst et al., 2019, Huang et al., 2022, Zhang et al., 2021) may help determine any changes that occur from early to late timeframes.

PSF is common, affecting 23-75% of stroke survivors (Nadarajah and Goh, 2015, Cumming et al., 2018, Alghamdi et al., 2021); half of whom report it as one of their worst symptoms (Van Der Werf et al., 2001). The estimated prevalence of fatigue after stroke is dependent on terminology (Falconer et al., 2010), with studies using rating scales yielding higher prevalence's than those which ask people if they are tired or fatigued (De Groot et al., 2003). Males and females also express fatigue differently which can influence prevalence estimates (Falconer et al., 2010). Despite this, 43% of stroke survivors report PSF as an unmet need (McKevitt et al., 2011). Symptoms of fatigue may begin at any time after stroke, with those developing symptoms early (months) at higher risk of remaining fatigued longer term (18-months) (Lerdal and Gay, 2013). Indeed, symptoms may continue for up to 6 years after stroke (Elf et al., 2016). Evidence suggests patients experiencing transient ischaemic attack (TIA) may also develop fatigue (Moran et al., 2014), however it is more common after stroke (Winward et al., 2009). Fatigue in the acute stage after stroke may be a protective response by the body to aid recovery and restoration (Choi-Kwon and Kim, 2011), which if persists, becomes pathological, with its aetiology multifaceted. PSF is associated with poorer general

health and greater levels of pain, anxiety, lower mental health and mortality (Glader et al., 2002, Mead et al., 2011). It is an independent predictor of increased dependency and institutionalised care and prevents many stroke survivors from returning to work (Lerdal and Gay, 2013, Glader et al., 2002, Andersen et al., 2012, Staub and Bogousslavsky, 2001). In addition, PSF prevents participation in rehabilitation and is associated with depression and poorer health-related quality of life (HRQoL) (Chen et al., 2015). One study found that 62% of patients with PSF did not suffer premorbid fatigue (Drummond et al., 2017). The fact fatigue is such a novel experience for many stroke survivors may explain why patients report poor knowledge about the condition and struggle to understand and adapt to its symptoms (White et al., 2012a). Understanding why PSF develops may be an important step in helping stroke survivors with symptom management.

1.3 Mechanisms of post-stroke fatigue

Fatigue is a prevalent symptom in many neurological (e.g., multiple sclerosis) and non-neurological (e.g., cancer) diseases (Nagaraj et al., 2013, Banipal et al., 2017). While the nature and time course of fatigue may vary across these populations (Schepers et al., 2006a, Spratt et al., 2012), the clinical similarities suggest that there may be common underlying mechanisms. A physiological classification can divide fatigue into central and peripheral entities depending on where in the body it develops (Figure 1) (Davis and Walsh, 2010). Central fatigue is thought to originate from the central nervous system (CNS) (brain, spinal cord) while peripheral fatigue originates from within the peripheral nerves and muscle fibres.

1.3.1 Central fatigue

Central fatigue may be related to neural interruptions in the basal ganglia, thalamus and frontal Cortex (Chaudhuri and Behan, 2004), key players in motor control, motivation and attention (De Doncker et al., 2018, Chaudhuri and Behan, 2000). Fatigue appears more prevalent when stroke affects subcortical regions of the brain (e.g., the cerebellum or basal ganglia) (De Doncker et al., 2018, Chaudhuri and Behan, 2000). Indeed, an MRI study of 334 stroke patients by Tang et al. (2010) found that people diagnosed with fatigue were significantly more likely to have infarcts in their basal ganglia than any other brain region (Tang et al., 2010). Studies also highlight independent links between stroke lesion burden (Glader et al., 2002), stroke severity (Winward et al., 2009), lesion laterality (Manes et al., 1999) and the development of

PSF, cumulatively supporting the notion that alterations in neurochemistry are an implicated mechanism of PSF. Studies using transcranial magnetic stimulation (TMS) (a non-invasive form of brain stimulation) (Eldaief et al., 2013) have shown higher resting cortical motor thresholds among patients with PSF compared to stroke patients without fatigue. This suggests that patients with fatigue require higher levels of cortical stimulation to produce a motor response. Such patients also demonstrate greater levels of central activation failure (CAF) during motor tasks (e.g., isometric biceps hold), indicating sub-optimal recruitment of motor units during normal motor activation, which can lead to an increased perception of effort during tasks (Gruet et al., 2013). Thus, a deficit in corticomotor excitability appears to be important in central PSF. However, a study by Winward et al. (2009) found significantly higher levels of fatigue in patients with minor stroke who had little or no residual neurological deficit compared to TIA patients at 6-months. This was independent of factors such as depression, anxiety, comorbidity or medications. This suggests that central factors may not be the only mechanisms behind the development of PSF.

1.3.2 Peripheral fatigue

The literature highlights factors such as inflammation, physical deconditioning and impaired peripheral cellular energetics as the main pathways manifesting peripheral fatigue (Figure 1) (Ponchel et al., 2015).

1.3.2.1 Inflammation

Evidence supports the role of inflammation in fatigue in various clinical populations including chronic fatigue syndrome (Montoya et al., 2017), cancer (Bower and Lamkin, 2012), and multiple sclerosis (MS) (Heesen et al., 2006). Fatigue is also a well-established symptom in immunological disorders such as rheumatoid arthritis (RA) (van Steenbergen et al., 2015), and systemic lupus erythematosus (SLE) (Tarazi et al., 2019), and is also common after viral infections (Yamato and Kataoka, 2015). An extensive literature indicates that people with these conditions exhibit increased systemic production of pro-inflammatory markers, such as C-reactive protein (CRP) tumor necrosis factor-alpha (TNF-α), interleukin-6 (Il-6), interleukin 1 (IL-1) and interferon-gamma (IFN-γ) (Liu et al., 2012, Yang et al., 2019, Shrivastava et al., 2013, Wang et al., 2018). Pharmacological treatments aimed at inhibiting the action of proinflammatory cytokines (e.g., TNF-α or IL-6 inhibitors) are effective in reducing fatigue in patients with rheumatoid arthritis and systemic lupus erythematosus (Yount et al., 2007, Illei

et al., 2010, Wendling et al., 1993, Druce et al., 2015). Furthermore, injection of inflammatory mediators such as IL-6 induces fatigue in healthy populations (Papanicolaou et al., 1998, Späth-Schwalbe et al., 1998).

Enhanced pro-inflammatory cytokine activity has been linked to the development of PSF (Ponchel et al., 2015). During the acute phase of stroke, pro-inflammatory cytokines (e.g., TNF-α, IL-1) are released into the cerebral ischaemic core and the peri-infarct area, activating microglia, neutrophils and macrophages (De Doncker et al., 2018, Barrington et al., 2017). Inflammatory cell activation within the brain is associated with the release of inflammatory cytokines in peripheral tissues (e.g., kidneys, adipose tissue, skeletal muscle) (Chiba and Umegaki, 2013, Shichita et al., 2012). For example, a study of 20 stroke patients by Hafer-Macko et al. (2005) found increased levels of TNF-α in paretic leg muscle biopsies compared to controls. Studies have found elevated levels of systemic inflammatory markers and mediators among patients with PSF including CRP (McKechnie et al., 2010), cortisol, and vascular cell adhesion molecule-1 (VCAM-1) (Becker, 2016). Most studies exploring the relationship between inflammation and PSF have focused on the acute stage of stroke recovery (Wen et al., 2018). For example, Ormstad et al. (2011) found a significant positive correlation between acute serum levels of interleukin (IL)-1β and the severity of PSF (measured using the Fatigue severity scale, FSS) at 6-months post-stroke. There is less information on whether PSF in the later stages of stroke recovery is also associated with increased pro-inflammatory activity in the peripheral circulation (Wu et al., 2015a, Becker, 2016). A cross-sectional study of 70 chronic stroke patients by Gyawali et al. (2020) explored the relationship between PSF (measured using the fatigue assessment scale, FAS) and the proinflammatory cytokines IL-6 and CRP. The investigators found a significant relationship between the level of fatigue reported on the FAS and serum levels of IL-6 and CRP. However, this relationship was no longer statistically significant once cardiovascular covariables were introduced into a linear regression model (Gyawali et al., 2020). The idea that elevated physiological concentrations of proinflammatory cytokines in the central and peripheral nervous system contribute to PSF has been described by researchers as ‘inflammation-induced sickness behaviour’ (Dantzer and Kelley, 2007).

1.3.2.2 Physical deconditioning

One hypothesis is that PSF might be triggered by reduced physical fitness early after stroke (Lewis et al., 2011). The term physical fitness encompasses cardiorespiratory fitness (CRF), muscle power (rate of generation of force), and muscle strength (maximal force produced during a contraction) (Lewis et al., 2011). The effects of reduced physical fitness may lead to increased perception of effort and fatigue during physical activity (Billinger et al., 2014). CRF is the ability of the heart, lungs, and vascular systems to deliver oxygen to active muscles during physical activity and is usually expressed peak oxygen uptake (VO_{2peak}) in clinical populations including stroke (Saunders et al., 2020). VO_{2peak} is the highest value of oxygen (O_2) attained on an incremental exercise test (Chambers and Wisely, 2019). It is a valid measure of aerobic endurance and is typically assessed using cardiopulmonary exercise testing (CPET) (Taylor et al., 2015). This will be discussed in more detail in Chapter 4. CRF confers endurance, which is the ability to perform physical activity for an extended period before experiencing fatigue (Saunders et al., 2020). Physical activity improves physical fitness, optimises vascular risk factors and is a key recommendation for patients after stroke (Sacco et al., 2006, Sharman et al., 2015). Inactivity after stroke is common (Rand et al., 2009, Bernhardt et al., 2004, English et al., 2014, Kunkel et al., 2015) and is associated with reduced aerobic fitness (Ivey et al., 2005), muscle strength (Gerrits et al., 2009, Horstman et al., 2008, Harris et al., 2001) and power (Saunders et al., 2008). Low CRF after stroke is associated with poor psychological health (Kunkel et al., 2015), loss of independence and decreased ability to perform activities of daily living (ADLs) (Shephard, 2009). CRF in people with stroke is 26-87% of that of healthy age-and gender-matched individuals (Smith et al., 2012). Deconditioning of muscles involves a reduction in muscle mass, increase in muscle fat content, a shift from slow-twitch to fast-twitch fatigable fibres, increased inflammatory mediator content of the muscle and a contraction of the capillary bed (Billinger et al., 2012a). Loss of muscle mass and reduced aerobic fitness is also associated with decreases in the content and function of mitochondria in older adults (Peterson et al., 2012, Johnson et al., 2012, Short et al., 2005, Lanza et al., 2008). Physical deconditioning and PSF are inter-related: people with fatigue tend to live more sedentary lives which results in a vicious cycle of further reductions in physical fitness and a subsequent worsening of fatigue (De Doncker et al., 2018, Lewis et al., 2011).

Biological consequences of reduced physical fitness after stroke are more severe in the paretic limbs (English et al., 2010). This was demonstrated in a cross-sectional study by Ryan et al.

(2002) who found that skeletal muscle atrophy and fat content (measured using dual x-ray absorptiometry and computerised tomography, CT) was higher in the paretic limbs of deconditioned chronic stroke patients (47 males, 13 females, >6 months post-stroke) compared to the non-affected limbs.

A cross-sectional study of 66 community-dwelling stroke patients by Lewis et al. (2011) investigated the relationship between PSF (measured using the vitality score of the 36-item Short-Form Health Survey version 2) and two indices of physical fitness; lower limb extensor power (LLEP) and walking economy (oxygen uptake during ambulation and measure of aerobic fitness, VO_{2peak}). The investigators found that increased fatigue was significantly associated with reduced LLEP in the unaffected leg (R= -0.38, P=0.003, n=58). No association was found between PSF and walking economy (R= -0.024, P = 0.86, n = 60) (Lewis et al., 2011). Furthermore, a systematic review found no significant associations between measures of physical activity or fitness and PSF (Duncan et al., 2012). However, it remains plausible that reduced physical activity and physical fitness can have a negative influence on fatigue after stroke. This will be discussed in more detail in Chapter 4.

Recently a study of 23 inpatients with subacute stroke by Oyake et al. (2021) demonstrated that PSF severity as measured using the fatigue severity score (FSS) was associated with longer *time constant* VO_2 kinetics (which reflects the ability of the body to adapt from rest to a new steady state of submaximal exercise) (Spearman's Rho = 0.530; P = 0.009), rather than VO_{2peak} (Spearman's Rho − 0.264; P= 0.224). Thus, PSF may be related not only to perturbations in the respiratory and cardiovascular responses to activity but also to the steady state energy capabilities of tissues.

1.3.2.3 Impaired cellular bioenergetics

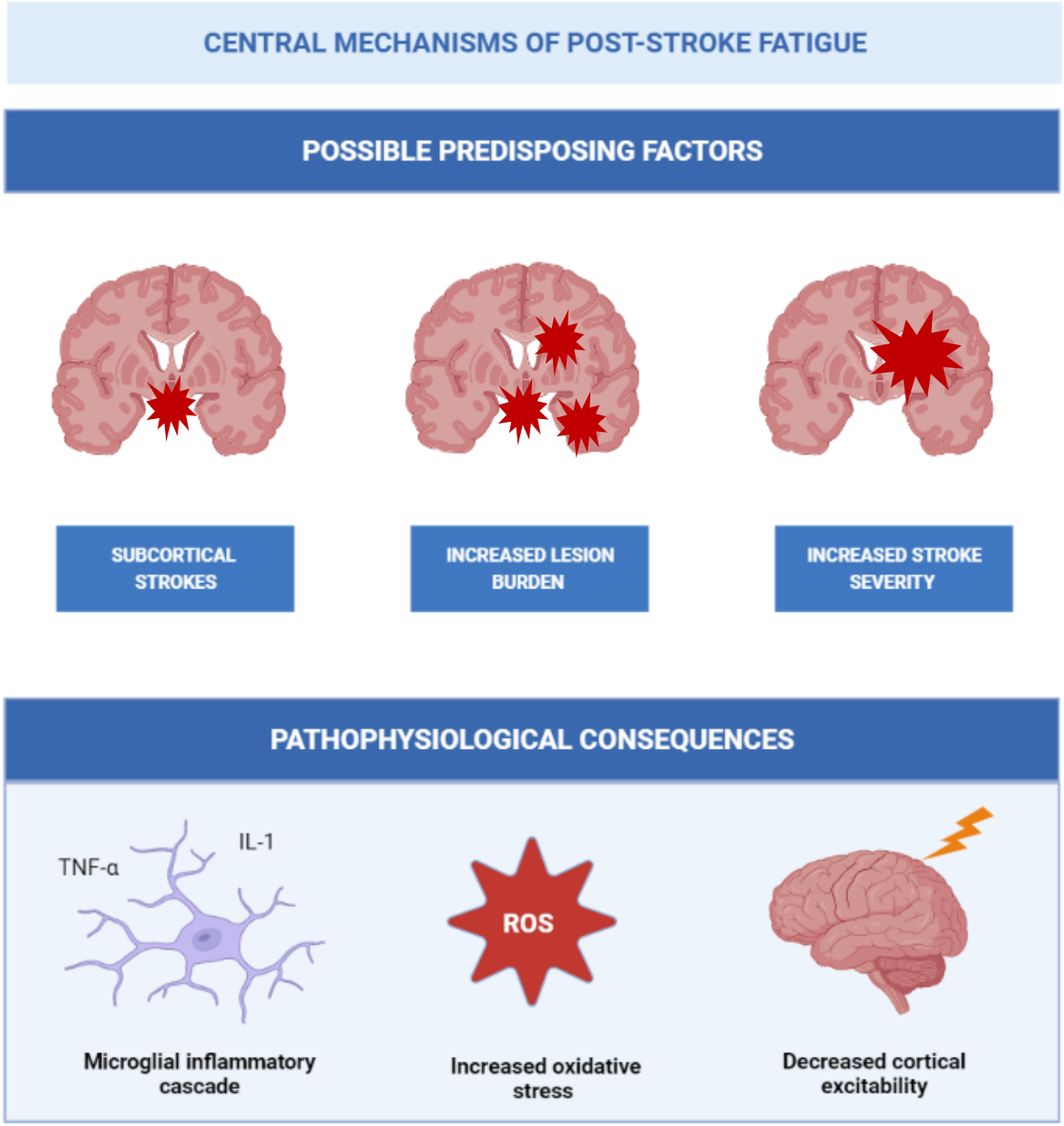
Impaired mitochondrial energy metabolism is associated with inflammation and fatigue in disease conditions like cancer (Chae et al., 2018), chronic fatigue syndrome (Morris and Maes, 2013), and frailty (Wawrzyniak et al., 2016) and could plausibly be implicated in the development of PSF. Klinedinst et al (2019) recently conducted a cross-sectional study of 20 stroke survivors whose platelet oxygen consumption rates (OCR) were measured at rest (a measure of the platelets ability to adjust to energy demands). They found that increased self-reported fatigue scores (measured using the Fatigue Assessment Scale) were associated with

increased OCR (increased resting OCR suggests the efficiency of energy production may be less – more oxygen required for the same energy requirement) and thus may be related to alterations in mitochondrial and cellular bioenergetics (Klinedinst et al., 2019).

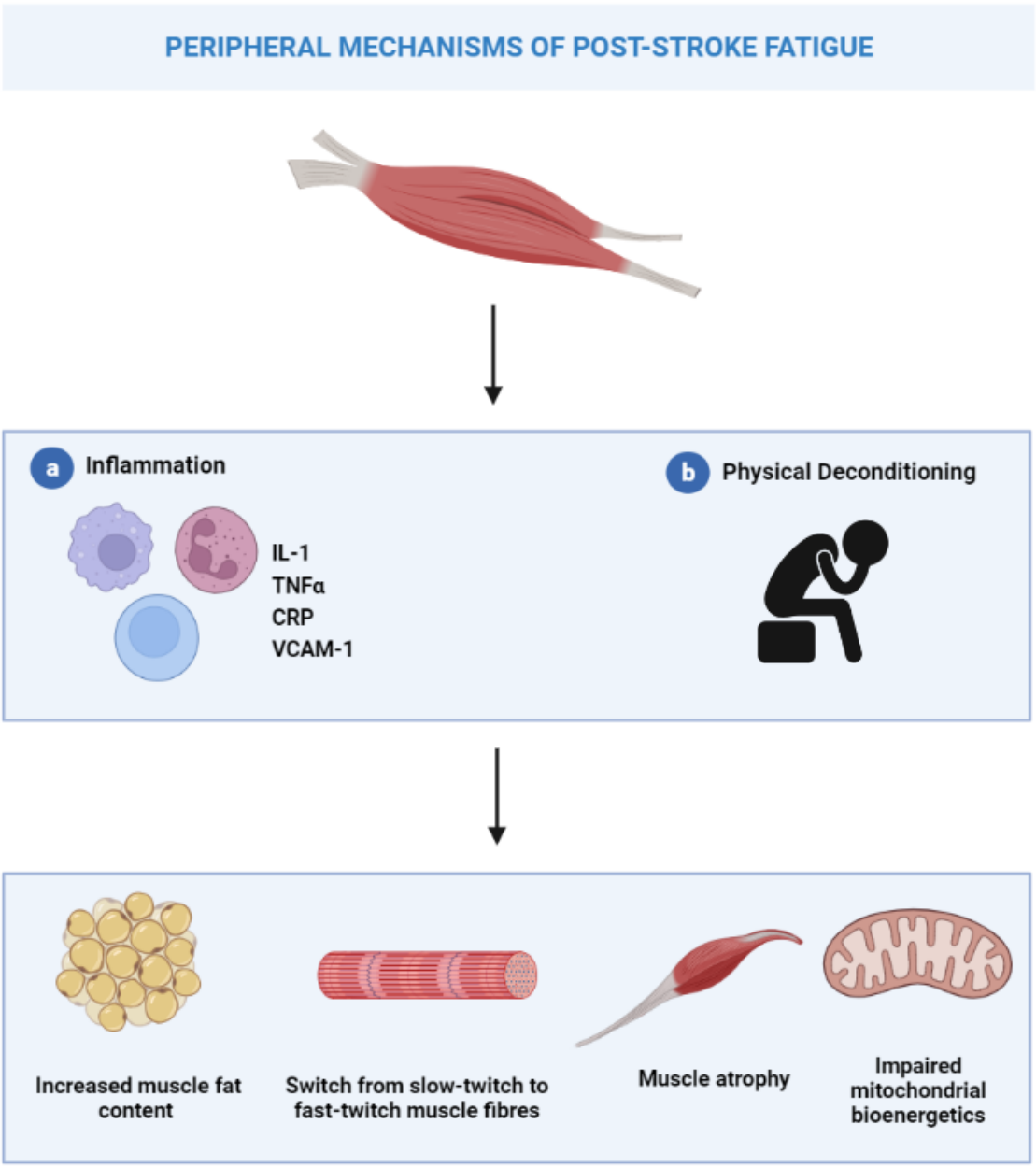
1.3.2.4 Differentiating central and peripheral causes of fatigue

Recognised investigations to differentiate between central and peripheral causes of PSF are lacking. If we consider the concept of peripheral fatigue being caused by the accumulation of metabolites such as lactate, inorganic phosphate (Pi), hydrogen (H^+) ions and reactive oxygen species (ROS), that cause the perception of fatigue (Wan et al., 2017, Sundberg and Fitts, 2019) and central fatigue being caused by central activation failure (CAF) (Kuppuswamy et al., 2015) then EMG biomarkers may vary in each condition. In CAF with enduring activity the primary driver of fatigue is a reduction in corticomotor excitability and in general this results in a gradual reduction in the number of motor units recruited in a muscular activity (Kuppuswamy et al., 2015). CAF can be quantified using the interpolation twitch technique (ITT) which is the gold standard to evaluate non-invasively the ability to maximally activate motor units (Shield and Zhou, 2004). The ITT involves percutaneous electrical stimulation of the muscle nerve during maximal voluntary contraction (MVC). The stimulation produces an increase in the force torque (i.e., superimposed twitch) as it recruits muscle fibres that were not recruited during the maximal effort. An increase in force elicited by the superimposed stimulation suggests a deficit in voluntary activation (VA) (i.e., the larger the superimposed twitch, the weaker the VA). A reduction in %VA due to exercise is considered central fatigue (Millet et al., 2012). Peripheral fatigue can be investigated by stimulating the muscle in a relaxed state before, during or after the fatiguing exercise. This is usually done by progressively increasing the intensity of the electrical stimulus until increasing the intensity does not increase the electrical or mechanical responses (i.e., optimal intensity) (Millet et al., 2012). Studies using surface EMG (SEMG) have shown that mean or median power spectrum frequencies decrease during sustained MVC (Kranz et al., 1985, Moritani et al., 1986). During maximal effort, a decrease in muscle fibre conduction velocity (MFCV) has often been reported which contributes to this decline of power frequency (Zwarts et al., 1987, Arendt-Nielsen and Mills, 1988). The decline in MFCV reflects the accumulation of metabolic by products during the fatiguing exercise (Brody et al., 1991, Masuda et al., 1999, Zwarts and Stegeman, 2003) and thus can be used as a measure of peripheral fatigue. A study by Schillings et al. (2003) investigated the contribution of central and peripheral factors of fatigue during a 2-min

sustained MVC of the biceps brachii in 20 healthy subjects. In this study, MFCV was determined using SEMG and peripheral fatigue was quantified by comparing force development following electrical stimulation on the motor endplate *before* and *after* the contraction. To measure force loss due to central factors, the investigators used superimposed electrical simulation *during* contraction. The investigators found voluntary force during the MVC decreased by 38% with peripheral factors contributing to 89% of this decline. This was indicated by a decline in MFCV during the first minute of contraction which then levelled off. The further decline in voluntary force (12%) could be attributed to an increase in CAF during the second half of the contraction (Schillings et al., 2003). These findings demonstrate the contribution of both central and peripheral fatigue in the decline of voluntary force. They also show how peripheral fatigue seems to dominate, but central factors become more prominent during longer sustained MVCs.


CENTRAL MECHANISMS OF POST-STROKE FATIGUE
POSSIBLE PREDISPOSING FACTORS
SUBCORTICAL STROKES
INCREASED LESION BURDEN
INCREASED STROKE SEVERITY
PATHOPHYSIOLOGICAL CONSEQUENCES
TNF-α
IL-1
Microglial inflammatory cascade
ROS
Increased oxidative stress
Decreased cortical excitability

(A)



(B)

Figure 1. Proposed central (A) and peripheral (B) mechanisms of post-stroke fatigue. *Figure made by author using Biorender (BioRender).*

1.4 Risk factors for post-stroke fatigue

Studies have postulated numerous risk factors for PSF development including emotional disorders, pre-stroke fatigue, sleep disorders, comorbid disease and neurological disability (Schepers et al., 2006a, Ponchel et al., 2015).

1.4.1 Sociodemographic factors

Few studies have reported on demographic factors associated with PSF, including a higher prevalence in females that mirrors fatigue in the general population (Watt et al., 2000, Schepers et al., 2006a) , but no clear relation with ethnicity, marital status or educational level (Ponchel et al., 2015). Several studies have however reported an association between PSF and older age (Glader et al., 2002, Schepers et al., 2006a, Feigin et al., 2012).

1.4.2 Emotional disturbances

Post-stroke depression (PSD) is experienced by at least one-third of stroke survivors (Hackett et al., 2005). In many patients, PSD co-exists with PSF (Wu et al., 2014) and is associated with greater fatigue severity and impact on daily living (Schepers et al., 2006a). There is evidence to suggest that both pre- and post-stroke depression independently predict development of PSF (Naess et al., 2012b, Duncan et al., 2015). Fatigue is also a manifestation of depression with many diagnostic tools listing it as a main diagnostic criteria (Demyttenaere et al., 2005). However, evidence supports the notion of PSF and PSD being two distinct clinical entities. First, PSF is more prevalent than PSD with studies in stroke outpatient settings estimating only 38-57% of patients with PSF also had PSD (Ingles et al., 1999, Van Der Werf et al., 2001) . Second, pharmacological studies have shown that antidepressant therapies (e.g., Fluoxetine) do not effectively prevent or treat PSF (Karaiskos et al., 2012, Choi-Kwon et al., 2007b). It is important that researchers control for the presence of depression to allow greater accuracy in prevalence estimates and enhance investigation of targeted treatments for PSF (Van De Port et al., 2007). Some studies have also found an association between anxiety and PSF, however anxiety has been less frequently assessed (Duncan et al., 2015).

Like PSD, apathy is a frequent occurring symptom after stroke affecting 30% of patients (van Dalen et al., 2013, Caeiro et al., 2013). Apathy is defined as a reduction in goal-directed activity in the cognitive, emotional, behavioural or social domains of a patient's life and may be difficult

to differentiate from PSF due to similarities in their behavioural manifestation (i.e., less energy) (Robert et al., 2018). Symptoms of apathy include loss of motivation, reduced levels of physical activity, difficulty in maintaining activity or conversation, and loss of interest in their health and wellbeing (Marin, 1990, Tay et al., 2021, Ho et al., 2021a, Tay et al., 2020). Post-stroke apathy is increasingly recognised as a consequence of neurobiological changes triggered by stroke. For example, apathy has been associated with damage to the frontal subcortical circuit of which the anterior cingulate is associated with goal directed behaviour (Levy and Dubois, 2006, Tekin and Cummings, 2002). It has also been associated with damage to the basal ganglia which also plays a role in goal directed behaviour (Levy and Dubois, 2006). Preliminary findings indicate that PSF and post-stroke apathy are not correlated and do not interact (Douven et al., 2017), although further work is needed to optimise ways to differentiate these two distinct entities.

1.4.3 Pre-stroke fatigue and comorbid disease

Though many patients report fatigue as a new symptom since their stroke, studies have found that pre-stroke fatigue is a significant predictor of PSF (Egerton et al., 2015). A study of 265 patients with first-ever ischaemic stroke found that pre-stroke fatigue (fatigue lasting longer than 3-months) significantly predicted early (< 2 week) PSF (Wang et al., 2014). Pre-stroke fatigue is also associated with longer term (18 months) PSF (Lerdal et al., 2012), as well as the clinical severity of PSF (Choi-Kwon et al., 2004). It appears this association may be even stronger than the effects of other contributing factors (depression, functional dependency) (Choi-Kwon et al., 2004). However, a limitation of these studies is that data on pre-stroke fatigue must be collected retrospectively, which introduces recall bias that may affect the accuracy of results (Althubaiti, 2016). Fatigue as a symptom also complicates many vascular conditions such as hypertension (Van Der Werf et al., 2001), diabetes (Naess et al., 2005), and ischaemic heart disease (Eijsden et al., 2012), which are common among patients suffering stroke. However, the contribution of these conditions to PSF is inconsistent and unclear. Medications often prescribed following stroke, including beta-blockers (McKelvie et al., 1991) and sedatives (Braley et al., 2015) are well known to cause fatigue but evidence on how they contribute to PSF is lacking.

1.4.4 Sleep disturbances

Approximately 50-70% of stroke survivors experience obstructive sleep apnoea (OSA) (Tosun et al., 2008, Ifergane et al., 2016), a condition whereby the upper airway repeatedly narrows during sleep, interrupting one's progression through to the restful phase of sleep (Davis et al., 2013). Clinical symptoms of OSA include snoring, morning headaches, excessive daytime somnolence, lack of energy and fatigue (Chervin, 2000, Sharma and Culebras, 2016). OSA is a modifiable risk factor for stroke (Davis et al., 2013) and associated with vascular risk factors such as hypertension, diabetes, atrial fibrillation and heart failure (Davis et al., 2013, Lavie et al., 2000). Studies link the severity of OSA with death and dependency following stroke (Turkington et al., 2004). While the main mechanism of OSA related fatigue differs to that of PSF, the accompanying rise in systemic inflammation (Ifergane et al., 2016) and autonomic dysregulation (Sharma and Culebras, 2016, Nagata et al., 2008) may contribute to a central fatiguing effect. PSF has also been found to be associated with other sleep disorders such as insomnia and frequent wakening during the night (Wu et al., 2015b).

1.4.5 Physical and cognitive disability

Studies have suggested that fatigue is closely related to the degree of physical disability. Appelros et al. (2006) investigated the predictors of pain and fatigue in 253 patients with first-ever stroke (Appelros, 2006). In this study, a total of 135 (53%) patients had fatigue and were examined at baseline and after 1-year and found PSF was significantly associated with greater disability (Modified Rankin Scale), more so than depression, sleep disturbances, stroke severity or cognitive state. Indeed, relating to cognition, a recent systematic review failed to find significant associations between PSF and selective attention, executive function, memory, language, and overall cognitive assessment scores (Ponchel et al., 2015). This may be confounded however by the reliability of self-reporting of fatigue in patients with cognitive impairment (Hilari et al., 2007, Engelter et al., 2006, Barrett, 2009).

1.5 Measuring post-stroke fatigue

Currently, fatigue is most commonly measured using patient-reported outcome measures (PROMS) which ask individuals if they are experiencing fatigue, sleepiness or tiredness (Gawron, 2016). None of the scales were developed specifically for fatigue after stroke however they have been validated in stroke populations (Choi-Kwon and Kim, 2011, Lerdal et al., 2009, Elbers et al., 2012, Mead et al., 2007). One of the most commonly used is the fatigue

severity scale (FSS), developed by Krupp *et al*. (1989) to measure fatigue in patients with systemic lupus erythematosus (SLE) and multiple sclerosis (MS), and has subsequently been used in other chronic conditions with high sensitivity and reliability (Learmonth et al., 2013, Whitehead, 2009). Other self-report scales used to measure fatigue are listed in *Table 1*. Interpretation of changes in PROM scores is of concern in studies evaluating the effects of interventions aimed at reducing fatigue (Boyce et al., 2014). Estimates of minimal clinically important differences (MCID), defined as the smallest change in a score that the patient would perceive as beneficial (Jaeschke et al., 1989), help with the evaluation and interpretation of PROM scores. Self-reported measures of fatigue provide a real understanding of the impact of fatigue on ones health status and well-being from the patient's perspective (Reeves et al., 2018). Most PROMs are reliable, reproducible, easy to complete, and allow for efficient data collection (Reeves et al., 2018, Reeve et al., 2013, Mead et al., 2007). They can also assess a broad array of fatigue domains (Gawron, 2016) (*Table 1*). However, self-reported measures of fatigue are subjective and can be influenced by the participant's mood or their desire to answer "correctly'' (Gawron, 2016). The scale used to measure fatigue in this study will be discussed in detail in subsequent chapters. The scales included in *Table 1* were selected as they appear to be the most commonly used fatigue scales in the literature.

Table 1. Scales used to assess fatigue and the dimensions they measure.

	Dimensions						
Scale	***Physical fatigue***	***Mental fatigue***	***General fatigue***	***Fatigue severity***	***Motivation***	***Physical activity***	***Impact on ADLs***
Fatigue severity scale (FSS)	✓	✓		✓	✓	✓	✓
Multidimensional fatigue inventory (MFI-20)	✓	✓	✓		✓	✓	
Modified Fatigue Impact Scale (FIS)	✓	✓			✓		✓
Checklist Individual Strength – subscale fatigue (CIS-*f*)	✓	✓	✓	✓	✓	✓	
Fatigue Assessment Scale (Zedlitz et al.)	✓	✓	✓	✓	✓	✓	
Visual Analogue Scale -fatigue (VAS-F)			✓	✓		✓	
The Short Form (36) Health Survey (SF-36) – vitality component			✓	✓			
Profile of Mood States – Fatigue subscale (POMS-F)			✓	✓			

Source: Lerdal et al. (2009). ADLs = Activities of daily living.

1.6 Current treatments for post-stroke fatigue

Relatively little research focused on the prevention and treatment of PSF has been conducted, although interest in this area has increased over the last 10 years. Studies that have been undertaken have investigated a wide range of interventions, reflecting the etiological complexity of this condition. Such interventions include pharmacological, psychological, physical activity-based, and electrical treatments in the main and are summarised in *Table 2.* To formulate Table 2 the following databases were searched: PUBMED, MEDLINE, The Cochrane Library, and Web of Science. Subject heading and free text terms relating to stroke (e.g., ischaemic stroke, haemorrhagic stroke, subarachnoid haemorrhage), fatigue (lethargy, tiredness, exhaustion) were used to produce a search strategy for OVID MEDLINE, which was adjusted using Boolean operators for the other databases. Articles involving human study that were full text and English language were used in the critical analysis irrespective of which part of the stroke continuum they related to (e.g., acute phase, rehabilitation phase, longer term). References of included articles were also analysed for additional information sources. A second researcher reviewed the references and the accuracy of the table.

Table 2. Summary of randomised controlled trials investigating the efficacy of various interventions for the prevention and treatment of post-stroke fatigue.

Author	Country	Design	Participants (intervention)	Criteria	Fatigue scale	Type of intervention	Results/ Comments
Prevention							
West et al. (2019)	Denmark	Quasi-RCT	90 (44)	Acute stroke patients admitted to rehabilitation unit	MFI-20	Naturalistic light intervention using multi-coloured LED lights that vary irradiance depending on time-of-day vs usual rehabilitation lighting	Significant reductions in perceived fatigue at discharge from the intervention unit.
Dennis et al. (2019)	UK	Multicentre, double-blind, placebo-controlled RCT	3,127 (1,564)	Acute stroke patients 2 – 15 days after stroke onset	SF-36 vitality	Fluoxetine 20mg orally once daily for 6 months vs placebo	No significant reductions in vitality measures or the number of patients meeting fatigue criteria
Treatment							
	Anti-depressants and mood stabilisation						
Choi Kwon et al. (2007a)	South Korea	Placebo controlled double-blind RCT	83 (40)	Stroke patients attending outpatient clinic with PSD, PSEI or PSAP	FSS & VAS-f	Fluoxetine (anti-depressant) vs placebo	No difference in the number of people with fatigue and in fatigue severity between

							treatment and placebo group at 3 and 6 months.
Karaiskos et al. (2012)	Greece	Open-label RCT	60 (20:20:20)	Diagnosis of first-ever stroke within the last 12 months	FSS	Duloxetine vs citalopram vs sertraline	PSF = secondary outcome Significant improvement in depression and anxiety but not fatigue in all three treatment groups at 3 months
Johansson et al. (2012b)	Sweden	Placebo controlled double-blind randomised cross-over trial	12 (12)	Stroke or TBI >12 months earlier (but no more than 10 years) Subjects had recovered from neurological symptoms but suffered debilitating mental fatigue for at least one year	MFS	Monoaminergic stabiliser (-) -OSU6162 vs placebo	Significant improvement in MFS score at 4 weeks
	Stimulants and neuroprotectants						

Bivard et al. (2017)	Australia	Double-blind, placebo- controlled, crossover RCT	36 (18)	≥ 3-months post-stroke MFI score ≥ 60	MFI-20	Oral Modafinil 200mg daily for 6-weeks vs placebo	Significant improvements in MFI scores with Modafinil
Poulsen et al. (2015)	Denmark	Double-blind, placebo controlled RCT	41 (21)	Stroke within 14 days mRS ≤ 3 MFI-20 ≥ 20	MFI-20	Modafinil 400mg daily for 90 days (200mg daily of > 65 years) vs placebo	Modafinil did not result in significant improvement in MFI-20 but did improve FSS
Ogden et al. (1998b)	New Zealand	Placebo controlled Double-blind RCT	31 (16)	SAH ≥ 3 months prior	Self-reported experience of fatigue	Tirilazad mesylate vs placebo	PSF = secondary outcome Significantly lower level of fatigue reported in treatment group compared to control at 10 days
	Vitamin and Qi supplementation						
Guo et al. (2012)	China	Placebo controlled parallel RCT	90 (30:30:30)	Ischaemic stroke with qi deficiency syndrome	FSS	Traditional Chinese medicine and rehabilitation therapy vs usual care	No significant improvement in FSS score at 4 weeks.
Gurak et al. (2005)	Russia	Placebo controlled parallel RCT	30 (15)	Stroke or myocardial infarction	MFI-20	Enerion (synthetic derivative of vitamin B1) vs placebo	Significant reduction in total MFI (16%, *P*=0.01)

Liu et al. (2016)	Taiwan	Double-blind RCT	64 (29)	Hemorrhagic- or infarction-type stroke ≥ 3 months prior PSF (BFI ≥4)	BFI	Oral Astragalus Membranaceus 2.8g TDS for 28 days vs placebo	Large reductions in fatigue score extending to 2 months follow up.
	Psychological therapies						
Johansson et al. (2012a)	Sweden	Placebo controlled cross-over RCT	16 (12)	Stroke or TBI >12 months earlier Aged 30-65 Mental fatigue (MFS>10)	MFS	Mindfulness-based stress reduction vs usual care	Significant improvement in MFS score at 8 weeks
Nguyen at al. (2017)	Australia	Feasibility RCT	15 (9)	History of stroke Aged 16-70 years FSS ≥ 4 Poor sleep (PSQI>5)	FSS	Cognitive behavioural therapy (8 sessions) alongside aerobic exercise prescription vs usual care	Significantly lower fatigue and depression scores in the intervention group as well as improved HRQoL
Clarke et al. (2012)	New Zealand	Single-blind, parallel RCT	16 (9)	History of stroke FSS >3.9	FSS	Fatigue management group (FMG) – 6 sessions, 60 min weekly: education, sleep / relaxation, exercise /	No significant benefit of FMG over general stroke education in reducing fatigue.

						nutrition, mood vs general stroke education	
	Exercise and other physical therapies						
Zedlitz et al. (2012)	Netherlands	Single-blind RCT	83 (38)	Stroke > 4 months before recruitment CIS-f ≥40) Aged 18-70 years Able to walk independently	CIS-f	CBT and graded activity training vs CBT alone	Improvement in PSF after CBT. Improvement greater when combined with graded activity training (e.g. walking, strength training)
Zhou et al. (2010)	China	Parallel RCT	128 (64)		SSQOL-energy	Electro- acupuncture (EA) combined with cupping **vs** medication (compound aminobutyric acid vitamin E, magnesium gluconate + sertraline)	Significant increase in baseline SSQOL-energy score in both groups at 5 weeks. Improvement significantly greater in EA + cupping group
Brown et al. (2013)	USA	Double-blind RCT	32 (15)	Ischaemic stroke with evidence of OSA (eligible if stroke within 7 days	FSS	Continuous Positive airway pressure (CPAP) vs usual care	PSF = secondary outcome Slight improvement in FSS score in active CPAP treatment group compared

				of sleep apnoea assessment)			to sham CPAP at 3 months.
Aaronson et al. (2016)	Netherlands	Cross-over RCT	36 (20)	Stroke between 1-16 weeks prior and diagnosis of OSA	CIS-20	Nocturnal CPAP (min 1 hr / night) titrated to achieve AHI < 5, for 4 weeks vs usual care	No significant improvement in fatigue scores or in neurological or functional recovery
	Electrical therapies						
Zhou et al. (2010)	China	Parallel RCT	128 (64)		SSQOL – energy	Electro-acupuncture (EA) combined with cupping vs medication (compound aminobutyric acid vitamin E, magnesium gluconate + sertraline)	Significant increase in baseline SSQOL-energy score in both groups at 5 weeks. Improvement significantly greater in EA + cupping group
De Doncker et al. (2021b)	UK	Double blind RCT	30 (20)	First-ever stroke >3 months prior FSS-7 ≥ 4	FSS	2 x 20-minute sessions of Anodal tDCS separated by 10 minutes of rest vs sham stimulation	Significant reduction in FSS at 1 week post intervention in the tDCS group but not at 4 weeks
Dong et al. (2021)	China	Double blind RCT	60 (30)	Stroke > 3 months prior and within 1 year	FSS	20-minute sessions of anodal tDCS 6 x a week for	Significant reduction in FSS at 4 week follow up in the active group vs

	FSS >36 Aged 18-65 years	4 weeks alongside routine rehabilitation vs sham.	sham group but not at 8 weeks follow up.

Abbreviations: RCT = Randomised controlled trial; MFI-20 = Multidimensional fatigue inventory-20; SF-36 = The short-form 36 health survey; FSS = Fatigue severity scale; VAS-f = Visual analogue scale-fatigue; PSF = Post-stroke fatigue; MFS = Mental fatigue scale; SAH = Subarachnoid haemorrhage; BFI = Brief fatigue inventory; HRQoL = Health related quality of life; FMG = Fatigue management group; CIS-f = Checklist individual strength-subscale fatigue; CBT = Cognitive behavioural therapy; SSQoL – energy = Stroke specific quality of life scale – energy domain; EA = Electro-acupuncture; OSA = Obstructive sleep apnoea; CPAP = Continuous positive airway pressure; tDCS = Transcranial direct current stimulation.

1.6.1 Prevention of post-stroke fatigue

Natural and artificial light therapy has been utilized in various non-pharmacological treatments of psychological, emotional, and behavioural disorders to balance the circadian rhythm (internal biological process that regulates the sleep-wake cycle) (Shirani and St. Louis, 2009, Onega and Pierce, 2020). Blue light therapy has been shown to be effective in alleviating fatigue in patients with traumatic brain injury (TBI) (Sinclair et al., 2014, Connolly et al., 2021). Stroke patients admitted for rehabilitation are mostly indoors and not exposed to natural daytime light. One study has focused primarily on preventing fatigue after stroke using naturalistic lighting for an inpatient rehabilitation unit (West et al., 2019). In this study, 90 patients with acute stroke were randomised to either a rehabilitation unit using multi-coloured LED lights that vary irradiance according to the time of the day (n=44) or a one that used standard lighting throughout (n=46). Discharge fatigue scores (Multidimensional fatigue inventory, MFI-20) were significantly lower among those rehabilitated in the unit using naturalistic light compared to usual lighting (-20.6%, P=0.025), and sleep quality (Pittsburg Sleep Quality Index) appeared improved albeit not statistically (-13%, P=0.36).

Another study that focussed on the prevention of PSF was a study by Dennis et al. (2019) who conducted a large RCT evaluating early initiation (2-15 days after stroke onset) of fluoxetine (an antidepressant) 20 mg daily for 6 months on functional recovery after stroke (fluoxetine on functional outcomes after acute stroke, FOCUS trial) . The investigators found no difference in the primary outcome of the modified Rankin Score (mRS) between the treatment and placebo groups. They also found no difference in fatigue (measured using the SF-36 vitality sub-component) between the two groups at 6-months.

1.6.2 Pharmacological treatments

1.6.2.1 Antidepressants and mood stabilisers

Affective disorders are commonly associated with PSF, and antidepressant therapy including fluoxetine, citalopram, sertraline and duloxetine have been studied in randomised controlled trials (RCTs) although none have demonstrated improved levels of fatigue over control groups (Karaiskos et al., 2012, Choi-Kwon et al., 2007b). In a double-blind, cross-over trial, Johansson

et al. (2012b) studied the effects of a monoaminergic stabiliser (-OSU6162), that acts primarily on dopaminergic pathways to stabilise mood, on patients with mental fatigue (measured using the Mental Fatigue Scale, MFS) after stroke (n=6) and traumatic brain injury (TBI) (n=6). In this study, half of the participants started on the active drug and the other half on the placebo for 4-weeks. At the end of the 4-weeks period, participants who started on the active substance were changed to placebo for an additional 4-weeks. The investigators found the molecule resulted in significant improvements in mental fatigue compared to placebo (35% reduction in MFS score after -OSU6162 compared to placebo, *P*=0.031), although the study suffered from a small sample size (n=12; 6 stroke patients) and high risk of bias as there was no control group.

1.6.2.2 Stimulants and neuroprotectants

Modafinil is a wakefulness-promoting agent that excites monoaminergic pathways, stimulating the release of dopamine, histamine, serotonin, norepinephrine and orexin in the brain (Gerrard and Malcolm, 2007). It exerts neuroprotective effects by increasing anti-oxidative processes and reducing free-radical formation (Xiao et al., 2004). Modafinil has been studied in 2 RCTs of patients with clinically significant PSF. The Modafinil in Debilitating Fatigue after Stroke (MIDAS) phase 2 trial randomised 36 patients at least 3 months post stroke to either Modafinil (200mg daily) or placebo for 6 weeks, and found significantly lower mean MFI-20 scores at end of treatment (-7.38, 95% CI, −21.76 to −2.99 *P*= <0.001) in the Modafinil group (Bivard et al., 2017) although this magnitude of change fell short of that considered clinically meaningful (Nordin et al., 2016).

Poulsen *et al*. (2015) similarly randomised 41 patients with PSF, only earlier (< 2 weeks post stroke), to a higher dose of Modafinil (400mg daily) or placebo for 90 days (Poulsen et al., 2015). At day 90, they failed to see a significant difference in the primary outcome of fatigue (MFI-20). However, the intervention group exhibited significantly reduced median scores on the FSS-9 compared to the placebo group (36 vs 49 points respectively, *P*=0.019). The intervention group also exhibited significantly reduced median scores on the reduced form of the FSS (FSS-7) compared to placebo (22 vs 37.5 points, *P*=0.042). Although this finding seems promising, the FSS was a secondary outcome measure, and the study was not powered to detect significant differences in the outcome measures.

Tirilazad mesylate is lipid soluble steroid hypothesised to help cell membrane stabilisation and free radical scavenging (Kassell et al., 1996). In a double-blind trial by Ogden *et al.*(1998a) patients were given either the active treatment (n=9) or vehicle (100 ml of sterile solution) (n=9) for 10 days after subarachnoid haemorrhage (SAH). Debilitating fatigue was measured using an interview-like questionnaire (e.g., are you experiencing low energy, do you feel exhausted for hours to days after an activity, are you falling to sleep in unusual situations). Questions were answered with either a yes or no and the two groups were compared with Chi squared tests (χ^2). The proportion of patients with debilitating fatigue at 3-months was significantly lower in the Tirilazad mesylate group compared to placebo with all nine vehicle-treated patients reporting fatigue as a problem, compared to four patients in the drug-treated group (χ^2 — 6.92, $df = 1$, $P < 0.01$). Participants in the treatment group also scored significantly better on the attention index (measures concentration, psychomotor speed, and sustained attention) compared to the vehicle-treated group ($\chi^2 = 5.56$, $df = 1$, $P < 0.02$). However, again the sample size was small, outcome measures crude, and the intervention was not specifically designed for PSF.

1.6.2.3 Vitamin and Qi supplementation

Vitamin deficiencies have been associated with PSF and its severity. In an observational study of first ever lacunar stroke, PSF severity was greatest among those with vitamin B12 deficiency (Huijts et al., 2012), postulating vitamin supplementation as a potential treatment. Gurak et al. (2005) reported lower MFI-20 scores in patients with PSF receiving Enerion, a synthetic derivative of vitamin B1, compared to control (-16%, P=0.01). Chinese herbal medicines that aim to supplement and tonify *Qi* (vital energy of your body) have long been used to treat conditions such as fatigue. A commonly used compound, *Astragalus Mebranaceous* is reported to contain flavonoids and polysaccharides that result in anti-oxidant and immunoregulatory effects (Ding et al., 2017). A RCT by Liu et al. (2016) reported significantly reduced fatigue (Brief Fatigue Index, BFI) in those allocated oral *astragalus* compared to placebo both at end of treatment (mean difference -8.03, P=0.01) and at 2-months follow up (mean difference –9.47, P=0.05) (Liu et al., 2016). A subsequent systematic review and meta-analysis of Chinese herbal medicines for PSF reported significant benefits from treatment with various *Qi* supplements among 16 studies (Xu et al., 2019). However, all the studies of vitamin and Qi supplements suffer from inadequate sample size and methodological deficiencies (e.g., lack of

placebo and insufficient information on randomisation or blinding) that mean definitive efficacy data for such interventions is still required.

1.6.3 Psychological treatments

Psychological therapies have been effective in cancer related fatigue (Armes et al., 2007) and chronic fatigue syndrome (White et al., 2011) and is a promising therapy for PSF. A pilot RCT (n=15) of Cognitive Behavioural Therapy (CBT), a talking therapy that aims to reduce symptoms of depression and anxiety disorders by changing the way you think and behave, demonstrated significant reductions in FSS-7 compared to usual care (mean difference -1.92; 95% CI -0.24 to -3.6) among 15 patients with PSF (Nguyen et al., 2019). Whether CBT affects fatigue directly or indirectly through its effects on mood and sleep is uncertain (Driessen and Hollon, 2010, Williams et al., 2013). Group cognitive therapy was also associated with reductions in PSF severity (Checklist Individual Strength – subscale fatigue, CIS-f) when delivered weekly for 12 weeks, however, reductions in PSF were greater when it was combined with graded physical activity (Zedlitz et al., 2012). Mindfulness-based stress reduction has also been shown to help reduce mental fatigue amongst patients with stroke and TBI (Johansson et al., 2012a), while a 6-week fatigue management program including education on sleep, relaxation, exercise, nutrition, and mood was no better than general stroke education at fatigue reduction (Clarke et al., 2012). Wu et al. (2017) have recently co-designed a psychological fatigue management manual that appears feasible, acceptable and effective for patients with PSF, but requires testing against a control group.

1.6.4 Physical treatments

1.6.4.1 Exercise

There are several mechanisms by which physical exercise may plausibly improve PSF. For example, studies have shown how exercise can increase cerebral blood flow (CBF) by activating the sympathetic nervous system (SNS) (Dishman et al., 2006). A study of 8 healthy volunteers by Williamson et al. (2003) found that central command during handgrip exercise independently increases regional CBF (measured using single-photon-emission computed tomography) in insular and anterior cingulated regions (Williamson et al., 2003). Ischaemia/reperfusion injury (I/R injury) to these brain areas have been associated with subjective feelings of impaired energy (Manes et al., 1999). In MS, fatigue development is associated with atrophy of frontal and posterior parietal cortices (Calabrese et al., 2010), while

in aging humans aerobic fitness reduces tissue loss in these brain regions (Colcombe et al., 2003). Exercise can ameliorate physical deconditioning through increasing muscle activation and recruitment, lengthening muscle contraction, enhancing insulin sensitivity and vascular morphology (Billinger et al., 2012a), ultimately leading to improved CRF, muscle strength and mobility (Pang et al., 2005b, Moore et al., 2010). Further, exercise can modulate neurotransmitter pathways involving dopamine, noradrenaline and serotonin to enhance alertness and cognition (Lin and Kuo, 2013).

No RCTs have investigated the role of exercise alone in reducing fatigue, however the aforementioned study by Zedlitz *et al.* (2012) demonstrated greater reductions in fatigue severity when cognitive therapy was combined with graded exercise, compared to cognitive therapy alone. Studies evaluating the effects of exercise programs in stroke patients without fatigue have demonstrated improvements in the vitality subscale of the SF-36 (Kirk et al., 2014, Aidar et al., 2016), energy subscale of the stroke specific quality of life scale (SS-QOL) (Yoo and Yoo, 2011), as well as measures of sleep quality (Flansbjer et al., 2008). However, results are inconsistent with others reporting no significant changes in fatigue following exercise (Duncan et al., 2012). In addition, it is not clear whether exercise targets peripheral or central causes of fatigue. Overall, there is a paucity of trial evidence in this area that requires addressing with future study design.

1.6.4.2 Continuous Positive Airway Pressure

Sleep disorders, including OSA, commonly complicate PSF (Ho et al., 2021b, Lerdal et al., 2011). Continuous positive airway pressure (CPAP) therapy is a type of non-invasive ventilation (NIV) that delivers oxygenated air into the airways through a mask during sleep and is the gold standard treatment for OSA (Spicuzza et al., 2015). CPAP has been trialled in 2 RCTs of patients with fatigue and OSA diagnosed post stroke, but unfortunately was not found to improve fatigue scores compared to controls (Aaronson et al., 2016, Brown et al., 2013).

1.6.5 Electrical therapies

1.6.5.1 Electroacupuncture and cupping

Electroacupuncture is the delivery of small amounts of electric current through acupuncture needles inserted into specific parts of the body. It is thought to increase somatosensory stimulation from affected areas, potentially affecting cortical reorganisation and neuronal excitability, and has been widely used to treat neurological conditions in China (Liu et al., 2015). Zhou et al. (2010) demonstrated improved scores on the energy sub-domain of the SSQOL in patients with PSF randomised to 5 weeks of electroacupuncture and cupping to the lumbar spine (n=64) compared to vitamin supplementation and sertraline (n=64) (Zhou et al., 2010). Interest in electrical stimulation for neuroplastic motor recovery is growing (Meyers et al., 2018), and this may be an interesting avenue for future research.

1.6.5.2 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation method that can regulate cortical excitability and output. It is relatively low cost, easy to use and safe (Brunoni et al., 2012). A single dose of tDCS lasting a few minutes can have cortical excitatory effects that last hours with repeated doses incurring longer lasting effects (Nitsche et al., 2008). Studies involving tDCS of the prefrontal (motor) cortex have shown promise in fatigue states associated with MS (Saiote et al., 2014). De Doncker et al. (2021b) recently demonstrated that a single dose of tDCS (2 x 20 minute sessions separated by a 10 minute break) was associated with significantly greater reductions in FSS-7 amongst patients with PSF compared to sham control, at 1 week post intervention but not at 1 month (De Doncker et al., 2021b). Another study randomised 60 patients with PSF to either 4 weeks of daily tDCS (20 minutes per day, 6 x per week) delivered in hospital compared to sham stimulation and demonstrated safety and acceptability as well as greater reductions in FSS in the intervention group at 4 weeks but not at 8-week follow-up (Dong et al., 2021). Thus, tDCS appears to be safe and effective at reducing fatigue severity but may not result in long lasting changes.

1.6.6 Knowledge gaps

What is evident is that both clinicians and patients feel ill equipped to manage PSF, which is not a benign condition, and impacts negatively on clinical outcomes. There is still a great deal

we do not understand about PSF including the mechanism of development which may differ according to time point along the journey of the stroke survivor. This is a key factor that future research needs to address for more targeted and effective treatments to be developed. A greater understanding of the pathophysiological mechanisms may also uncover biomarkers that may hopefully correlate with not only the diagnosis, but the clinical severity of PSF, enabling a more profiled approach to treatment and the ability to monitor responses to treatments.

We still do not have a clear understanding as to what causes PSF, whether central mechanisms lead to cognitive phenotypes, while peripheral mechanisms lead to distinct physical phenotypes, or whether a mixture of phenotypes are at play in many individuals with PSF. The interventional studies to date are small and vary in methodological quality but some have shown promise despite the significant variation in the types of treatments. An intervention that may benefit a multitude of mechanisms that may promote PSF may become an effective treatment for PSF, especially if it is safe, cheap and self-delivered.

1.7 Remote ischaemic conditioning

Remote ischaemic conditioning (RIC) is a strategy whereby brief, reversible episodes of ischaemia and reperfusion, typically via the cyclical application of a blood pressure (BP) cuff to a limb inflated to above systolic pressure (mmHg), confers systemic protection against ischaemia/reperfusion (I/R) injury in remote organs or tissues (Heusch et al., 2015, Ayodele and Koch, 2017). The term I/R injury refers to the harmful effects of both oxygen deprivation (ischaemia) and reoxygenation (reperfusion) when blood flow to a tissue is compromised, and then restored (Carden and Granger, 2000). Ischaemia triggers both local and systemic inflammatory responses such as increased mast cell and macrophage activation, increased platelet-leukocyte aggregation, and an upregulation of proinflammatory gene products (e.g., cytokines) (Eltzschig and Collard, 2004, Carden and Granger, 2000). Cellular effects of prolonged ischemia include altered membrane potentials, disrupted ion distribution (e.g. calcium, sodium), reduced adenosine triphosphate (ATP) synthesis, cellular acidosis, cellular swelling and death (Eltzschig and Collard, 2004). While restoration of blood flow is necessary to prevent irreversible injury to an ischaemic tissue, reperfusion can exacerbate the cellular responses to ischemia and can cause both local and remote organ dysfunction (Carden and Granger, 2000, Abela and Homer-Vanniasinkham, 2003, Eltzschig and Collard, 2004). Reperfusion of ischaemic tissue leads to increased production of reactive oxygen species

(ROS) and alters mitochondrial calcium metabolism (Meng-Yu et al., 2018). This leads to mitochondrial calcium overload, further increase in ROS and oxidative stress (Meng-Yu et al., 2018). Oxidative stress promotes endothelial dysfunction leading to inflammation, platelet aggregation, loss of vasodilation and cardiovascular complications (Higashi et al., 2014).

The concept of 'ischaemic preconditioning' (IPC) as a protective phenomenon was first described by Murry et al. (1986) who found that four cycles of 5-minute (min) episodes of ischaemia by occlusion of the circumflex artery, separated by 5-min reperfusion, protected canine hearts from a subsequent prolonged 40-min circumflex artery occlusion. Myocardial infarct size was 75% lower in IPC treated animals compared to controls. These findings were extended when Przyklenk et al. (1993) found that brief episodes of circumflex artery occlusion using the same IPC protocol, also significantly reduced myocardium infarct size when canine hearts were subjected to a one-hour coronary artery occlusion of a different arterial territory (anterior descending artery) – *remote ischaemic conditioning (RIC).* Experimental studies later found that applying transient episodes of sub-lethal ischaemia and reperfusion to remote organs (e.g. small intestine, kidney) (Gho et al., 1996) or even a limb (Birnbaum et al., 1997, Oxman et al., 1997) also had a cardioprotective effect. There is a wealth of experimental animal data demonstrating clear cardioprotective benefits with RIC (You et al., 2019, Kerendi et al., 2005, Bromage et al., 2017). Studies show that the threshold for protection is greater in older animals (Boengler et al., 2009) and animals with comorbidities (e.g. diabetes, hypertension), with such animals requiring a more robust conditioning signal (Heusch et al., 2015). However, most pre-clinical, experimental studies use young, healthy animals who are free from any comorbidities (Ludman et al., 2010)The use of animal models has improved our understanding of RIC and the potential mechanisms underlying ischaemic conditioning (discussed in chapter 1.8), however the translation of results obtained for animals to humans has been limited. In laboratory experiments most variables are tightly controlled, and animals are treated according to a strict protocol. Furthermore, as mentioned previously animal models typically use young, healthy, genetically similar animals, however such homogeneity does not exist in the human population (Casals et al., 2011). To optimise preclinical research and improve the chances of translating animal findings to humans, multi-morbid animal models and larger models (e.g., nonhuman primates) that more adequately represent the human phenotype are needed (McCafferty et al., 2014) As well, in animal studies ischaemic preconditioning requires an intervention to be applied directly to the target organ (e.g., heart) which may not be feasible in clinical settings (Lim and Hausenloy, 2012a). This may explain why the translation of

preclinical findings on the cardioprotective effect of RIC into the clinic has been largely disappointing (Ludman et al., 2010, Bosnjak and Ge, 2017). To ensure the proper reporting of experimental data and to limit bias in the conduct of preclinical studies, it is recommended to follow established guidelines such as CAMARADES (The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) (Ma et al., 2020, Hirst et al., 2014, Macleod et al., 2004). However, several articles on preclinical studies have failed to mention details of studies such as blinding, allocation concealment and randomisation that may result in higher levels of unconscious bias (Hansen et al., 2021).

As well as protecting the heart, RIC can protect other vital organs such as the kidneys (Wever et al., 2011) liver (Tapuria et al., 2008), lungs (Xia et al., 2003) and brain (Candilio et al., 2013) against subsequent I/R injury. Jensen et al. (2011) found that hind limb RIC (4 x 5 min episodes of ischaemia) before temporarily suspending blood flow by hypothermic circulatory arrest, significantly reduced cerebral injury, neuronal damage and brain lactate levels in pigs. In another study, Kitagawa et al. (1990) looked at the effect of direct IPC on neuronal cell death in the hippocampus of the gerbil through brief episodes of common carotid artery (CCA) occlusion/reperfusion. The investigators found that 2 x 2 min episodes of bilateral CCA occlusion at one-day intervals two days before a lethal 5-min neuroischaemic insult resulted in a greater protective effect, with a significantly higher neuronal density in the CA1 region of the hippocampus (defined as the number of surviving pyramidal neurons per 1 mm length of the CA1 region) after conditioning compared with controls (188.5 ± S.E.M 3.6 vs 12.9 ± 2.2, respectively, $P<0.01$). A single 2-min episode was associated with a lower level of cerebral protection, whereas 2 x 1 min episodes undertaken only 12-hours before did not provide neuroprotection. These findings suggest the duration and timing of RIC is critical to its effectiveness. Researchers call this the 'ischaemic tolerance phenomenon.' Kitagawa et al. (1990) said that ischaemic tolerance develops when ischaemic stress is sufficient to disrupt cellular metabolism and alter gene expression, but not enough to cause cell death. Using mice, Ren et al. (2008) tested whether different protocols of ipsilateral hind limb RIC by femoral artery occlusion (FAO) (Cossarizza et al., 2019) protected against focal cerebral ischaemia from a subsequent bilateral CCA occlusion (30-min) and permanent occlusion of the left distal middle cerebral artery (MCA) (Harrison et al.). RIC with 3 x 15 min FAO (and 15-min reperfusion) immediately before ischaemia significantly reduced infarct volumes from 47.5 ± 7.6% (controls) to 9.8 ± 8.6%, and to 24.7 ± 7.3% after 2 x 15 min FAO. However, RIC with only 2 x 5 min FAO offered no protection. These findings highlight the differing ischaemic tolerances of varying tissue types, with muscle, bone and skin cells of the limb having greater

ischaemic tolerance than neuronal cells in the brain. RIC activates at least two distinct timeframes of protection: the acute effect occurs immediately and lasts 2-hours (Ren et al., 2008), while the second window of protection (i.e. delayed protection) occurs 12-24 hours later, lasting up to 48-72 hours (Kuzuya et al., 1993).

1.8 Mechanisms of remote ischaemic conditioning

The mechanisms underlying RIC are not fully understood but are postulated to involve neural, humoral and systemic inflammatory pathways (Baig et al., 2021, Xia and Ji, 2019). The mechanisms underlying RIC can be considered as three interconnected events: (1) initial events taking place at the distal tissues (limbs) in response to the RIC stimulus (2) transmission of a protective signal to a target organ or tissue (neuronal, humoral, systemic mechanism) (Galán-Arriola et al., 2021) and (3) events ococuring at the target organ or tissue which protects it from future I/R injury (Figure 2) (Hess et al., 2015, Lim and Hausenloy, 2012b).

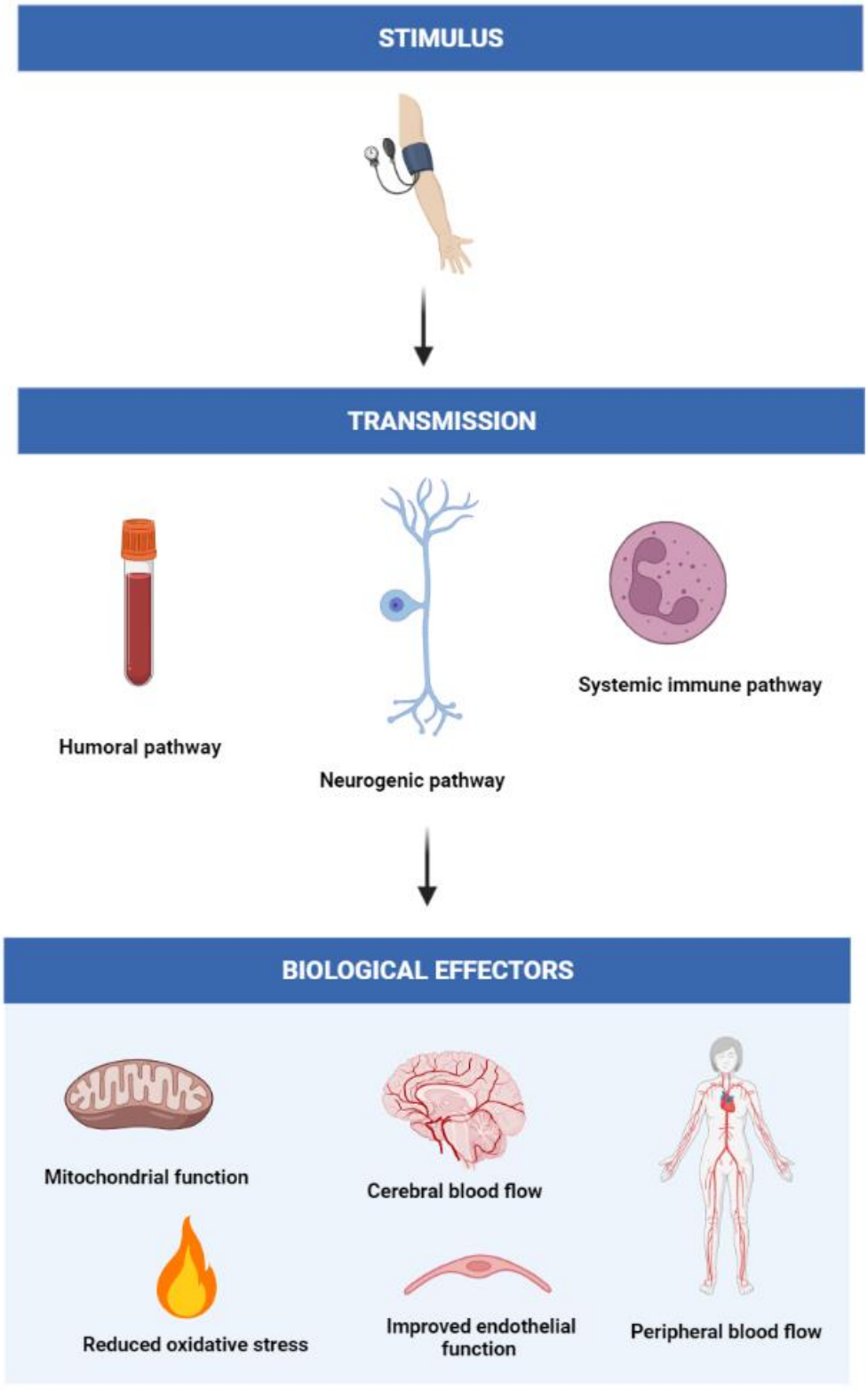


Figure 2. Proposed mechanisms of remote ischaemic conditioning. *Figure modified from Baig et al. (2021) using Biorender (BioRender) (author of this thesis is a co-author on the paper).*

1.8.1 Conditioning stimulus

Application of a conditioning stimulus (e.g., blood pressure cuff) stimulates the local release of autocoids (active biological substances producing physiological effects locally or through transport to other parts of the body) such as adenosine, bradykinin and opiods into the surrounding tissue (Liem et al., 2002, Schoemaker and van Heijningen, 2000, Hausenloy and Yellon, 2008). Experimental studies have found that pre-treating rats with Hoechst 140 (HOE-140), a bradykinin receptor antagonist, 8-(*p*-sulfophenyl)theophylline (8-SPT), an adenosine receptor antagonist or naloxone, an opiate receptor blocker, all abolish the cardioprotective effect of RIC (Schoemaker and van Heijningen, 2000, Liem et al., 2002, Shimizu et al., 2009). Uncertainty exists as to whether these molecules (collectively or individually) play a role in signal initiation or transmission, or if they have direct effects on target organs. Indeed, they may impart effects at a number of these steps (Heusch et al., 2015).

Another proposed mechanism of RIC involves the upregulation of hypoxia inducible factors (HIF) in response to hypoxia caused when the conditioning stimulus is applied to the limb (Chen et al., 2018). There are three types of HIFs (HIF-1, HIF-2 and HIF-3) which play a key role in regulating tissue oxygen homeostasis under hypoxic conditions by regulating the expression of key genes (e.g. the erythropoietin gene) (Xia and Ji, 2019). HIF binding to the erythropoietin (EPO) gene promotes erythropoiesis (process which produces red blood cells) increasing oxygen delivery to tissues (Haase, 2012, Jelkmann, 2011) and exerting neuroprotective effects (Xia and Ji, 2019). Support for the role of HIFs in the conditioning response comes from studies that have found increased levels of HIFs during RIC therapy (Yang et al., 2018, Cai et al., 2013, Albrecht et al., 2012). It is unclear how HIFs induced by RIC performed on a remote limb exerts protective effects on distal organs or tissues, since HIFs are transcriptional factors that cannot be excreted from cells (Xia and Ji, 2019). HIF-regulated gene expression must occur locally in the ischaemic limb where the conditioning stimulus was applied (Xia and Ji, 2019). Since the type of HIFs expressed in cells differ from cell to cell, it is presumed that the expression profiles of HIF-regulated genes differ depending on where the RIC stimulus is applied (e.g. limbs or kidney) (Xia and Ji, 2019). HIF-induced effects in the target organ (e.g., ischaemic brain tissue) are likely caused by HIF-regulated gene expression in the conditioned limb, rather than the direct effects of HIFs on the ischaemic brain tissue (Cai et al., 2013).

Another theory is that transient hypoxia in the limb when the conditioning stimulus is applied stimulates the release of extracellular vesicles (exosomes) by the hypoxic tissue cells which may act on the ischaemic target organ to reduce tissue damage (Giricz et al., 2014, Xiao et al., 2017). Exosomes play a neuroprotective role by transporting microRNAs, which are involved in gene expression, to ischaemic tissues (Zhang et al., 2018a, Zhao et al., 2019a).

1.8.2 Neural mechanisms

According to the neural hypothesis of RIC, the local release of adenosine and bradykinin in the remote organ or tissue stimulates nociceptive fibres which activates a neural pathway projecting to the target organ (Schoemaker and van Heijningen, 2000, Liem et al., 2002, Hausenloy and Yellon, 2008). The autonomic nervous system (ANS), spinal cord and somatosensory system are all believed to be involved in this protective signalling pathway (Heusch et al., 2015). Support for the role of the ANS in RIC comes from the finding that ganglionic blockades such as trimetaphan (Loukogeorgakis et al., 2005) and hexamethonium (Gho et al., 1996, Schoemaker and van Heijningen, 2000, Malhotra et al., 2011, Wei et al., 2012) block the cardioprotective effect of RIC. Further, studies have demonstrated that bilateral vagotomy, spinal cord dissection and limb denervation all block the protective effect of RIC if performed prior to conditioning (Basalay et al., 2012, Donato et al., 2013, Steensrud et al., 2010). Similarly, stimulation of the vagus nerve, either directly (Donato et al., 2013, Mastitskaya et al., 2012) or via transcutaneous means (Merlocco et al., 2014), has been found to mimic the cardioprotective effect of RIC. Interestingly, some studies have shown how RIC is only effective when it includes a period of reperfusion (Gho et al., 1996). This suggests that a neural pathway is activated during the reperfusion phase of RIC. Alternatively, it may mean that reperfusion is required for a humoral factor to be released to the circulation, needed in addition to the neurogenic pathway.

1.8.3 Humoral mechanisms

The humoral hypothesis of RIC postulates that blood-borne factors mediate RIC, conveying a protective signal from the remote organ or tissue to the target organ (Hess et al., 2015, Lim and Hausenloy, 2012b). Studies have shown that treating isolated, naïve hearts with coronary effluent obtained from conditioned hearts (Dickson et al., 1999) or dialysate from animals who have had limb RIC (Shimizu et al., 2009, Steensrud et al., 2010, Redington et al., 2012), protects the recipient heart from subsequent I/R injury. It is possible that the release of humoral

factors is induced by peripheral nerves (Redington et al., 2012). Support for this theory comes from a study by Jensen et al. (2012) who found that cardioprotection was lower in naïve hearts treated with dialysate from conditioned patients who had diabetic peripheral neuropathy, compared to naïve hearts treated with dialysate from diabetic patients with intact peripheral nerves. Furthermore, occlusion of venous outflow from a conditioned limb (femoral vein in a hind limb) also blocks the cardioprotective effect of RIC (Lim et al., 2010). Thus, during reperfusion of the remote organ or tissue conditioned, these protective humoral factors are 'washed away' towards the target organ (Gho et al., 1996, Weinbrenner et al., 2002, Lim and Hausenloy, 2012b). The exact nature and identity of these 'protective factors' is unclear but a number of putative agents have been proposed on the basis of experiments in cardiac injury models and are listed in *Table 3*.

Table 3. Proposed humoral mediators of remote ischaemic conditioning.

Humoral mediators	Role
Heat Shock Protein (HSP70) **(Dubey et al., 2015, Mashaghi et al., 2016)**	• Improves protein integrity, prevents protein aggregation and regulates protein metabolism. • Inhibits cellular apoptosis. • Protects neurons from oxidative stress.
Adenosine **(Kristiansen et al., 2005, Mubagwa et al., 1996b)**	• Vasodilator. • Activates ATP-sensitive K+ (K_{ATP}) channels → prevents excessive mitochondrial calcium ion (Ca^{2+}) influx → preserves mitochondrial integrity + enables ATP synthesis. • Regulates energy metabolism. • Improves glucose metabolism.
Bradykinin **(Sharma et al., 2015)**	• Vasodilator. • Activates number of pathways including protein kinase C (PKC) pathway (plays an important role in gene expression and cell proliferation). • Activates K_{ATP} channels. • Reduces oxidative stress, increases blood flow.
Nitrite/Nitric oxide (NO) **(Förstermann and Münzel, 2006, Forstermann et al., 1994, Huang, 2003, Hess et al., 2015)**	• Vasodilator. • Increases blood flow. • Protects mitochondria from oxidative stress. • Inhibits endothelial cell activation and leukocyte adhesion. • Inhibits platelet adhesion and aggregation. • Prevents smooth muscle cell proliferation and thickening of arterial walls. • Prevents atherosclerosis.
Stromal cell-derived factor 1 (SDF-1) **(Hess et al., 2015, Janowski, 2009)**	• Chemokine activated by hypoxia. • Involved in stem cell trafficking and cell proliferation. • Neuromodulator.
MicroRNA-144 **(Li et al., 2014, Przyklenk, 2014)**	• Regulates gene expression. • Promotes cell proliferation. • Inhibits apoptosis. • Involved in cell differentiation. • Induces autophagy signalling. • Promotes pro-survival kinase signalling.
Endogenous opioids (Schultz et al., 1995, Randhawa and Jaggi, 2017, Aggarwal et al., 2019)	• E.g., endorphins, dynorphins and enkephalins. • Interact with G-protein coupled opioid receptor system. • Act on cardiac opioid receptors. • Activates cardiac K_{ATP} channels. • Modulates heart rate and vascular function.

These factors may act directly on target organs or may influence the host's immune response and as such may also play a role in the systemic mechanisms of RIC. Although we have discussed the neural and humoral pathways of RIC as separate entities, researchers have shown how the two pathways are not mutually exclusive and together mediate RIC. For example, Steensrud et al. (2010) found that the cardioprotective effect observed when naïve rabbit hearts were perfused with dialysate from rabbits who had limb RIC was abolished when they dissected the rabbit's femoral nerves before conditioning. Similarly, a cardioprotective effect was observed when Redington et al. (2012) treated naïve hearts with plasma dialysate from rabbits who had direct femoral nerve stimulation without limb RIC. These findings suggest that protective humoral factors released in response to a RIC stimulus rely on intact neural pathways, and that such substances can be released into the bloodstream and mimic the effect of RIC through direct stimulation of peripheral nerves.

1.8.4 Systemic response

Animal studies have also shown how RIC suppresses the expression of genes involved in the intrinsic apoptosis pathway (Caspase 3 and Caspase 8) and also reduces the activation of TNF-related apoptosis-inducing ligand (TRAIL) death receptors, key mediators of apoptosis (Xu et al., 2017). Research using healthy human participants has shown how RIC can influence the host's immune and inflammatory response. Microanalyses of human DNA following limb RIC have found an upregulation of anti-inflammatory genes (e.g. HSP70 and Calpastatin) and the downregulation of pro-inflammatory genes (e.g. CD11b and TLR4) in human leukocytes (Konstantinov et al., 2004). Further, a study of 31 healthy volunteers by Kharbanda et al. (2001) found that RIC of the upper limb before a prolonged occlusion of the brachial artery prevented neutrophil activation as measured by neutrophil adhesion molecule expression and platelet-neutrophil complexes. Reduced levels of proinflammatory cytokines (TNF-α, IL-1β, IFN-$_{\gamma}$) have been found in animal studies of myocardial (Pilz et al., 2019, Zhang et al., 2018b) and cerebral infarction (Du et al., 2020). One way RIC inhibits proinflammatory cytokine release is via inhibition in nuclear factor kappa beta (NF-$_{k}$B) activation (Shin et al., 2014, Kim et al., 2019b, Pearce et al., 2021), an important transcription factor in inflammatory disease. Together these findings suggest that RIC limits I/R injury and protects tissue function by modulating inflammatory cell recruitment, activation and cellular apoptosis.

1.8.5 Signal transduction at the target organ

Irrespective of the pathways through which the effects of RIC are transmitted, they ultimately lead to changes in the target organ that alter cellular bioenergetics and tissue perfusion for example. Such changes that may explain the effects of RIC on subsequent ischaemic injury or indeed physical performance (discussed in following sections).

1.8.5.1 Mitochondria

Mitochondria are fundamental for cellular metabolism and energy supply in the form of adenosine triphosphate (ATP) (Sharma et al., 2009). They also play central roles in cell signalling, calcium homeostasis and the regulation of reactive oxygenation species (ROS) and apoptosis (Gouspillou and Hepple, 2016, Brookes et al., 2004). I/R injury impairs mitochondrial integrity, reduces mitochondrial cytochrome C oxidase (respiration enzyme) and reduces activity in the electron transport chain (ETC) (Paradies et al., 2004, Boengler et al., 2018, Murphy and Steenbergen, 2008). The ETC is composed of protein complexes (CI-CV) bound to the inner mitochondrial membrane and is the site of ATP production via oxidative phosphorylation (i.e., aerobic metabolism) (Zhao et al., 2019b), therefore disruptions in ETC activity can lead to reductions in ATP. It also causes increases in ROS production (Chen et al., 2008) and ultimately leads to cell death (Murphy and Steenbergen, 2008), and loss of muscle mass and function (Gouspillou and Hepple, 2016). ATP can also be produced in an oxygen-independent manner (i.e., anaerobically) via glycolysis (Bonora et al., 2012), however this process produces less ATP and results in the accumulation of lactate.

Studies have shown how most pathways triggered by RIC converge on the mitochondria and that these are essential for the protection afforded by ischaemic preconditioning. For example, humoral factors (e.g., bradykinin, adenosine, opioids) stimulate G-protein coupled receptors on the mitochondrial cell surface, which activates intracellular kinase signalling and the opening of ATP-dependant mitochondrial potassium ($MitoK_{ATP}$) channels (Hausenloy and Yellon, 2008, Ong et al., 2015). $MitoK_{ATP}$ channels of the inner mitochondrial membrane (Heusch, 2015), have been implicated as key mediators of the protective phenomenon (Yellon and Downey, 2003). $MitoK_{ATP}$ channel activation plays a crucial role in the development of ischaemic tolerance by enhancing the antioxidative ability of the ischaemic organ/tissue during I/R injury (Wu et al., 2011b). Studies show that selective $MitoK_{ATP}$ inhibition using pharmacological antagonists (e.g., 5-hydroxydecanoate or glibenclamide) abolishes the

cardioprotective effect of RIC (Moses et al., 2005, Pell et al., 1998, Konstantinov et al., 2005). The precise mechanism by which the opening of MitoK_{ATP} channels provide protection is unknown, however proposed mechanisms include: (1) enhanced mitochondrial respiration and reduced ROS production (Ferranti et al., 2003), (2) reduction in the rate of ATP hydrolysis thereby reducing ATP depletion rate (Dos Santos et al., 2002), (3) activation of pro-survival kinases (Yellon and Downey, 2003), and (4) decrease in calcium (Ca^{2+}) uptake and inhibition of mitochondrial permeability transition pore (mPTP) opening (Costa et al., 2006, Ong et al., 2015, Carreira et al., 2005). It is not clear whether the mitoK_{ATP} channel exerts its effects in the preconditioned organ or limb vs the ischaemic target organ or tissue under investigation (Hausenloy and Yellon, 2008). The mPTP is a non-specific channel of the mitochondrial inner membrane which mediates cell death in the setting of I/R injury by uncoupling oxidative phosphorylation (i.e., disruption of ATP production), which causes ATP depletion and mitochondrial swelling (Hausenloy and Yellon, 2008). In animal models and clinical trials of acute myocardial I/R injury, preventing mPTP opening using cyclosporin A (an immunosuppressant) at the onset of reperfusion has been shown to reduce myocardial infarct size (Hausenloy et al., 2014, Chiari et al., 2014). The precise mechanism through which ischaemic preconditioning inhibits mPTP opening and exerts its protective effects is not clear. Postulated mechanisms include: (1) ischaemic preconditioning modulates mitochondrial Ca^{2+}, phosphate accumulation, intracellular pH changes and oxidative stress, factors which are known to influence mPTP opening (Halestrap and Richardson, 2014, Hausenloy et al., 2003), or (2) RIC activates a signalling pathway by modulating the same factors highlighted above, or by interacting with components of the mPTP (Hausenloy et al., 2003).

Experimental models demonstrate how ischaemic preconditioning provides protection by preventing I/R injury induced impairment of the mitochondrial ETC in cardiac and skeletal muscle (Thaveau et al., 2007, Mansour et al., 2012, Leung et al., 2014). Leung et al. (2014) found that treating naïve rabbit hearts with RIC effluent before a prolonged episode of I/R injury preserved mitochondrial membrane integrity (preserved complex I and III respiration) and reduced cytochrome C release. A study by Addison et al. (2003) investigated the mechanisms underlying the infarct sparing effect of RIC on skeletal muscles in pigs and found slower rates of ATP depletion in the RIC group compared to controls. Similarly, using a mouse model Thaveau et al. (2007) found that hind-limb RIC (3 x 10 min) before 5-hours of limb ischemia prevented I/R injury induced impairments in mitochondrial complexes I and II in rat skeletal muscle. A reduction in the rate of ATP depletion after RIC may also be caused by

reduced ATPase activity (enzyme that catalyses ATP hydrolysis) which reduces cellular demand in the organ or tissue (Vander Heide et al., 1996, Vuorinen et al., 1995).

A clinical study of 60 patients (30 RIC, 30 control) undergoing isolated CABG surgery by Slagsvold et al. (2014) evaluated the effect of a single dose of upper limb RIC (3 x 5 min cuff inflation to 200 mmHg separated by 5-min reperfusion) on mitochondrial respiration compared to controls (blood pressure cuff remained deflated). Mitochondrial respiration was assessed in situ by obtaining right atrial biopsies before and after aortic cross-clamping. In the RIC group, maximal mitochondrial respiration was preserved throughout the surgical procedure, however it significantly reduced (-28%; $P<0.05$) in the control group after aortic clamping.

Proposed signalling pathways recruited by RIC that converge on the mitochondria to confer cardioprotection are shown in Figure 3. There is a lot pre-clinical and clinical evidence from cardiac populations supporting preservation of mitochondrial resilience and recovery after RIC (Ramachandra et al., 2020). However, could this help in peripheral muscles with peripheral fatigue? Details of the signalling pathways that may either directly or indirectly make the mitochondria more resilient to states of ischaemia or energy deficiency are provided in Chapter 5.

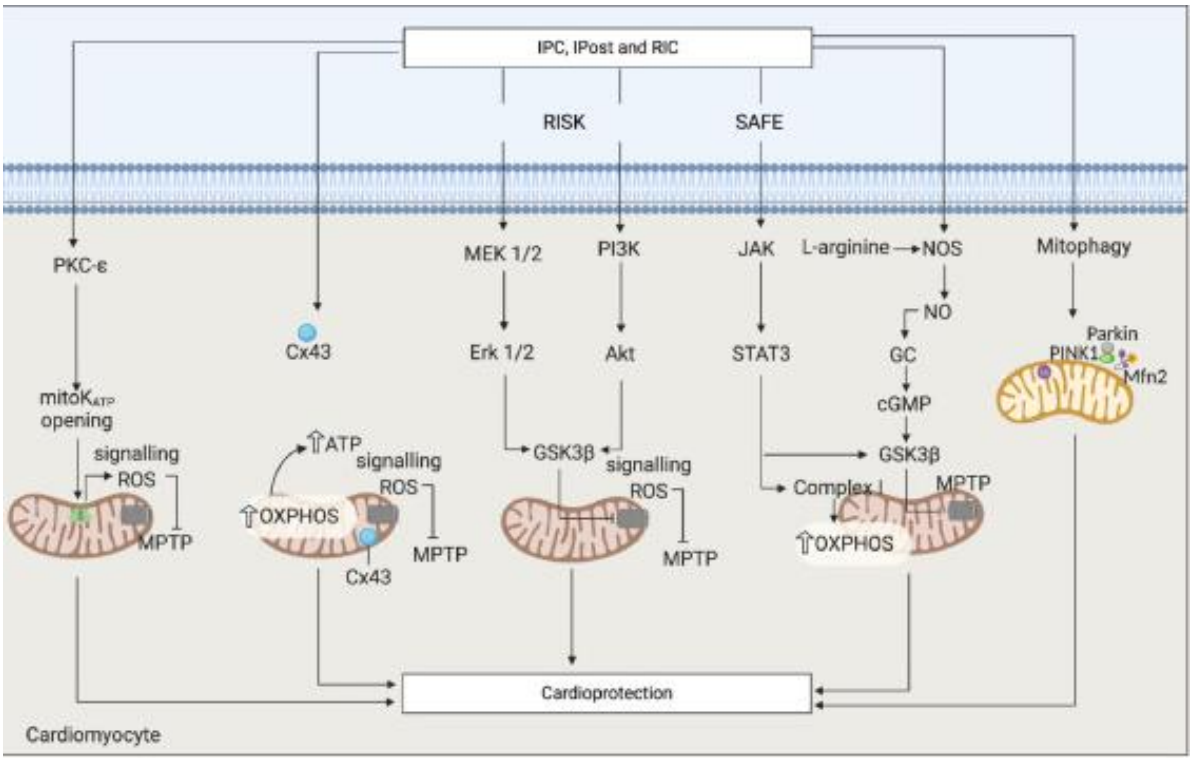


Figure 3. Figure detailing the proposed signalling pathways recruited by RIC that converge on the mitochondria to confer cardioprotection. *Figure obtained from Ramachandra et al. (2020) published under terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).*

1.8.5.2 Vascular bed and changes in blood flow

Vascular nitric oxide is produced by endothelial cells in a reaction catalysed by endothelial nitric oxide synthase (eNOS) (Vallance and Hingorani, 1999). I/R injury impairs endothelial regulation of eNOS and thus the ability to regulate microvascular perfusion (Yang et al., 2016). However, RIC appears to protect against endothelial I/R injury in patients (Manchurov et al., 2020), and healthy volunteers (Loukogeorgakis et al., 2005, Kharbanda et al., 2001), and these effects can be long lasting if RIC is repeated over several weeks (Jones et al., 2015). Manchurov et al. (2020) investigated the effect of RIC (4 x 5 min cuff inflation to 200 mmHg) on endothelial dysfunction when performed before primary percutaneous coronary intervention (PCI) in 173 patients (86 treatment, 87 control) with ST-Elevation Myocardial Infarction (STEMI) (underwent PCI within 24 hours of symptom onset). Endothelial function of the brachial artery was measured using the endothelium-dependent flow-mediated dilatation

test (FMD) (non-invasively measures endothelial response to occlusion-induced hyperemia using ultrasound) on the 2-7th day after hospital admission. In this study, the investigators found that the percentage of patients with endothelial dysfunction was significantly lower in patients who underwent RIC before PCI compared to controls (underwent PCI without previous RIC) (43.1% vs. 75.8% respectively, *P*=0.0001). The incidence of heart failure was also significantly lower in the RIC group compared to controls (1.5% (n=1) vs 9.7% (n=6); *P*=0.045). An earlier study by Loukogeorgakis et al. (2005) investigated *in vivo* the effect of upper limb RIC (3 x 5 min cuff inflation to 200 mmHg) on endothelial function of the brachial artery (measured using FMD) of the contralateral (non-dominant) arm in 16 healthy volunteers (12 males, 4 females). RIC was applied to the dominant arm immediately, 4-hours, 24-hours and 48-hours before 20-min prolonged ischemia and reperfusion (IR) on the contralateral limb. The researchers continuously monitored blood flow velocity through the brachial artery using pulsed-wave doppler and FMD was assessed before the 20-min ischaemia and 20-min after reperfusion. In this study, the researchers found that the reduction in FMD observed after IR (8.7 ± 1.1% before IR, 4.9 ± 1.2% after IR; *P*< 0.001), did not occur when IR was preceded by RIC (8.0 ± 0.8% after IR; *P*= NS). The protective effect of RIC on endothelial function was not observed after 4-hours (4.9 ± 1.1% after IR; *P* < 0.001), however it was observed after 24-hours (8.7 ± 1.1% after IR; *P*= NS) and 48-hours (8.8 ± 1.4% after IR; *P*= NS). These findings demonstrate how RIC may provide protection against endothelial I/R injury and how there are two phases of protection: early (occurs immediately and lasts up to 4-hours) and late (activated at 24-hours and lasts at least 48-hours) (Loukogeorgakis et al., 2005).

RIC has been shown to improve coronary (Shimizu et al., 2007, Ma et al., 2006, Kono et al., 2014, Lau et al., 2018) and cerebral blood flow (CBF) (Kitagawa et al., 2018, Zhao and Nowak, 2006, Dawson et al., 1999) in experimental and clinical studies. A study by Khan et al. (2018) used a mouse model to show how chronic RIC for either 1 or 4 months after bilateral carotid artery stenosis (BCAS), significantly improved CBF and increased angiogenesis (formation of new blood vessels). It was also associated with improvement in cognitive outcomes (spatial and working memory assessed using the NOR test and Y-maze test, respectively). In a clinical study, Meng et al. (2012) demonstrated improved cerebral perfusion (single-photon emission computed tomography, SPECT) in 68 stroke patients treated with bilateral arm RIC (5 x 5 min cuff inflation to 200 mmHg) twice daily for 300 days. The release of vasoactive substances (NO, adenosine) in response to RIC may also enhance skeletal muscle perfusion (Andreas et al., 2011b, Jeffries et al., 2018a). In a randomised, cross-over trial Andreas et al. (2011b)

investigated whether lower limb RIC (3 x 5 min + 10 min reperfusion) 4 hours before 20-min ischaemia (cuff inflated around thigh to 200 mmHg for 20 mins) followed by interrupted reperfusion (i.e., stenosis) (reperfusion interrupted by deflating cuff to 30 mmHg below systolic BP) improved skeletal muscle oxidative metabolism in 23 healthy subjects. In this study, nuclear magnetic resonance (NMR) imaging and spectroscopy was used to measure phosphocreatine (PCr) (involved in ATP production) and oxygen signals (blood oxygen level-dependant signals, BOLD). RIC administered 4-hours before ischaemia significantly increased the maximal PCr reperfusion signal after post-ischaemic stenosis compared to post-ischaemic stenosis alone (55±16 vs 46±15 respectively, $P<0.05$). RIC also mitigated the peak BOLD signal following post-ischaemic stenosis during reperfusion to 108 ± 13% of the baseline value ($P = 0.029$ vs. no RIC). These findings demonstrate how RIC may improve energy metabolism and oxygenated blood delivery to skeletal muscles during the post-ischaemic period. In another study of 20 healthy male volunteers (mean age = 26 years), Jeffries et al. (2018b) found that bilateral leg RIC (4 x 5 min) for 7-days increased oxidative capacity (assessed using near-infrared spectroscopy during short bursts of arterial occlusion) of the leg skeletal muscle and also improved microvascular oxygenated blood flow. These findings suggest that RIC stimulates cellular processes to help aid skeletal muscle recovery after ischaemia. The positive effect of RIC on blood flow, mitochondrial function and energy metabolism makes it an attractive treatment option for vascular conditions (stroke, peripheral artery disease, and ischaemic heart disease), as well as a treatment to enhance muscle function and repair, particularly in deconditioned states.

1.9 Clinical applications of remote ischaemic conditioning

RIC has been used clinically in the context of coronary artery disease (Pei et al., 2014, Corcoran et al., 2018, Rahman et al., 2010, Shahvazian et al., 2017), cerebrovascular disease, (Koch et al., 2011, Meng et al., 2012) and peri-procedurally (e.g. surgery) (Cheung et al., 2006, Li et al., 2010), to mention just a few.

1.9.1. Hyper-acute and acute stroke

A small number of experimental and clinical studies have investigated the use of RIC in acute ischaemic stroke (Hougaard et al., 2014, Zhao et al., 2018a, Kitagawa et al., 2018). A study by Hougaard et al. (2014) assessed whether pre-hospital RIC is an effective adjunctive therapy to

intravenous alteplase (rtPA) in patients with acute ischaemic stroke. Patients with suspected ischaemic stroke (n=443) eligible for thrombolysis (symptom onset less than 4.5. hours prior to hospital admission) were randomised to receive either upper limb RIC (4 x 5 min episodes of cuff inflation to 25 mmHg above systolic blood pressure up to a maximum of 200 mmHg, n=247) or no RIC (controls, n=196) during transportation to hospital. Magnetic resonance imaging (MRI) was performed to confirm stroke diagnosis before treatment with rtPA and at 1-month follow-up. A single dose RIC was safe and well tolerated. There was no difference in penumbral salvage (defined as mismatch between MRI perfusion and diffusion weighted imaging not progressing to infarct), final infarct size at 1-month, and clinical outcome measures (National Institutes of Health Stroke Scale, NIHSS) at 3-months, though the study was inadequately powered to detect significant differences in clinical outcomes. However, after adjustment for baseline severity of hypoperfusion, voxel wise analysis demonstrated that pre-hospital RIC significantly reduced tissue risk of infarction (likelihood ratio test P=0.0003). These findings give credence to the target organ effects previously discussed and suggest that pre-hospital RIC may have an early neuroprotective effect.

1.9.2 Secondary stroke prevention

Chronic RIC has been investigated in stroke populations particularly with respect to secondary prevention in patients with symptomatic intracranial arterial stenosis (SIAS) (Meng et al., 2012, Meng et al., 2015a). Meng et al. (2012) found that 300 days of twice daily bilateral arm ischaemic preconditioning (BAIPC) (5 x 5 min cycles of cuff inflation to 200 mmHg) reduced the incidence of recurrent stroke from 26.7% (controls) to 7.9% (P<0.01). To assess whether BAIPC is safe and effective in the elderly population, the same investigators conducted another trial whereby patients aged 80-95 years with SIAS (n=58) were randomised to either receive 180 days of BAIPC (n=30) or sham (n=28). In this study, Meng et al. (2015a) found BAIPC significantly reduced plasma C-reactive protein, interleukin-6 and leukocyte count (biomarkers of inflammation), as well as reducing plasminogen activator inhibitor-1 and fibrinogen (biomarkers of coagulation) (all P<0.01). Compared to controls the incidence of recurrent strokes (confirmed by magnetic resonance diffusion weighted imaging) was significantly lower in the BAIPC group (2 infractions and 7 TIAs in the BAIPC group vs 8 infarctions and 11 TIAs in the sham group at day 180, P<0.05). Furthermore, compared to controls BAIPC had no adverse effects local skin integrity (no skin breakage, skin discoloration, edema or tenderness to palpation), heart rate or blood pressure. These findings show RIC is safe, non-invasive and

effective in reducing stroke recurrence and plasma biomarkers of inflammation and coagulation in very elderly patients with SIAS. Chronic RIC may induce distinct adaptations that influence stroke recovery and recurrence (Doeppner et al., 2018, Khan et al., 2018).

1.10 Application of ischaemic preconditioning in physical performance

While the bulk of research has concentrated on the effects of ischaemic conditioning on subsequent ischemia, the benefits may extend beyond this.

1.10.1 Elite athletes and healthy volunteers

For example, studies in healthy volunteers and athletes have shown that ischaemic conditioning can enhance muscle strength and exercise performance. De Groot et al. (2009) tested whether a single dose of leg RIC improves maximal performance on a leg cycle ergometer in 15 well-trained cyclists. The investigators performed 3 x 5 min bilateral leg RIC (cuff inflated to 220 mmHg) just before the cycling test. Compared to controls, RIC resulted in a 1.6% increase in maximal power output (W) (P=0.05) and a 3% increase in maximal oxygen consumption (VO_{2max}) (P=0.003), corresponding to an increase in muscle force and aerobic fitness. Such improvements (VO_{2max}) are comparable with those seen in athletes after 4 weeks of altitude training (Stray-Gundersen et al., 2001, Levine and Stray-Gundersen, 1997). In another study, Patterson et al. (2015) investigated whether a single dose of lower limb RIC (4 x 5 min cycles of cuff inflation to 220 mm Hg) reduced the rate of muscle fatigue (measured using electromyography of the vastus lateralis muscle) during a repeated-sprint cycle test involving twelve 6-second (s) sprints, compared to sham (cuff inflation to 20 mmHg). Muscle fatigue was measured by calculating the change in median electromyography (EMG) frequency in the vastus medialis muscle across the twelve sprints. While leg conditioning did not reduce rates of muscle fatigue, it did induce a 2-4% increase in mean and peak power output in the first three sprints. This is comparable with the improvement in power output seen after the use of other ergogenic aids used during exercise such as analgesics (Foster et al., 2014). However, across a range of sports, RIC has been trialled with conflicting findings, results of which are summarised in *Table 4*.

Table 4. Studies on the effect of RIC on enhancing exercise performance and muscle strength in healthy volunteers and athletes.

Author	Sport type	RIC protocol	Findings
De Groot et al. (2009)	Cycling	Single dose of 3 x 5 min ischaemia (cuff inflated to 220mmHg) and 5 min reperfusion in both legs.	• 1.6% increase in maximal power output (P=0.05). • 3% increase in VO_2 max (P=0.003).
Patterson et al. (2015)	Cycling	Single dose of 4 x 5 min ischaemia (cuff inflated to 220mmHg) and 5 min reperfusion in both legs.	• 2-4% increase in the mean and peak power output. • No increase in VO_2 max. • No improvement in fatigue.
Crisafulli et al. (2011)	Cycling	Single dose of 3 x 5 min ischaemia (cuff inflated to 50mmHg above systolic BP) and 5 min reperfusion in both legs.	• 4% increase in maximal power output. • Increase in total exercise time and max HR. • No increase in VO_2 max.
Jean-St-Michel et al. (2011)	Swimming	Single dose of 4 x 5 min ischaemia (cuff inflated to 15mmHg above systolic BP) and 5 min reperfusion in both arms	• Mean improvement in maximal swim time (P=0.04). • Improvement in swim time relative to personal best (P=0.02).
Lisbôa et al. (2017)	Swimming	Bilateral 4 x 5 min episode of ischaemia and 5 min reperfusion in both arms or legs (cuff inflated to 220mmHg for legs and 180mmHg for arms) 1, 2 or 8 hours before swim test.	• 1% improvement in swim time at 2h (P=0.002). • 1.2% improvement in swim time at 8h (P<0.001). • No improvements at 1h.
Williams et al. (2018)	Swimming	Single episode of 4 x 5 min ischaemia (cuff inflated between 160 to 228 mmHg) and 5 min reperfusion in both thighs. 2 or 24 hours before swim test.	• No improvement in swimming performance at 2h or 24h.
Tocco et al.(2015)	Running	3 x 5 min ischaemia (cuff inflated to 50mmHg above systolic blood pressure, SBP) and 5 min reperfusion in both legs. 5 minutes before run test.	• No improvement in VO_2 max. • No improvement in running speed.

Surkar et al. (2020)	Strength training	Two weeks of repeated upper limb RIC received over 8 visits (5 x 5 min 20 mmHg above systolic BP) combined with strength training or sham (10 mmHg below diastolic BP)	• RIC group had significantly greater wrist extensor strength gains compared to the sham (9.38 ± 1.01 lbs versus (6.3 ± 1.08 lbs respectively, *P* = 0.035). • RIC group had significantly greater % change in EMG amplitudes compared to sham (31.0% vs. 8.6% respectively, *P* = 0.023)
Tanaka et al. (2021)	Cycling	• Repeated bilateral lower limb RIC for 2 weeks (6 days/weeks) (5 x 5 min cuff inflation to 220 mmHg)	• No improvement in VO_{2peak} during ramped cycling test. • No improvement in time to task failure on knee extensor MVC task.

Abbreviations: VO_2 max = Maximal oxygen consumption; EMG = Electromyography; MVC = Maximal voluntary contraction.

Although the relative improvements seen in some of these studies appear small, they are likely to be significant in this elite population where marginal gains are difficult to achieve. The relative improvements in a clinical population may be much greater. The inconsistencies seen across studies may be due to a number of methodological differences. Firstly, conditioning protocols differ in their timing (how long before a measured exercise activity), frequency of cycles, and which limb is conditioned (arm vs leg). Indeed, the limb conditioning preference may be important when considering the athletic discipline (e.g. swimming vs cycling) as the level of force contributed by different limbs can vary across different sport modalities (Deschodt et al., 1999). Further, the dose of ischaemia delivered may vary significantly between the arm and leg, and between protocols involving unilateral and bilateral conditioning. Secondly, variation in resting systolic blood pressure (SBP) and the volume of connective tissue surrounding vessels (Loenneke et al., 2012) may mean that the degree of true ischaemic conditioning varies from person to person with any cuff inflation pressure. While the standard inflation pressures used to deliver RIC range 200-250mmHg, improvements in physical

performances have been demonstrated with lower pressures (30 mmHg above SBP for arm, and 50 mmHg above SBP for leg) (Sharma et al., 2014).

1.10.2 Clinical populations

Relatively little attention has been paid to whether RIC improves strength and physical performance in clinical populations. A study by Hyngstrom et al. (2018) found that RIC significantly improved knee extensor muscle strength in the paretic leg of 10 patients with chronic stroke. In this study, patients performed isometric maximum voluntary contractions (MVCs) of the knee extensor using a Biodex dynamometer. Patients then underwent a single dose of RIC (5 x 5 min cuff inflation to 225 mmHg) on the paretic leg before repeating the MVCs. Nine out of 10 patients experienced a mean increase in MVC of 10.6 ± 8.5 newton metres (Nm) (P = 0.001), coupled with a 30.7 ± 15% increase in the magnitude of *vastus lateralis* muscle activation, measured with EMG. After sham RIC (cuff inflation pressure = 25 mmHg) treatment there was no difference in knee extensor MVC (mean difference post-sham RIC: 1.3 ± 2.9 Nm, P = 0.65). Later the same investigators examined whether RIC (5 x 5-min) in the paretic leg every 48-hours for 2-weeks, improves walking speed (assessed using the 10-metre walking test) and knee extensor strength and fatigability in the paretic leg of 22 chronic stroke survivors (Durand et al., 2019). To measure muscle fatigability patients completed an endurance task on the Biodex dynamometer and had to maintain a target torque of 30% of the paretic leg knee extensor MVC (submaximal contraction). In this study, the researchers found that compared to sham (cuff inflation = 10 mmHg) participants who received repeated RIC maintained a submaximal isometric contraction for longer (397±203 seconds pre-sham vs. 355±195 seconds post-sham; P=0.46; 278±163 seconds pre-RIC vs. 496±313 seconds post-RIC, P=0.004). In addition, walking speed significantly increased in participants in the RIC treatment group (0.86±0.21 m/s pre-RIC vs. 1.04±0.22 m/s post-RIC; P<0.001) but not sham (0.92±0.47 51 m/s pre-RIC vs. 0.96±0.46 m/s post-RIC; P=0.25) (Durand et al., 2019). These findings demonstrate how both single and repeated doses of RIC can have beneficial effects on muscle strength and fatigue in people with stroke, and thus has the potential to be used as a novel therapy.

1.11 Rationale for the study

PSF affects over half of stroke survivors and is associated with poor neurological recovery and affects rehabilitation outcomes. Evidence suggests the cause of fatigue is multidimensional and

may result from impairments in mitochondrial function, high levels of central and peripheral inflammation after stroke and/or physical deconditioning. Although psychological therapies and neurostimulants are promising targets for PSF, evidence is limited by small sample sizes. In addition to small sample sizes, the existing therapies (drugs, psychological therapies, neurostimulation e.g., tDCS) may exhibit side effects or may not be readily available (e.g., tDCS needs a lab). Limb RIC is a simple, non-invasive, easy-to-use intervention with many potential mechanisms of action (neural, humoral, systemic) and end organ effects that may counteract mechanisms that promote the development of PSF (Figure 4).

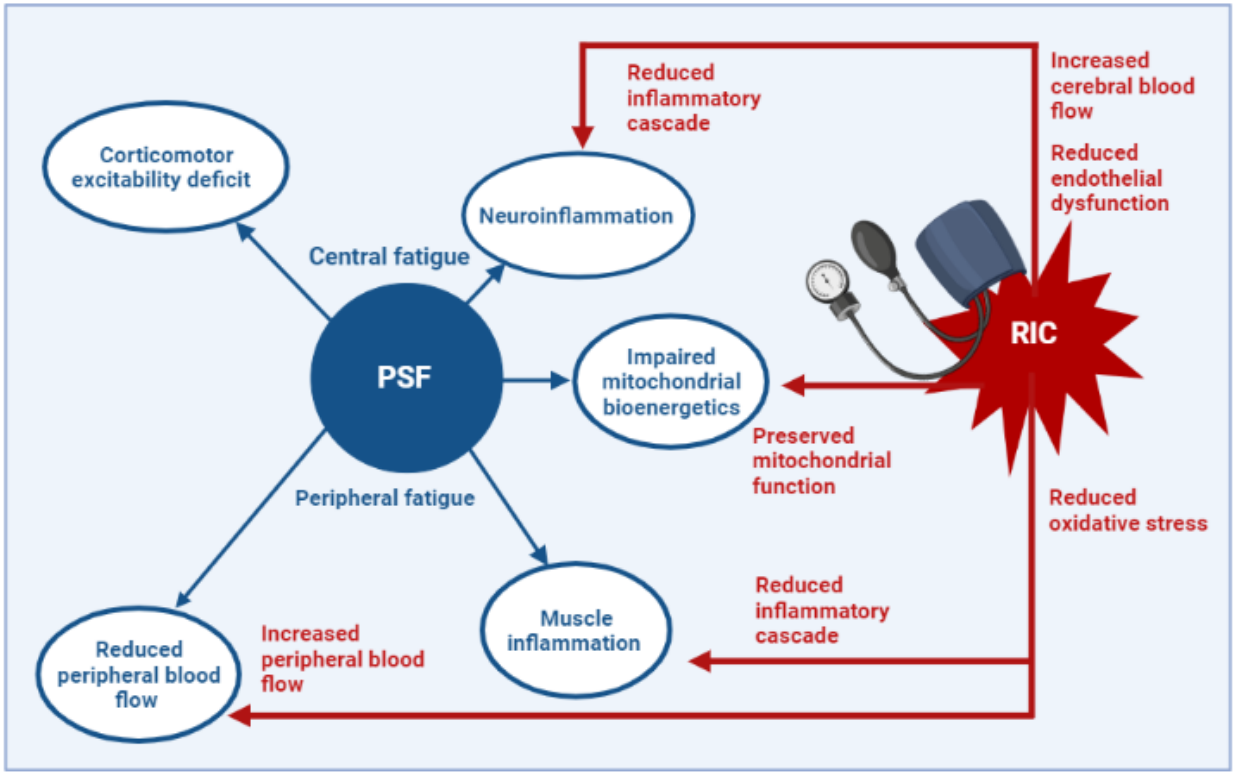


Figure 4. Potential overlap in the mechanisms of post-stroke fatigue (PSF) and remote ischaemic conditioning (RIC). Figure created by author using Biorender (biorender.com).

For example, RIC may enhance mitochondrial reliance and help repair potential impairments in mitochondrial function associated with PSF (Hausenloy et al., 2003, Yellon and Downey, 2003, Hausenloy and Yellon, 2008, Slagsvold et al., 2014). RIC may downregulate pro-inflammatory mediators and upregulate anti-inflammatory mediators and thus reduce some of the high levels of inflammation seen in patients with PSF. Furthermore, RIC may enhance skeletal muscle perfusion (Jeffries et al., 2018a, Andreas et al., 2011b) and improve the effects

of physical deconditioning observed after stroke (low muscle mass and function, poor tissue perfusion), therefore potentially improve perceived fatigue. The versatile nature of RIC has triggered a high level of interest in the research world, and my project aimed to fill gaps in knowledge base and explored new clinical areas for use (e.g., post-stroke fatigue). Before a definitive study into whether RIC is efficacious for PSF we need to evaluate its safety and acceptability and assess compliance and feasibility of both researcher-delivered and self-delivered RIC.

1.12 Study aims and hypotheses

1.12.1 Aims

1. To assess whether it is safe for stroke patients to undergo chronic RIC for a period of 6-weeks by identifying any adverse events (AE) or serious adverse events (SAE) related to the intervention.

2. To assess acceptability of, and compliance with, 6-weeks of researcher or self delivered RIC among stroke patients with fatigue by reviewing symptom diaries and monitoring diaries.

3. To establish if it is feasible to undertake a randomised controlled trial (RCT) of RIC to reduce fatigue by assessing the number of eligible participants, monitoring recruitment and retention rates, and calculating the percentage of completed outcome measures.

4. To explore the effectiveness of RIC on symptoms of post-stroke fatigue.

5. To explore whether chronic RIC improves walking distances/speed.

6. To explore whether PSF severity is associated with cardiopulmonary exercise test (CPET) parameters (e.g., VO_{2Peak}, ventilatory anaerobic threshold (VAT), minute ventilation/carbon dioxide production (VE/VCO_2) slope, and respiratory exchange ratio, RER).

7. To explore whether it was feasible to undertake ^{31} Phosphorus Magnetic Resonance Spectroscopy (^{31}P-MRS) to assess skeletal muscle metabolism in stroke patients with fatigue and investigate whether indices of mitochondrial oxidative capacity are related to the severity of PSF.

1.12.2 Hypotheses

1. RIC for a period of 6-weeks is safe, acceptable, and feasible in stroke patients with PSF.

2. RIC reduces fatigue severity (measured using the fatigue severity scale).

3. RIC improves peak oxygen consumption (VO_{2Peak}), time to ventilatory anaerobic threshold (VAT) and minute ventilation/carbon dioxide production (VE/VCO_2) slope.

4. ATP in skeletal muscle measured using ^{31}P-MRS is associated with post-stroke fatigue and physical function methods like six-minute walk test (6MWT) and maximal isometric voluntary contraction (MVIC).

5. There are differences between ATP, phosphocreatine and inorganic phosphate between stroke affected and non-affected legs.

6. RIC improves mitochondrial function and thus results in increased levels of tissue ATP, increased levels of phosphocreatine and reduced levels of inorganic phosphate.

CHAPTER 2. FEASIBILITY AND ACCEPTABILITY OF REMOTE ISCHAEMIC CONDITIONING FOR POST-STROKE FATIGUE

2.1 Rationale for pilot studies

The fundamental role of a pilot study is to analyse the feasibility of trial methods and procedures to be used in a larger scale study (Porta, 2014, Thabane et al., 2010). They are designed to examine the feasibility of recruitment, randomisation, retention and outcome assessment procedures (Leon et al., 2010). Pilot studies also address methods of randomisation, blinding and rates of treatment fidelity (extent to which a treatment was given as intended) and compliance (Sim, 2019). They can also be used to assess the safety of novel interventions and/or explore the application of an intervention (Thabane et al., 2010). Pilot studies play an important role in health and translational research in gathering preliminary support for the planning and justification of RCTs which are used to test the effectiveness of interventions (Lancaster et al., 2004). Pilot studies are essentially a small scale test of the methods and procedures to be used in a larger trial. Although pilot studies assess the feasibility of trial methods and procedures, they are not the same as feasibility studies and the terms 'pilot' and 'feasibility' are often misused. Feasibility studies are preliminary pieces of research done before a large trial to answer the question 'can this be done?' and whether researchers should proceed with it in the future. Although pilot studies ask the same question, they also have a specific design feature: in a pilot study a future study is conducted on a smaller scale (Eldridge et al., 2016). There is ample evidence from previous clinical trials that chronic RIC is feasible in clinical populations (stroke, heart failure) (Meng et al., 2012, Meng et al., 2015b, Vanezis et al., 2018), therefore we assumed feasibility and went straight for the pilot study to test the trial procedures.

The main focus of this pilot study was to investigate the safety, acceptability and feasibility of a 6-week programme of RIC in stroke patients with PSF, along with an assessment of compliance with the intervention. We also explored a potential efficacy signal between RIC and several secondary outcome measures including fatigue, walking distance/speed, cardiopulmonary exercise testing parameters, and ^{31}Phosphorus-Magnetic Resonance Spectroscopy (^{31}P-MRS) of the legs. The potential efficacy of RIC will be discussed in

following chapters. Consolidated standards for reporting trials (CONSORT) guidelines followed for this chapter can be found in the appendix (Appendix 1).

2.2 Methodology

2.2.1 Ethics approval and funding

Ethical approval for this study was granted by the Northwest - Preston Research Ethics Committee (Ref 18/NW/0401) (IRAS Project ID: 245385). All trial documentation including consent forms, participant invites, participant information sheets, template GP letters, symptom and monitoring diaries, interview guides and questionnaires, were submitted for approval. The research reported in this thesis was funded by the Ryder Briggs Trust, Sheffield Biomedical Research Centre (BRC), and Sheffield Teaching hospitals stroke research fund. ClinicalTrials.gov Identifier NCT03794947.

2.2.2 Design

This study was a pilot, single-blind, randomised, placebo-controlled trial.

2.2.3 Setting

Baseline and follow-up assessments and RIC protocols were undertaken at the Assessment and Rehabilitation Centre (ARC), Nether Edge Hospital, Sheffield, the Stroke Unit at Royal Hallamshire Hospital (RHH), or the Facility of Health and Wellbeing, Sheffield Hallam University (SHU). In exceptional circumstances (due to COVID-19 restrictions or working full-time), participants were given the option to self-administer the intervention independently at home.

2.2.4 Sample and recruitment process

Adults (aged > 18 years) who had experienced an ischaemic or haemorrhagic stroke at least 6-weeks prior and had symptoms of debilitating fatigue (Fatigue severity scale-7 $\geq$ 4) were eligible to participate in the study. Participants were recruited from stroke follow-up clinics at RHH and ARC, between January 2019 and August 2021. Since March 2018, stroke patients attending their routine follow-up appointments at RHH were screened for fatigue using the FFS-7 as part as routine clinical practice (Appendix 2). If patients were happy to be contacted

about the study in the future, they were asked to tick a 'yes' box at the bottom of the questionnaire. Patients who scored above the threshold for fatigue (mean FSS-7 ≥ 4) and were willing to be contacted were sent an invitation letter (Appendix 3) and participant information sheet (PIS) (Appendix 4) in the post. An aphasia accessible PIS was also sent to participants with aphasia (Appendix 5). Individuals who were interested in participating in the study were asked to contact a member of the research team to arrange a mutually convenient time to have their first study visit. Stroke patients identified as having fatigue during their 6-month review at ARC (prospectively and retrospectively) were asked by members of the rehabilitation team if they are happy to be contacted about the study. Invitation letters and study information sheets were posted to those who gave their consent. One participant found out about the study online and reached out to the research team to express their interest.

2.2.5 Sample size

A sample size of at least 34 participants was chosen as we estimated a 30% drop out rate (non-conservative estimate) (National Research Council, 2010), and were aware that a minimum of 24 participants (12 in each arm) is considered the lowest number required to prove feasibility of an intervention and to get a reasonable spread of data (i.e., precision about the mean and variance) and to be confident with how representative the sample is of the population studied (Julious, 2005, Lancaster et al., 2004).

2.2.6 Inclusion criteria

- Adults (aged > 18 years) who have had an ischaemic or haemorrhagic stroke at least 6 weeks prior.
- Symptoms of debilitating fatigue for at least 4 weeks.
- Fatigues severity score (FSS-7) ≥ 4.

2.2.7 Exclusion criteria

Participants were unable to participate in the study if they met one or more of the following exclusion criteria:

- History or presence of significant peripheral vascular disease in the upper limbs.
- History or presence of complex neuropathic pains or peripheral neuropathy in the arms.
- Presence of lymphoedema in the arms.

- Presence of skin ulceration to the arms.
- Hospitalisation for cardiovascular or cerebrovascular disease within the last 4 weeks.
- Uncontrolled arrhythmia, hypertension, diabetes or angina.
- Third degree heart block or progressive heart failure.
- Acute aortic dissection, myocarditis, or pericarditis.
- Acute deep vein thrombosis, pulmonary embolism or pulmonary infection.
- Suspected or known dissecting aneurysm.
- Uncontrolled visual or vestibular disturbance.
- Systolic blood pressure (SBP) >180 mmHg
- Known or suspected cause of fatigue e.g., obstructive sleep apnoea (Epworth > 15), depression (PHQ-9 > 14).
- Modified Rankin Score > 4.
- Other neurological disorders that cause fatigue (e.g., Multiple Sclerosis, Parkinson's Disease, Myasthenia Gravis)

Exclusions were either aimed at maintaining the safety of the participants e.g., peripheral vascular disease, lymphoedema, skin ulceration, uncontrolled arrhythmia, or excluding those that may not respond to RIC e.g., peripheral neuropathy, high blood pressure; or exclude confounders e.g., mood, sleep apnoea.

2.2.8 Eligibility screening and informed consent

In line with the ethics approval the principal investigator (PI) checked interested participant's electronic health records to ensure they did not meet any obvious exclusion criteria. Participants then met with the researcher and were given the opportunity to ask any questions about the study and study procedures. If participants were happy to continue, they signed a consent form (Appendix 6). The researcher recorded the participant's medical history (stroke type, medications) and socio-demographic details (age, gender, ethnicity). Participants were informed that their General Practitioner (GP) would be notified via a letter (Appendix 7) about their participation in the study and of any concerns about their medical condition which arise as a result of their participation. The researcher then began the baseline data collection of clinical outcome measures (e.g., fatigue, mood) to ensure participants met eligibility criteria (e.g., FSS-7 $\geq$ 4, PHQ-9 < 14) before continuing with other outcome measure assessments.

Participants were only enrolled onto the study if they had the capacity to give informed consent, assessed according to the Mental Capacity Act (2005) (Nicholson et al., 2008).

2.2.9 Randomisation

Participants were randomised in a 1:1 ratio, by an online block allocation system (Sealed Envelope Ltd, 2017) to receive either the RIC or sham intervention (details of intervention given below). Participants were stratified by their modified Rankin Scale (mRS) score according to dependency (mRS 0-2 and mRS 3-4). The mRS score was obtained during the baseline assessment. Randomisation was completed by the chief investigator after the participant's baseline assessment.

2.2.10 Blinding

This was a single-blinded study. Participants were blinded to treatment allocation at baseline and at follow-up. Participants were aware there was a chance they would be randomised to a 'dummy' intervention; however, care was taken to ensure the patient information sheet described the intervention as an inflation of the cuff, without mention of the pressures that would be expected. The researcher who performed the conditioning protocols was aware of treatment allocations, while a second researcher blind to treatment allocation completed the face to face follow up assessments. Baseline assessments were performed prior to randomisation.

2.2.11 Withdrawal of participants

Participants were advised that they could withdraw from the study at any time without giving a reason and without this affecting their clinical care. Participants who withdrew from the study did not complete further questionnaires or assessments but with their consent, data already collected were used to determine feasibility criteria for the study.

2.2.12 Duration of the study

We recruited participants between January 2019 and August 2021 and followed them up for 6-months.

2.2.13 Intervention protocol

2.2.13.1 Remote ischaemic conditioning

A manual sphygmomanometer (SECA®) was used to perform the conditioning protocol. The active RIC treatment group involved inflating a blood pressure cuff around the participant's arm to 200 mmHg for 5 minutes and then deflating for 5 minutes. This was repeated 4 times (one dose = 40 minutes), three times weekly for 6-weeks. Participants could choose whether RIC was delivered to their left or right arm (preferably not their stroke affected side). RIC was either delivered by the researcher at one of the research sites, or it was self-administered by the participant independently at home. The option of home delivery was only made available during the pandemic (details provided in Section 2..2.21 'Changes to Protocol'). Participants who opted for home delivery were trained on how to inflate the manual sphygmomanometer to the determined pressure via an individualised education session. They then underwent an assessment of their ability to independently apply and deliver the intervention according to pre-determined criteria of competency (Appendix 8). Family members who accompanied participants on their study visits were also trained on how to deliver the conditioning protocol. An online education session was provided to carers who required it. Individuals who were able and willing to perform the intervention with competency were issued a manual sphygmomanometer and a digital timer to take home for 6-weeks. Transport was arranged for participants who required it to get to and from study visits. Alternatively, we reimbursed travel expenses or car parking charges that occurred through taking part in the study up to a maximum of £20 per visit.

2.2.13.2 Sham protocol

The sham protocol involved inflating a blood pressure cuff around the participant's upper arm (humerus) to 20 mmHg for 5 minutes and then deflating for 5 minutes. Again, this was repeated 4 times (total of 40 minutes), three times weekly for 6-weeks. This cuff pressure was chosen as the most common sham protocol used in prior RIC studies is between 10 – 30 mmHg (Lisbôa et al., 2017, Hyngstrom et al., 2018, England et al., 2017, Bailey et al., 2012, Walsh et al., 2016, Lindsay et al., 2017a, Zarbock et al., 2015). Inflating the cuff to 20 mmHg does not induce arterial occlusion but does give a 'pressure effect' (Groothuis et al., 2003). It was recognised that it may be difficult to ensure participant blinding due to the pressure difference with cuff

inflations between the RIC and sham protocol. The sham protocol was designed to make the same noises that the true RIC intervention made and for the same length of time.

2.2.14 Primary outcome measures

To evaluate the safety and acceptability of delivering a 6-week programme of RIC in patients with stroke who have debilitating fatigue, compliance with the intervention, and study feasibility the following outcome measures were assessed:

2.2.14.1 Safety

To assess safety, any adverse event (AE) or serious adverse event (SAE) either related or unrelated to the RIC protocol were recorded. A SAE is any event that results in death, is life-threatening, requires hospitalisation or results in persistent or significant disability, or is a congenital anomaly or birth defect (Health Research Authority, 2021a). AEs or any unexpected symptoms were reported in a symptom diary (Appendix 9) given to each participant at the beginning of the study, which included free-text sections for participants to report any AEs or SAEs. AE report forms were completed for AEs and stored in the study site file. Symptom diaries were reviewed weekly at a research meeting with the research clinician. Participants who self-administered the intervention at home received weekly telephone calls to review any problems with intervention delivery, review AEs and intervention compliance, as well as ensure consent to continue the study. AEs were scrutinised to determine whether they were likely to be related to the intervention itself. The PI investigated any AEs in accordance with National Research Service guidelines (Health Research Authority, 2021b). Safety was defined as there being no SAE relating to RIC and less than 10 AEs in total across all participants. Safety was also assessed by review of blood counts at the end of the RIC treatment intervention (*Table 5*).

2.2.14.2 Acceptability

Acceptability was measured by asking participants to rate their experience of several expected side effects during the intervention sessions on a 5-point Likert scale (1 = none, 5 = extremely severe) using the symptom diaries provided. This included rating the level of discomfort, skin irritation/redness, pain, weakness after, and pins and needles. These expected side effects were explained to participants at the beginning of the study before they gave their informed consent.

Acceptability was defined as less than 1/3 of participants reporting moderate or greater discomfort (mean score ≥ 3 for overall discomfort) (*Table 5*). Eight participants in the active treatment group were also invited to participate in a semi-structured qualitative interview to allow a more in-depth exploration of the participant's expectations and experiences of the treatment. They were sequentially offered the opportunity to participate in the qualitative part of the study however this part of the study was optional. The interview took place either during the last scheduled study visit or at another mutually exclusive time between the participant and researcher. Details of how interviews transcripts were analysed are provided in Chapter 6.

2.2.14.3 Compliance

To measure compliance with the intervention the researcher recorded the number (%) of RIC/sham cycles successfully delivered each week (in-hospital RIC). Participants who self-administered the intervention independently at home (home-RIC) were given monitoring diaries to take home (Appendix 10). The monitoring diaries were used to record how many RIC cycles were completed each week, and whether any sessions were either missed (<18 sessions completed over 6-weeks) or incomplete (<40 min). Compliance was defined as more than 80% of the intended RIC cycles completed (*Table 5*).

2.2.14.4 Feasibility

Study feasibility was assessed by review of participant recruitment rates and retention rates, and number (%) of baseline and follow-up assessments completed. The total number of participants screened for eligibility and reasons for ineligibility was also examined. Study feasibility was defined as the ability to recruit 4 patients within the first 2 months, retention rates >80%, and completion of >80% of intended baseline and follow-up assessments (*Table 5*). Study feasibility was further evaluated using participant responses from qualitative interviews. The success criteria for safety, acceptability, compliance, and feasibility are shown in *Table 5*.

Table 5. Success criteria for primary outcome measures.

Aim/objective	Outcome measure	Success criteria
Safety	• Total number of AEs and SAEs • Review of blood counts at the end of RIC intervention	• No SAE directly related to RIC • Less than 10 participants experience any AE during the intervention period
Acceptability	• Mean score for overall discomfort on Likert Scale (0-5) • Qualitative interview responses	• Less than one third of patients report moderate or greater discomfort associated with RIC
Compliance	• Number (%) of completed RIC cycles	• More than 80% of the intended RIC cycles completed
Feasibility	• Recruitment rates • Retention rates • Number (%) of completed baseline and follow-up outcome measures	• Recruitment rates (4 patients recruited within the first 2 months) • Retention rates more than 80% • 80% of outcome measure assessments and follow up assessments completed

Abbreviations: AE = Adverse event; SAE = Serious adverse event.

2.2.15 Secondary outcome measures

All of the secondary outcome measures were assessed at baseline and at the end of the 6-week study period. Some were also assessed during the participant's 3-month and 6-month telephone follow-up. The secondary outcome measures and the timepoint they were assessed are shown in *Table 6*. More detail about each outcome measure and how they were assessed and analysed is provided in subsequent chapters.

Table 6. List of secondary outcome measures and timepoint they were assessed.

Secondary outcome measure	When were they assessed?	What it involves
FSS-7	Baseline, 6w, 3m, and 6m	• Self-report 7-item questionnaire • Measures severity of fatigue symptoms • 7-point Likert scale ranging from 1 (strongly disagree i.e., better fatigue) to 2 (strongly agree i.e., worse fatigue) • Completion time approximately 5 minutes
PHQ-9	Baseline, 6w, 3m, and 6m	• Self-report 9-item questionnaire • Depression screening tool • Scores range from 0 (no depression) to 27 (all symptoms occurring daily) • Completion time approximately 5 minutes
GAD-7	Baseline, 6w, 3m, and 6m	• Self-report 7-item questionnaire • Measures the severity of Generalised Anxiety Disorder (GAD) • Scores range from 0 (no anxiety) to 21 (severe anxiety) • Completion time approximately 2-3 minutes
EQ5D-5L	Baseline, 6w, 3m, and 6m	• Measures Health Related Quality of life (HRQoL) • Five dimensions including mobility, self-care, usual activities, pain/discomfort and anxiety/depression • Rate from 1 (no problems) to 5 (severe problems) • Completion time approximately 3 minutes
EQ-VAS	Baseline, 6w, 3m, and 6m	• Rate overall health on a scale of 0 (worst imaginable health) to 100 (best imaginable health). • Completion time approximately 1 minute
mRS	Baseline, 6w, 3m, and 6m	• Interview-like process completed by researcher • Measure of global disability • Single-item rating scale ranging from 0 (no symptoms) to 6 (death) • Single point change is clinically meaningful (Harrison et al., 2013). • Completion time approximately 2 minutes
BI	Baseline and 6w	• 10-item self-report questionnaire • Total score of 0-100 (5-point increments)

		• Measure of independence on Activities of Daily Living (ADL) • Higher score = greater independence • Completion time approximately 2-5 minutes
MoCA	Baseline and 6w	• 30-point questionnaire • Cognitive screening tool • Score < 25 out of 30 indicates mild or greater cognitive impairment • Completion time approximately 10 mins
6MWT	Baseline and 6w	• Physical assessment • Assesses functional exercise capacity • Measures distance walked in 6-minutes
CPET	Baseline and 6w	• Physical assessment • Global assessment of cardiovascular, metabolic and ventilatory responses to exercise • Conducted on a cycle ergometer • CPET derived variables include peak oxygen consumption (VO_{2Peak}), ventilatory anaerobic threshold (VAT) • Completion time approximately 40 minutes (average cycle time 8-10 minutes)
Blood samples	Baseline and 6w	• Blood samples taken at baseline and 6-weeks for safety evaluation of RIC • Blood tests included full blood count, renal function, liver function, coagulation profile, and inflammatory markers
^{31}P-MRS	Baseline and 6w	• Measures tissue metabolism i*n vivo* • Assesses mitochondrial function by quantifying phosphorus containing metabolites involved in ATP synthesis and utilization (e.g., γATP, PCr, Pi,) • Scan time approximately 50 minutes (20 minutes per leg plus preparation time) • Included for mechanistic evaluation
Qualitative semi-structured interviews	6w	• Conducted face-to-face or over the telephone • Conducted using a topic-guide • Audio recorded and transcribed • Completion time approximately 10 minutes

***FSS-7 = Fatigue severity scale-7; PHQ-9 = Patient health questionnaire; GAD-7 = General Anxiety Disorder questionnaire; EQ5D-5L = EuroQol- 5 Dimension; EQ-5D VAS = EQ-5D visual analogue scale; HRQoL = Health related quality of life; BI = Barthel Index of Activities of Daily Living; mRS = Modified Rankin Scale; MoCA = Montreal Cognitive Assessment; 6MWT = six-minute walk test; CPET = Cardiopulmonary exercise testing; VO_{2Peak} = Peak oxygen consumption; VAT = ventilatory anaerobic threshold; ^{31}P-MRS = ^{31}Phosphorus-Magnetic Resonance Spectroscopy; PCr = Phosphocreatine; Pi = Inorganic phosphate; ADP = Adenosine diphosphate; 6w = six weeks; 3m = three months; 6m = six months.**

2.2.16 Baseline data collection

For most participants, baseline data collection and assessments were divided into two visits. This was to make it more manageable for the participant considering the large number of outcome measures, and because some assessments needed to be carried out at different research sites. Visit one typically included eligibility screening, informed consent, self-reported clinical outcome measures (FSS-7, PHQ-9, GAD-7, EQ5D-5L, EQ5D-VAS, BI, MOCA), the 6MWT and blood sampling. The researcher recorded the participant's demographic details (age, sex, weight, height) and recorded their medical history (medications, medical conditions). Next, they checked the participant's vital signs (blood pressure, heart rate). Some participants also completed cardiopulmonary exercise testing in their first visit. This was dependant on where the first study visit took place. If visit one took place at Sheffield Hallam University, the site for cardiopulmonary exercise testing, then it was usually completed that same day. If the first study visit took place at RHH or ARC, then cardiopulmonary exercise testing was completed during a second visit (usually within one week of visit 1). Blood samples were taken to RHH for analysis. A total of eight participants were invited sequentially to undergo ^{31}P-MRS (details provided in Chapter 5), however this was optional. If participants agreed to have ^{31}P-MRS, an additional study visit was required. The sample size of eight for the sub-study was chosen due to resource limitations and was exploratory in nature. When all baseline assessments were complete, participants received a RIC demonstration to familiarise them with the procedure and intervention schedule and participants who opted for home-RIC were given their training session.

2.2.17 Follow-up data collection

At the end of the 6-week intervention period all baseline assessments were repeated. Follow-up assessments were completed as soon as possible after the 6-week intervention by a researcher blinded to treatment allocation. The researcher collected the symptom and monitoring diaries from participants so they could be analysed. At this point participants in the treatment group were invited to complete a qualitative semi-structured interview with the researcher (details provided in Chapter 6). Participants also completed telephone follow-up assessments at 3-months and 6-months.

2.2.18 Ethical considerations

Confidentiality

Participant information (socio-demographic, clinical, outcome measure assessment) were recorded on paper clinical research forms that were furnished with a unique ID number (e.g., RIC01). This was transferred to a password protected, encrypted laptop only accessible to the researcher entering data and performing statistical analyses. The participant's unique ID number was the only "identifier" linked to data collected. A separate enrolment log book linked these numbers to the participant's personally identifiable information and stored separate to the study data. In this way, the participants' data was "pseudo-anonymised" and no personally identifiable information was kept with the actual study data. The enrolment log and study data was stored in a locked office at the RHH. Interview topic guides and paper notes were also pseudo-anonymised and stored securely. All data was anonomysed and stored in line with the Data Protection Act 1998 (Mullock and Leigh-Pollitt, 2000).

Good clinical practice (GCP)

All members of the research team received good clinical practice (GCP) training and all study activities were conducted to the standards set out in the principles of GCP (Good clinical practice, 2020).

2.2.19 Statistical analysis of primary outcome measures

Baseline demographic and clinical characteristics for the treatment and sham group were reported and any differences between the two groups were highlighted. The main outcomes of the study were the descriptive statistics for safety, compliance, acceptability, and feasibility. This included:

- The number of AE and SAE reported.
- The mean rating of discomfort on the symptom diary.
- The number of complete or incomplete RIC/sham treatments each week.
- The total number of RIC/sham treatments received over the 6-week study period.
- The number of participants screened for eligibility.
- The number of eligible participants screened.
- The number of ineligible participants and reasons for ineligibility.

- The number of eligible participants recruited and randomised.

Details of how the secondary outcome measures were analysed are provided in subsequent chapters.

2.2.20 Patient and public involvement

In 2017 a local stroke and aphasia patient and public involvement (PPI) panel made up of 8 stroke survivors or carers was consulted about practical aspects of undertaking the study (e.g., how to approach eligible patients, how long after stroke to include patients, the feasibility of undertaking the required outcome measure assessments) as well as reviewing the associated documentation (e.g., consent forms, participant information sheets, invitation letters etc). They suggested spreading the outcome assessment measures over 2-3 visits to help with fatigue and suggested a practical way of trying to maintain treatment blinding in which participants were told that two cuff occlusion pressures were being tested but that they would not know which one they were allocated to. They suggested increasing the timeframe of eligibility to include patients suffering fatigue years after stroke. They also recommended outcome measure assessments were undertaken at the patient's optimal period of alertness. Often patients with PSF will know which part of the day they feel most energetic, and it should be these periods that are used for outcome measure assessments and that the research team should be flexible in accommodating for this. In January of 2020 we returned to the PPI panel (this consisting of 11 members) with a short presentation on the preliminary data obtained so far and presented the idea of self or caregiver delivered RIC for PSF (this was before a substantial amendment was submitted to include home-RIC in this study due to covid-19 restrictions). We were able to provide demonstrations of the self-delivered intervention and again received invaluable insights: they helped produce an easy access aphasia friendly intervention leaflet to aid adherence and fidelity and suggested having pre-set timers to aid self-delivery of the intervention.

2.2.21 Changes to protocol

After the trial began, some major changes were made to the study protocol. In June 2019 a substantial amendment was submitted and was approved in October 2019. All research activities were halted during this time. The changes made to the protocol included:

- The Facility of Health and Wellbeing at Sheffield Hallam University was added as a research site. This was due to practical difficulties with performing cardiopulmonary exercise testing at ARC. The lab at Sheffield Hallam University had appropriate staffing, insurance, and escalation policies to deliver this.

- The Multidimensional Fatigue Inventory (MFI) was replaced with the Fatigue Severity Scale (FSS-7). The FSS-7 is one of the most commonly used scale to assess fatigue and is validated in stroke populations (Choi-Kwon and Kim, 2011, Lerdal et al., 2009) It is also sensitive to detecting changes in fatigue and minimal clinically important differences (MCID) have been reported (Nordin et al., 2016, Rooney et al., 2019). We also found participants struggled to complete the MFI in clinics with their clinicians.

- The exclusion criteria were updated to include 'other neurological conditions that cause fatigue (e.g., Multiple Sclerosis, Parkinson's Disease, Myasthenia Gravis)'. We wanted to ensure we recruited participants whose fatigue was caused by their stroke and no other conditions.

- An upper limit for travel reimbursement was added (£20 per visit). This could be claimed via expenses or paid for pre-paid taxis.

- The PIS, study invite, and consent form were updated to reflect the changes to the protocol and to ensure participants had a good understanding of the trial before recruitment.

Due to COVID-19, in July 2020 a second substantial amendment was submitted (approved November 2020). The amendments included:

- Including the option for participants to self-administer the intervention independently at home in exceptional circumstances (patient works full-time, COVID-19 restrictions). The option of home delivery helped overcome potential difficulties with recruitment and in-hospital delivery of RIC because of covid-19. It also allowed us to explore whether it is feasible and acceptable for stroke patients with fatigue to deliver RIC independently at home for 6-weeks using a manual sphygmomanometer and whether it can help improve fatigue. This

information will help with the potential implementation of RIC into clinical practice in the future.

- The option of weekly home visits to deliver the intervention. Details of how a risk assessment was to be conducted before each home visit was added into the protocol. All home visits were to be subject to the University of Sheffield's risk assessment procedure. Although this change was approved the researcher did not complete any home visits. Again, this change was added to help overcome difficulties with recruitment as a result of covid-19.

- MRI perfusion of the brain and brain spectroscopy were replaced with ^{31}Phosphorus-Magnetic Resonance Spectroscopy of the legs. We have undertaken a small pilot study on the acute effects of RIC on cerebral perfusion and cerebral metabolism using MRI. However, we did not find large signals of effect and believed we were more likely to detect changes in the peripheral muscle rather than centrally. We believe using ^{31}Phosphorus-Magnetic Resonance Spectroscopy of the legs may help investigate mechanisms of action behind the effect of RIC on fatigue.

- Adding the option of telephone interviews if more convenient than face-to-face interview. Telephone interviews were audio recorded (hands free), transcribed and stored in the same way as interviews conducted face-to-face.

- Changes to the study documentation (PIS, patient invites, consent form, aphasia friendly PIS and study schedule) to reflect changes in the protocol. This included details of how participants would be trained on how to deliver the intervention independently at home and how they would have to meet pre-determined criteria of competency. The PIS also explained how participants who opted for home delivery would receive weekly telephone follow-ups to review any problems with intervention delivery, review adverse events and intervention compliance, as well as ensure consent to continue the study.

- We also increased the number of participants who would take part in a qualitative interview from 6 to 8.

- As a result of covid-19, protocol amendments and recruitment being slow, the study end date was extended to December 2021. The GANTT chart in the protocol was amended and all tasks were extended by 6 months to account for delays to the study.

- Overall, a 10-month period where recruitment was temporarily suspended occurred during the study period due to the pandemic and substantial amendments.

2.3 Results

2.3.1 Recruitment/Cohort

In total, 103 people with significant fatigue after stroke were identified and approached. Of the 103 individuals approached, 24 participants were recruited into the study and 22 participants subsequently completed 6-week intervention. Of the 24 participants, 12 were randomised into the intervention group and 12 to the control. The demographic and clinical characteristics of the recruited participants are shown in *Table 7*. Participants ranged in age from 35 to 73 years. Participants in the sham group were slightly older with a mean difference between the two groups of 6.3 years. Two thirds of the participants were male (16 males, 8 females) split evenly between groups (8 men, 4 females in both groups). Time since stroke was similar between the groups (38.5 months vs 39.8 months in treatment and sham, respectively), however, the sham group contained a greater proportion of people who had suffered intracerebral haemorrhage (41.7% vs 8.3%). Participants in the treatment group were heavier with a mean difference between the two groups of 7.6kg. The proportion of participants who received in-hospital RIC or home-RIC was reasonably equal (13 versus 11 participants, respectively). In the hospital RIC group, 7 out of 13 participants received active RIC and 6 participants received the sham treatment. In the home-RIC group, 5 participants were in the treatment group and 6 participants were in the sham group.

2.3.2 Participant flow

Figure 5 shows CONSORT flowchart, showing the flow of participants through the trial. Figure 5 provides a breakdown of the number of participants approached, numbers recruited and retained and reasons for ineligibility. Reasons for ineligibility included diagnosis of obstructive sleep apnea (OSA), high depression or anxiety scores, and mild fatigue (FSS-7<4). There is incomplete data on the number of participants screened as some participants were referred by

word of mouth. The CONSORT diagram in Figure 5 also details the reasons for refusal to participate in the study, including perceived study burden, lack of interest in the study and shielding from covid-19. Out of the 24 participants recruited, two participants (8.3%) withdrew from the study for non-intervention related reasons and did not complete further questionnaires.

Table 7. Characteristics of study participants by treatment group and location.

Characteristic	All	Treatment	Sham	In hospital RIC	Home RIC
n	24	12	12	13	11
Age, years (mean; SD)	58.6 (10.9)	55.5 (10.6)	61.8 (10.7)	59.0 (10.1)	58.2 (12.2)
Sex	16 M: 8 F	8 M : 4 F	8 M : 4 F	11 M : 2 F	5 M : 6 F
Height, cm (mean; SD)	172.8 (7.1)	174.3 (6.7)	171.3 (7.6)	172.1 (7.3)	173.6 (7.2)
Weight, kg (mean; SD)	86.3 (19.8)	90.0 (21.3)	82.4 (18.2)	90.2 (23.2)	82.1 (15.2)
BMI (mean; SD)	29.0 (7.7)	29.9 (8.5)	28.1 (6.9)	30.9 (9.8)	27.1 (4.0)
Ethnicity	Caucasian (83.3%) Black African (4.2%) Dutch (4.2%) Asian British (8.3%)	Caucasian (75.0%) Black African (8.3%) Dutch (8.3%) Asian British (8.3%)	Caucasian (91.7%) Asian British (8.3%)	Caucasian (76.9%) Black African (7.7%) Dutch (7.7%) Asian British (7.7%)	Caucasian (90.9%) Asian British (9.1%)
Stroke type (%) **Ischaemic** **Haemorrhagic**	 70.8% 29.2%	 91.7% 8.3%	 58.3% 41.7%	 76.9% 23.1%	 72.7% 27.3%
Time since stroke, months (mean; SD)	39.1 (14.3)	38.5 (14.7)	39.8 (14.5)	41.7 (10.8)	36.1 (17.6)
Comorbidities *n* (%)					

Hypertension	10 (42)	3 (25)	7 (58)	7 (54)	3 (27)
Hyperlipidaemia	3 (13)	2 (17)	1 (8)	2 (15)	1 (9)
Diabetes	2 (8.)	1 (8.)	1 (8)	1 (8)	1 (9)
Stroke/TIA	4 (17)	2 (17)	2 (17)	3 (23)	1 (9)
IHD	1 (4)	1 (8)	0 (0)	0 (0)	1 (9)
CCF	1 (4)	1 (8)	0 (0)	0 (0)	1 (9)
AF	2 (8)	2 (17)	0 (0)	1 (8)	1 (9)
Depression	7 (29)	4 (33)	3 (25)	3 (23)	4 (36)
Medications *n* (%)					
Antiplatelet	13 (54)	6 (50)	7 (58)	8 (62)	5 (46)
Anticoagulant	6 (25)	6 (50)	0 (0)	2 (15)	4 (36)
Antihypertensive	6 (25)	2 (17)	4 (33)	4 (31)	2 (18)
Statin	13 (54)	6 (50)	7 (58)	8 (62)	5 (46)
Antidepressant	6 (25)	3 (25)	3 (25)	3 (23)	3 (27)
***mRS (median; IQR)**	2.00 (1.25)	2.00 (1.00)	2.00 (2.00)	2.00 (1.75)	1.50 (1.25)
Independent (%) (score 0-2)	77.3	81.8	72.7	75.0	80.0
Dependent (%) (score 3-4)	22.7	18.2	27.3	25.0	20.0

RIC = Remote ischaemic conditioning; SD = Standard deviation; M = Male; F = Female; BMI = Body mass index; TIA = Transient ischaemic attack; IHD = Ischaemic heart disease; CCF = Congestive cardiac failure; AF = Atrial fibrillation; mRS = modified Rankin Scale. *mRS figures based on only the 22 participants who completed the 6-week intervention as it is used in later analysis.

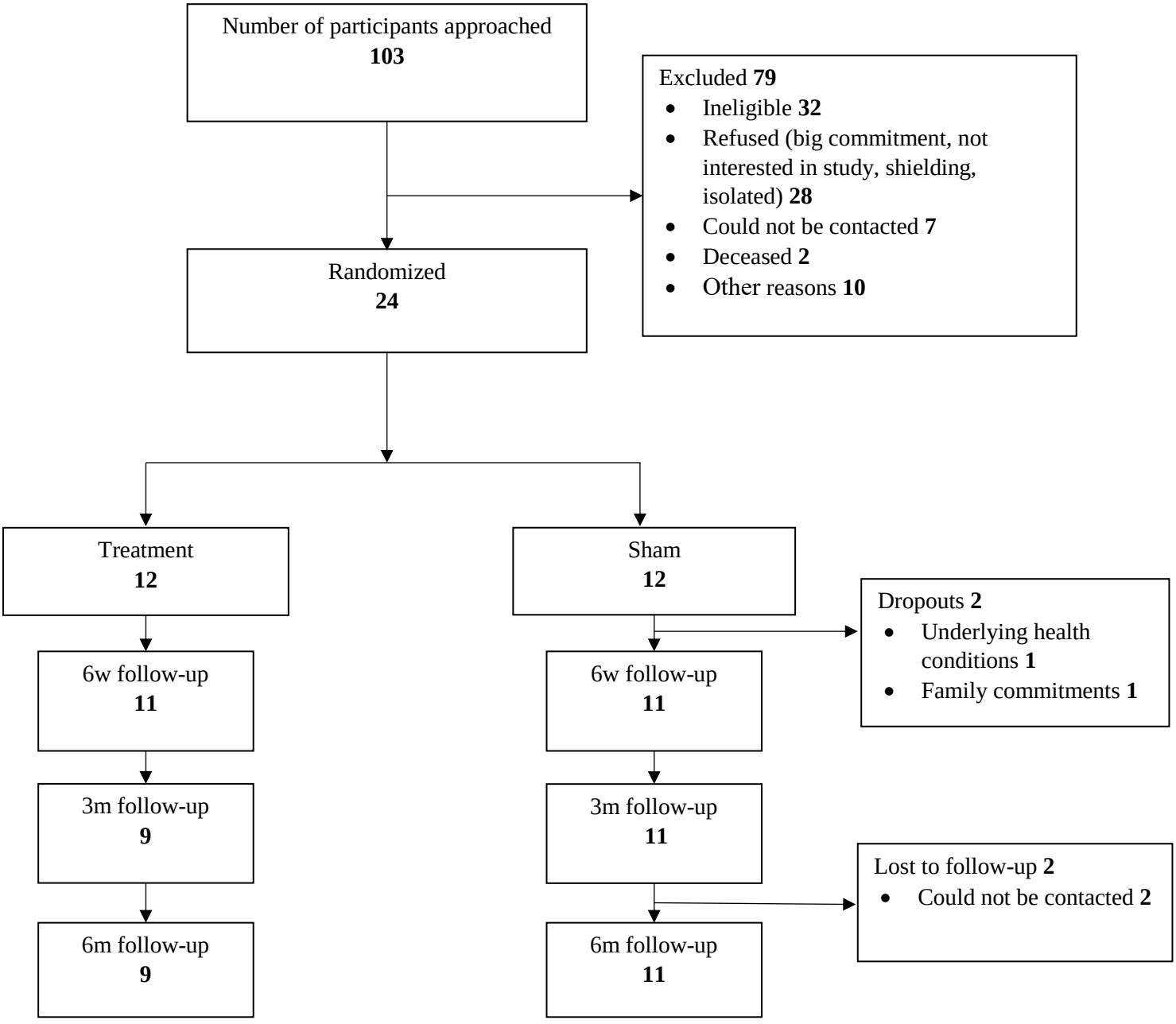


Figure 5. Consolidated Standards of Reporting Trials (CONSORT) diagram.

2.3.3 Primary outcome measures

2.3.3.1 Safety

Eight out of the 11 participants in the treatment group (73%) who completed the 6-week intervention reported AEs related to the intervention during the 6-week intervention period, including petechiae (microbleeds in the skin) (45%), headache (27%), dizziness (9%), swelling of the arm (9%), numbness of the arm (9%) and arm stiffness (9%) (*Table 8*). No participants in the sham group (0%) who completed the 6-week intervention experienced any AEs and no participants (0%) in the treatment or sham group experienced any SAE related to RIC/sham intervention. Out of all the AEs related to the intervention, 83% were experienced by participants in the hospital-RIC group (*Table 8*). Only two participants in the home-RIC group reported AEs related to intervention (1x petechiae, 1x headache) (*Table* 8). Two participants (both in the sham group) experienced AEs unrelated to the study intervention (musculoskeletal chest pain, hypoglycemia, dizziness) within 6-months of intervention cessation. Since less than 10 participants reported AEs (n=8) and no participants reported SAE directly related to the RIC protocol, the criteria for safety of RIC in stroke were met. Blood results were checked at baseline and at 6-weeks including full-blood count (FBC), renal function (urea and electrolytes, U&E) and liver functions (alanine aminotransferase, ALT) to ensure the intervention did not cause any unexpected abnormalities. One participant had a pre-existing abnormality in ALT at baseline, however this improved at follow-up, and one participant had incidentally found Gilberts syndrome which was communicated to the participant and their GP.

Table 8. Number of participants reporting adverse events related to the intervention in the treatment and sham group and hospital and home RIC group.

Adverse Event	**Treatment**	**Sham**	**Hospital RIC**	**Home RIC**
N	11	11	12	10
Petechiae *n* (%)	5 (45)	0 (0)	4 (33)	1 (10)
Headache *n* (%)	3 (27)	0 (0)	2 (17)	1 (10)
Dizziness *n* (%)	1 (9)	0 (0)	1 (8)	0 (0)
Swelling of arm *n* (%)	1 (9)	0 (0)	1 (8)	0 (0)
Numbness *n* (%)	1 (9)	0 (0)	1 (8)	0 (0)
Arm stiffness *n* (%)	1 (9)	0 (0)	1 (8)	0 (0)

***Table only includes data from the 22 participants who completed the 6-week intervention. There is a lack of data on safety or acceptability from the two participants who withdrew from the study.**

2.3.3.2 Acceptability

The mean score for overall discomfort on the symptom diary (Appendix 9) for all 22 participants who completed the 6-week intervention was 1.7 (± 0.9), reflecting mild discomfort (*Table 9*). The mean score for overall discomfort in the treatment group was 2.3 (SD ± 0.8), indicating mild-moderate discomfort. Only one participant in the treatment group (9.1%) reported moderate or greater discomfort associated with the RIC treatment (mean score = 4.5 which reflects severe discomfort). The mean score for overall discomfort in the sham group was 1.0 (± 0.0) (reflecting no discomfort) and no participants in the sham group reported moderate or greater discomfort (*Table 9*). The mean score for all the side effects listed on the symptom diary for the treatment/sham and hospital/home intervention group are shown in *Table 9*. Score for overall discomfort was slightly higher in the home-RIC group compared to the hospital-RIC group (*Table 9*). This was also the case for skin irritation, redness, weakness after and pins and needles (*Table 9*). Since less than one third of participants reported moderate or greater discomfort associated with the RIC treatment the criteria for acceptability were met. Responses from qualitative interviews about what participants felt about the treatment are provided in Chapter 6, however indicated good acceptability altogether and a willingness to continue the treatment should it be found to be effective.

Table 9. Mean score on each section of the symptom diary in the treatment and sham group and the hospital and home RIC group.

Symptom	Overall mean (mean; ±SD)	RIC group (mean; SD)	Sham group (mean; SD)	Hospital RIC	Home RIC
N	22	11	11	12	10
Overall discomfort	1.7 (± 0.9)	2.3 (± 0.8)	1.0 (± 0.0)	1.5 (± 0.5)	1.8 (±1.2)
Skin irritation/redness	1.3 (± 0.5)	1.5 (± 0.6)	1.1 (± 0.3)	1.1 (± 0.2)	1.4 (± 0.7)
Pain	1.5 (± 0.8)	1.9 (± 1.0)	1.0 (± 0.0)	1.2 (± 0.4)	1.8 (± 1.1)
Weakness after	1.1 (± 0.3)	1.2 (± 0.4)	1.0 (± 0.0)	1.0 (± 0.1)	1.2 (± 0.4)
Pins and needles	1.7 (± 0.9)	2.4 (± 0.9)	1.1 (± 0.1)	1.5 (± 0.5)	2.0 (± 1.2)

***Table only includes data from the 22 participants who completed the 6-week intervention. There is a lack of data on safety or acceptability from the two participants who withdrew from the study.**

2.3.3.3 Compliance

Out of the 24 participants recruited, 22 participants (91.7%) successfully completed the 6-week intervention, and 20 participants (83.3%) completed 100% of intended RIC/sham cycles (18 complete 40-minute sessions over the 6-week period). The two participants who either missed or had an incomplete session were both in the home-RIC group. As more than 80% of the intended RIC/sham cycles were completed, the criteria for compliance were met. Participants were told they could have the treatment any time of day, any day of the week, as long as they had three complete 40-minute sessions each week. Having this flexibility made it easier for participants to comply.

2.3.3.4 Feasibility

In total, 4 participants were recruited within the first 2 months. Out of the 24 participants recruited, all 24 participants (100%) completed baseline assessments. Two of the 24 participants withdrew from the study, leaving 22 participants (91.7%) who completed follow-up assessments at 6-weeks. The overall retention rate for the study was high (91.7%), despite challenges with recruitment due to covid-19. Twenty-one participants (87.5%) completed 100% of patient completed clinical outcome measures (FSS-7, PHQ-9, GAD-7, MRS, BI, EQ5D-5L, EQ-VAS and MOCA) at baseline and at 6-week follow-up. One participant could not complete the MOCA questionnaire due to aphasia.

In total, 17 participants (70.8%) completed cardiopulmonary exercise testing at baseline and at 6-week follow-up, and 19 participants (79.2%) completed a 6MWT at baseline and at 6-weeks. Reasons for non-completion of baseline or follow-up tests include withdrawal from the study, stroke impairments (spasticity, weakness), illness on the day of testing (breathlessness, cough), lack of access to research sites whilst protocol amendments were approved and covid-19 restrictions (discussed in chapter 4).

Eight out of an intended 8 participants (100%) underwent ^{31}P-MRS at baseline, however due to one participant withdrawing from the study, seven participants (87.5%) underwent ^{31}P-MRS at 6-weeks. Eight out of an intended 8 participants (100%) in the treatment group completed qualitative interviews.

Twenty out of 22 participants (90.9%) who completed the 6-week RIC/sham intervention completed telephone follow-ups at 3 months and 6-months. There were good completion rates for all baseline and follow-up outcome measures (>80%). Taking all of this into consideration, the criteria for study feasibility (4 participants recruited within the first 2 months, retention rate >80% and 80% of all outcome measure assessments and follow-up assessments completed) were met (*Table 5*).

2.4 Discussion

2.4.1 Safety and acceptability

Overall, RIC appeared to be safe and tolerable. The most common AEs related to the intervention experienced by participants in the active treatment group were skin petechiae and headache, similar findings to several previous studies (Meng et al., 2012, Meng et al., 2015a, Koch et al., 2011, Zhao et al., 2017, Mohammad Seyedsaadat et al., 2020). Less common AEs included dizziness, swelling of the arm, numbness of the arm and arm stiffness. Other side effects reported which were included in the symptom diary and highlighted as possible side effects, included skin irritation/redness and pins and needles, both of which were rated mild. Only one participant in the treatment group reported moderate or greater discomfort associated with the intervention, thus meeting the criteria for safety of less than one third of participants reporting moderate or greater discomfort. None of the participants requested the intervention to be stopped due to discomfort or AEs.

The proportion of RIC-related AEs was higher in the hospital RIC group compared to the home RIC group. This might be because in the hospital RIC group the researcher delivering the intervention was very vigilant and thorough when reporting AEs, whereas participants in the home RIC either did not want to make a ‘fuss’ or forgot to report the AE in the symptom diary. Although, the average scores on the symptom diary for overall discomfort, skin irritation, weakness, and pins and needles, were higher in the home RIC compared to the hospital RIC group. However, as highlighted in the results section one participant reported severe discomfort associated with the RIC intervention and this participant was in the home RIC group. Therefore, we believe they brought the average scores up in the home RIC group.

One participant in the RIC group said they thought the first dose of RIC was the most uncomfortable describing it as a 'shock' in their qualitative interview (see chapter 6). Most participants felt the treatment got easier over time, however the participant who reported moderate or greater discomfort did not feel as though it got easier. Despite this they completed all intended RIC cycles. They also described the treatment as 'not so painful but uncomfortable' in their qualitative interview (see chapter 6). Development of skin petechiae was more noticeable when the BP cuff was inflated over bare skin and could be ameliorated when applied over a thin sweater or long sleeve top without impairing the ability to facilitate RIC.

Exclusion criteria utilized in this study were aimed at either safeguarding participants and enhancing the likelihood of RIC efficacy and have been commonly used in many other RIC trials (Hougaard et al., 2014, Lavi et al., 2014, Hausenloy et al., 2019, Gonzalez et al., 2014, Hansen et al., 2019). Similarly, peripheral vascular disease or venous thromboembolism are consistent exclusion criterion used in previous studies RIC in stroke (Koch et al., 2011, Gonzalez et al., 2014). Although the intervention appears to be risky at face value (including tissue ischaemia), previous studies of RIC in stroke have not reported increased risk of vascular complications. For example, no studies have reported increased risks of venous thrombosis after the RIC treatment in stroke (Baig et al., 2021). Furthermore, a study conducted by Hansen et al. (2019) of 36 type 2 diabetic mellitus patients (mean age 70.7 years) which investigated repeated home-based upper limb RIC (4 x 5 min cycles of ischaemia followed by 5-min reperfusion for 12 weeks) in patients with known lower limb peripheral vascular disease (80% had peripheral symmetrical neuropathy) concluded the treatment was safe and feasible in this population. Treatment adherence was extremely good with compliance rates of 92.1% in the active treatment group and 82.1% in the sham group. Only three AEs related to the RIC intervention were reported were transient skin petechiae distal to cuff placement. However, the treatment was not found to be efficacious with no effect on tissue oxygenation (no change in transcutaneous tissue oxygen tension, $TcPO_2$), vascular (no change in aortic pulse wave velocity, PWV, or toe-brachial index), or neuronal function (measured using the electrochemical skin conduction test) in the lower extremities observed after RIC. Therefore, while these findings should provide some reassurance to researchers and clinicians in terms of the safety of including patients with peripheral vascular disease in clinical trials of RIC, peripheral neuropathy is associated with diabetes may be associated with reduced conditioning effects (Heusch et al., 2015, Hansen et al., 2019). We chose to exclude participants with a resting systolic blood pressure (SBP) >180 mmHg. This was to ensure inflating the cuff to 200

mmHg was sufficient to produce ischaemia in the limb receiving the conditioning stimulus. If the pressure is insufficient to cause ischaemia, then we are not truly assessing the safety and tolerability of the treatment. Some studies in the literature have instead inflated the BP cuff 20-50 mmHg above SBP instead of the standard 200 mmHg (Lavi et al., 2014, England et al., 2016, Gonzalez et al., 2014, Koch et al., 2011, England et al., 2019, Hougaard et al., 2014). However, the downside of this method is that the participant's blood pressure may vary between cycles affecting the standardisation of the protocol.

Clinical trials of limb RIC in stroke have demonstrated it is safe in patients with AIS, subarachnoid haemorrhage (SAH) and following reperfusion therapies such as carotid endarterectomy and mechanical thrombectomy (Gasparovic et al., 2019, Hougaard et al., 2014, Zhao et al., 2017, Che et al., 2019, Zhao et al., 2018a, Koch et al., 2011, Gonzalez et al., 2014, Mohammad Seyedsaadat et al., 2019, England et al., 2017), although treatment durations are generally short (<7 days). Zhao et al. (2018a) explored the safety and feasibility of unilateral upper limb RIC (4 x 5 min cycles of ischaemia to 200 mmHg) in patients with AIS with suspected large-vessel occlusion. Conditioning was performed before and immediately after recanalization (intravenous thrombolysis, IVT) of the occluded artery, and once daily for the subsequent 7 days. No SAEs related the RIC treatment occurred and only one participant reported skin petechiae. No participants had to discontinue the RIC treatment because it interfered with routine clinical managements and overall completion rates were good. Although some subjects failed to complete all intended cycles before recanalization due to lack of time between hospital admission and the patient receiving IVT. These findings are consistent with a recent study by An et al. (2020) who also investigated the safety of RIC in patients with AIS receiving IVT. In this study patients received bilateral RIC (5 x 5 min upper limb ischaemia to 180 mmHg separated by 3-min reperfusion) twice daily until discharge (average hospital stay 11.2 days). The control group underwent no inflations or deflations of the BP cuff. An et al. (2020) found that all patients in the RIC group (n=34 in the treatment and sham group), except for two (one was uncomfortable with the pressure, and another developed skin redness), received complete RIC sessions twice daily until discharge (maximum 14 days). This demonstrates how repeated RIC is well tolerated in these patients. There was however no significant difference in mortality rates at 3 months of treatment between the RIC and control group in this study (1 of 32 RIC patients vs 3 of 34 controls, $P = 0.614$).

Meng et al. (2012) investigated the effects of long-term home or hospital bilateral arm RIC (BAIPC) for 300 days (Meng et al., 2012) and found it was extremely well tolerated by all patients in both groups (no patients aborted the treatment early due to discomfort). Furthermore, no participants experienced local skin or vessel lesions and no patients died or had other vascular events during the study period. This is the longest duration of successful RIC therapy. A later study by the same investigators found BAIPC for 180 days in octo-and nonagenarians was safe with 83.3% of patients in the active treatment group completing the 180-day intervention without overt discomfort and 16.7% reporting mild discomfort but tolerated the treatment. AEs included transient skin petechiae (3/30 patients in the active treatment group) with no other tissue or neurovascular injury observed (Meng et al., 2015a). These findings demonstrate how long-term RIC is also safe in very elderly patients with stroke.

In our study, there is a chance that inflating the blood pressure cuff to 200 mmHg was not sufficient to induce ischaemia in every participant in the active treatment group, especially considering a couple of participants in the treatment group were overweight (mean weight in the treatment group = 90.0kg versus 82.4kg in the sham group). Many people with stroke have high blood pressure (Dawes, 2013) or atherosclerotic vessels (Hoshino et al., 2017), which may also require a higher pressure to induce ischaemia in the limb being conditioned. Going forward, if a larger trial investigating RIC for PSF is carried out, one way to ensure vascular occlusion may be to use a Doppler probe (or an equivalent technique), however this would be cumbersome and add complexity to the intervention. Excluding participants with a SBP > 180 mm Hg was done to ensure, as much as possible, that 200 mmHg was a sufficient pressure. However, unless we objectively measure this we will not know for certain whether ischaemia has been induced in the preconditioned limb.

2.4.2 Adherence

Overall, adherence with the intervention was very good with 91.7% of recruited participants completing the 6-week intervention and 83.3% completing all intended RIC/sham cycles. Compliance was slightly better in participants who received in-hospital RIC. One of the two participants who withdrew from the study, and the two participants who either missed a full day treatment or had an incomplete session, self-administered the intervention at home. One possible reason why compliance was better in the hospital-RIC group is that once participants

booked their weekly treatment sessions, they felt committed to turn up to their appointment. As well, for most participants we arranged transport to and from the hospital which made it easier for participants to attend their sessions. The researcher also sent weekly reminders to participants about their appointments and gave them the option to reschedule sessions if needed. One participant in the home-RIC group relied on family members or community carers (who received a virtual training session) to help deliver the treatment (attach the cuff, stop/start the timer), however sometimes carers did not have the time, or they did not join the virtual training session so were either unaware of the study or of the study procedures. Compliance was therefore lower in participants who relied on family or carers to help them administer the conditioning.

There is a lack of data on compliance in trials of chronic RIC in stroke, therefore it is difficult to know for certain whether stroke patients are able to carry out RIC consistently over weeks or months (Baig et al., 2021). For example, in the study by Meng et al. (2012) only 38 out of 51 patients (74.5%) randomised to receive twice daily bilateral arm RIC (5 x 5 min episodes of ischaemia to 200 mm Hg followed by 5-min reperfusion) successfully completed the study. A retrospective study by Zhao et al. (2021) investigated factors that influenced compliance in a study of 91 ischaemic stroke patients who received repeated bilateral upper limb RIC (5 x 5 min cycles) twice daily for 1-year. Out of 91 patients who completed the study, 56 had good compliance (defined as ≥80% RIC treatments completed). Factors that independently influenced patient compliance included physiological discomfort (comfort ratings higher in people with poor compliance) and the number of follow-up visits (number of follow-up visits was positively correlated with compliance). Longitudinal trials using larger samples of participants will help inform clinicians and researchers on likely compliance rates.

Overall, completion of the weekly monitoring diaries to record compliance with the intervention was very good in the home-RIC group. It is difficult to know for certain how accurate the logs are and whether participants were truthful in their answers, however after working with these participants for several weeks we believe it is likely they answered truthfully. Self-reported adherence methods (e.g., logbooks) is the most widely used method for assessing adherence to medication or device usage in clinical care and research. Advantages of self-report adherence methods include low-cost, ease of implementation and minimal patient burden (Stirratt et al., 2015). They can provide useful information on adherence determinants (e.g., understanding of medication regime or device application) and reasons for nonadherence

(Stirratt et al., 2015). However, there are questions about their validity and precision. If they are not completed correctly, they could lead to under- or overestimations of adherence rates (Stirratt et al., 2015, Farmer, 1999). The most difficult aspect of the intervention faced by participants in the home-RIC group was remembering to turn the timer on or remembering how many cycles they had completed. There is a chance that these participants sometimes either did not complete a full 5-min cycle or did not complete the correct number of cycles. This may have implications for the accuracy of the monitoring logs and compliance estimates. Nevertheless, adherence in the home-RIC group was very good demonstrating the feasibility of home-delivered RIC. In our study, the researcher kept a record of whether the participants in the hospital-RIC group had to reschedule or cancel any sessions. Two participants had to cancel a couple of sessions due to illness or family commitments. They either had an extra session the following week or had additional session added onto to the end of the 6-weeks. As long as they had a total of 18-sessions they met the criteria for compliance.

Home or carer delivered RIC has been shown to be safe and feasible in prior studies of RIC in stroke (Meng et al., 2012, Kate et al., 2019). In the study by Kate et al. (2019) they investigated the safety and feasibility of six cycles of self-or caregiver delivered bilateral upper limb RIC (inflated to 20mmHg above systolic BP to a maximum of 200 mmHg) compared to the popular four cycles in patients with AIS (n=30 in the six-cycles group; n=27 in the four-cycles group). The treatment began within 48-hours of symptom onset and participants were given a manual aneroid BP machine to take home and asked to perform RIC twice daily for 12-weeks. In terms of safety, no patients developed SAEs related to the treatment and only three (5.2%) developed petechial rash. In terms of feasibility, a total of 18 (66%) patients in the four-cycles group completed ≥50% sessions in 12-weeks, and 21 (69.7%) patients in the six-cycles group completed ≥50% sessions. Two thirds of patients completed at least 60 treatment sessions in 6-weeks in both groups. The main reason for discontinuation of the intervention was duration of the therapy. Other reasons included: treatment associated anxiety, pain during the intervention, petechial rash, rehospitalisation, and death. The investigators concluded that bilateral upper limb self-or caregiver delivered manual RIC was feasible and safe in both groups of patients with AIS, however it may be more feasible for a shorter duration (6-weeks) (Kate et al., 2019).

If home-RIC was an option at the beginning of the study, it is likely that recruitment rates would have been higher. Several people approached refused to take part in the study because of work commitments or perceived study burden. If they had the option to self-administer the intervention at home, they may have been more open to taking part in the study. For example, one participant in the home-RIC group said in their qualitative interview (see chapter 6) that they do not think they would have been able to manage coming into the hospital each week for treatment and were happy they could do it at home. Participants in the hospital-RIC/sham group were either retired, unable to work, or had an understanding employer who allowed them to start work later on the days they had an appointment. Regular visits to the hospital each week allowed a relationship to build between the participant and researcher, which was important for participant engagement. This personalised one-to-one care seemed to be valued by participants who enjoyed the time away from home and having someone to talk to. Participants in the home-RIC/sham group had less face-to-face contact with the researcher which some participants may have preferred. However, the option of home-RIC allowed participants to deliver the intervention at home over holiday periods which was important for recruitment.

Automated RIC devices (autoRIC CellAegis devices) are available and have been used in several of the large-scale trials (Gorog et al., 2021, Meng et al., 2012, Zhao et al., 2018a, Hansen et al., 2019). The electric devices automatically inflate the cuff (to 200 mmHg) and control the duration and number of RIC cycles (Belaoussoff et al., 2017). They also have a compliance monitoring system embedded in the cuff which monitors the number of cycles completed (Hansen et al., 2019). The most common way of inducing RIC, with a manual sphygmomanometer and a timer, is time-consuming and there are some concerns about its safety (e.g., leaving the cuff inflated in an obtunded or anesthetised patient) (Belaoussoff et al., 2017). Its delivery requires 40-45 minutes of a practitioner's time and is quite burdensome for participants. Automated devices offer a time saving solution and will make the clinical implementation of RIC more straightforward (Belaoussoff et al., 2017). However, automated devices are costly, and usually employ single use cuffs that are not yet validated for long term use (i.e., using the same cuff for inflation for a period of 30 days for example). The use of an automated RIC device in trials of repeated self-or caregiver RIC may help improve compliance rates. This is because the researcher will be able to monitor the number of cycles the participant completes each week using the wireless monitor unit connected to the monitoring system in the cuff and participants may feel a pressure to comply with a conditioning protocol if they know compliance will be checked electronically.

2.4.3 Feasibility

2.4.3.1 Recruitment and retention

Overall, the study methods were feasible in terms of recruitment, randomisation, baseline assessments and follow-up assessments and the recruitment criteria were met (four participants within the first two months). Recruitment total was not as good as one anticipated due to delays getting ethical approval, covid-19 restrictions, and substantial protocol amendments (see section 2.2.21 'Changes to protocol' and the covid impact form). However, despite these challenges we recruited appropriately and completion rate for baseline and follow-up assessments was >80% for most outcome measures.

At 3-month and 6-month follow up, the participant retention rate was 83.3%. We also recruited four participants within the first two months, fulfilling the criteria for feasibility. Due to covid-19, the recruitment period needed to be extended and one participant withdrew from the study because they classified as clinically vulnerable (asthma) and needed to shield from covid-19. Although recruitment was difficult, there was good adherence to the planned intervention in those participants who did receive the RIC/sham intervention, with a high level of detail provided in the qualitive interviews (discussed in chapter 6). While face-to-face assessments (baseline and 6-week follow-up) were largely successful, it was difficult to obtain telephone follow-up data from every participant. Two participants in the treatment group were either non-contactable or had severe aphasia which made telephone conversations difficult. Nevertheless, the retention rate at 3-month and 6-month follow-up was 90.9%. There is a lack of data on the number of participants screened as some participants were referred by word of mouth. The main reason for non-participation included ineligibility due to obstructive sleep apnoea, severe depression, mild fatigue, and old age, as well as perceived study burden. Although old age was not listed in the exclusion criteria, participants were excluded if the researchers felt they would be unable to complete the outcome measures (e.g., cardiopulmonary exercise test). We also did not want to invite particularly elderly individuals to participate during the pandemic due to risks associated with covid-19.

2.4.3.2. Outcome measures

We were able to assess the different outcome measures (patient-completed clinical outcome measures, 6MWT, CPET, ^{31}P-MRS, qualitative interviews) to determine which would be the

most suitable for a larger definitive RCT. The questionnaires were relatively easy to complete face-to-face as well as over the telephone for the 3m and 6m follow-ups. The questionnaires were explained to the participants before they completed them. Some participants preferred to complete the questionnaires by themselves, other asked for help reading the questions. For participants with aphasia, the researcher went through each question carefully to ensure participants understood the question and were happy with their response. It was sometimes useful to have a spare piece of paper for participants with aphasia to write down their thoughts and to help the researcher communicate with the participant effectively and sensitively. Two participants had moderate-severe aphasia. One of the participants was lost to follow-up and we believe that their aphasia was a barrier to participation in the telephone follow-up calls while face to face visits were restricted during the pandemic. Self-report measures of fatigue, mood and QoL provide a real understanding of how the participant is feeling from their own perspective. However, they can be influenced by confounding factors such as mood, age, and education level (Chang et al., 2019). More detail about the individual self-report measures including their strengths and limitations will be provided in Chapter 3.

At the beginning of the study, activity watches were given to all participants to record step counts during the study and the global physical activity questionnaire (QPAQ) was included as a secondary outcome measure. However, there were challenges with both. A couple of participants requested for the activity watch to be placed on their stroke-affected side so they could operate and attach the watch with their non-affected hand. However, limited movement on their stroke-affected side meant the watch did not always record their steps. Other problems with the activity watches included: participants forgetting to charge the watch battery, participants forgetting to wear the watch (particularly at weekends), and technical difficulties. Regarding the GPAQ, which assesses levels of physical activity at work, for travel, and for leisure (Bull et al., 2009, Cleland et al., 2014), most of the questions were either not relevant to our participants or were difficult to answer. Many of our participants were retired or had limited mobility and the questionnaire requires a lot of calculation (summing up commuting, leisure activities, sedentary behaviour) which participants tended to guess. As a result of these challenges we made the decision to remove the GPAQ and activity watches from the list of secondary outcome measures.

Regarding cardiopulmonary exercise testing, the main barrier was access to the exercise lab at Sheffield Hallam University. On several occasions research activities at the site were halted

due to protocol amendments, covid-19 restrictions, and risk-assessment updates. This meant two participants were unable to have their follow-up CPET and were therefore excluded from the analyses. Covid-19 restrictions meant staff numbers were limited which meant we were restricted to the days and times we could book the exercise lab. Overall, participants who completed CPET managed very well. A couple of participants required help getting onto the bike but were okay once they were positioned correctly. The researchers had to put a resistance band around the knee of one participant and pull it outwards to stop it hitting off the bike when cycling. This participant had to terminate the test early and was excluded from analyses. More details about cardiopulmonary exercise testing will be provided in Chapter 4.

Blood samples were not always easy to obtain. Some participants either had small or deep veins or were not adequately hydrated (although advised to arrive to their appointments in an euhydrated state). Other problems included finding a suitable clinical space to take the blood samples.

The 6MWT is a simple and reliable measure of functional exercise capacity in stroke (Dunn et al., 2015, Kosak and Smith, 2005), however lack of space at RHH meant that sometimes the 6MWT west was completed on a 9-metre corridor at Sheffield Hallam University. The more frequent need to turn around on a shorter corridor may have negatively impacted the total distance walked in participants who were more impaired as they tended to struggle with this part of the test. A cross-sectional study of 26 chronic stroke patients investigated the effect of turning directions (turning to stroke affected side and unaffected side) and different walkway distances (10, 20 and 30-m) on distance walked on the 6MWT (Ng et al., 2011). Walkway length had a significant effect on distance covered (e.g., 30m walkway distance 265.47±94.16 m vs 10m walkway distance 227.32±79.07 m, $P<0.001$), whereas turning direction did not significantly affect distance walked. These findings demonstrate how using a 9m walkway in our study may have significantly shortened the distance walked. However, a 10m course is commonly administered by physiotherapists in primary care where space is limited and is a valid test (Beekman et al., 2014). Another factor which may have impacted participant performance on the 6MWT is the need to wear a face covering during the pandemic. One participant expressed difficulty regulating their breathing during the test and requested to remove their facemask. This may have slowed down their walking pace and reduced overall distance walked during the test. Participants were permitted to use their walking aid. Results from the 6MWT will be discussed in chapter 3.

Regarding the qualitative interviews, a high level of detail about individual experiences with the treatment were provided. Before Covid-19, interviews were conducted face-to-face. They were conducted using a topic guide and only took approximately 10-minutes to complete (details provided in chapter 6). The interviews were friendly and informal, and participants were very happy to participate. In June 2020, a substantial amendment was submitted which included the option for interviews to be conducted over the telephone. Telephone interviews were also easy to complete.

Despite the pandemic we were able to recruit all intended eight participants into the ^{31}MRI phosphorous spectroscopy (^{31}P-MRS) sub study. Overall, participants coped well with the protocol including measurement of their maximal voluntary isometric contraction (MVIC) of their tibialis anterior (stroke affected and non-affected leg) and dynamic protocol in the MRI scanner (details provided in Chapter 5).

2.5 Strengths and limitations

This pilot study has several strengths. We now have a good idea which outcome measures were easy to collect (e.g., self-report measures, qualitative interviews, ^{31}P-MRS) and which outcome measures were more difficult to complete (GPAQ, activity monitors). We also have a better understanding of what would be feasible to perform in a larger trial.

There was frequent review of participants by the researcher, so we are confident we did not miss any AEs or SAEs. We are also confident that there was no cross-over of interventions (e.g., participants in the sham group inflating the cuff to a higher pressure than prescribed). The symptom diaries were all completed, and AEs reported match other studies looking at the safety of RIC (Meng et al., 2012, Meng et al., 2015a, Koch et al., 2011, Zhao et al., 2017, Mohammad Seyedsaadat et al., 2020). Another strength is the high adherence rates in the hospital and home-RIC group.

With regards to recruitment, we met the recruitment criteria which was recruit 4 participants within the first two months. However, this may be because we had a dedicated PhD student working on the study. If we had staff from multiple research sites working with stroke research nurses on recruitment the results may have been different. A limitation is that we did not keep

an accurate screening log of all the participants that had fatigue that were approached or whether they were eligible or not eligible. However, we tried to keep the inclusion and exclusion criteria such that it was as inclusive as possible excluding only patients who it might be unsafe or ineffective to perform RIC on and who could realistically perform study activities.

Although the plan was for the researcher undertaking face-to-face follow-up assessments to be blinded to the treatment allocation, this was not always possible due to covid-19 restrictions and its impact on recruitment and follow-up. Approximately two thirds of participants had blinded assessment. We proved that blinded follow-up assessment was possible before the pandemic, however we wanted to expose participants to least number of researchers possible to reduce their risk of covid-19 infection.

We did not ask people whether they thought they were in the treatment or sham group therefore we do not know if blinding was successful. However, in other studies that have investigated whether people can identify whether they are treated with intervention or sham conditions they have not been able to identify (England et al., 2019).

Furthermore, inadvertently there was a greater number of participants who had suffered intracerebral haemorrhage in the sham group compared to the treatment group (41.7% vs 8.3%). This may be due to the fact that stroke type was not factored into randomization. However, we are not aware of any supporting literature or data to show that patients that have intracerebral haemorrhage have higher levels of fatigue after stroke than those who have ischaemic stroke (Ingles et al., 1999, Lynch et al., 2007, Eijsden et al., 2012, Duncan et al., 2015, Miller et al., 2013, Schepers et al., 2006a, Kutlubaev et al., 2013).

2.6 Conclusion

In summary, repeated RIC for 6-weeks is safe and acceptable in stroke patients with debilitating fatigue. Only one participant in the treatment group reported moderate or greater discomfort associated with the RIC intervention, no SAEs related to the treatment were reported, and all AEs were mild and transient. There was excellent treatment adherence in the hospital and home-RIC/sham group and high completion rates for all secondary outcome measures at baseline and follow-up despite covid-19 related challenges. Furthermore, despite recruitment challenges during the pandemic we recruited >70% of the intended number of participants and

met all recruitment success criteria. Outcome measures that were unsuccessful include activity monitors and GPAQ. There were a number of challenges with cardiopulmonary exercise testing including access to the exercise lab. Some participants also struggled to get on the bike due to stroke-specific impairments. Therefore, cardiopulmonary exercise testing using bicycle ergometry may be unfeasible in a larger trial. Alternative methods to objectively assesses CRF should be considered (discussed in chapter 4). Successful outcome measures included patient completed clinical outcome measures and ^{31}P-MRS. Although there were a few challenges with the 6MWT including the availability of an adequately sized corridor due to covid-19 restrictions in hospitals (discussed in chapter 3), it is a simple and reliable measure of exercise tolerance that could be feasibly utilized in future trials.

CHAPTER 3. EFFECT OF REMOTE ISCHAEMIC CONDITONING ON POST-STROKE FATIGUE AND WALKING DISTANCE

3.1. Introduction

The objective of this chapter was to explore the correlation between fatigue (FFS) and physical function (6MWT), investigate potential differences in these outcome measures between groups (RIC vs sham) and to find estimates of effect sizes for both treatment arms. We evaluated the results and appropriateness of each outcome measure to determine what measures would be suitable for use in a larger efficacy RCT. Other patient reported outcome measures (e.g., PHQ-9, GAD-7, EQ5D-5L) were included to examine whether any potential effects or associations are confounded by mood and QoL. This small pilot study was not powered to detect any differences in outcomes as this was not the purpose but rather to explore the application of RIC for fatigue and exercise tolerance after stroke and examine trial feasibility.

As discussed in Chapter one, PSF is among the most prevalent symptoms after stroke (Skånér et al., 2007) and is a common cause of concern for patients, caregivers, and healthcare professionals (Hinkle et al., 2017, Acciarresi et al., 2014). PSF limits participation in exercise (Choi-Kwon et al., 2004) and rehabilitation (Lerdal and Gay, 2013) and has a significant negative impact on QoL (Chen et al., 2015, Glader et al., 2002, Naess et al., 2012a). PSF may have central (e.g., corticomotor excitability deficit) and /or peripheral (inflammation, physical deconditioning, impaired cellular energetics) origins (De Doncker et al., 2018, Paciaroni and Acciarresi, 2019). While several interventional studies have been conducted and have added to our current knowledge of PSF (Aali et al., 2020), such studies have been small and thus far none have led to changes in clinical practice. RIC may represent a simple, low-cost, and safe procedure for reducing fatigue symptoms after stroke.

Since the discovery of RIC as a protective phenomenon over 30 years ago (Murry et al., 1986), the bulk of research has focused primarily on the clinical utility of RIC to protect against subsequent ischaemia in the context of cerebrovascular disease (Meng et al., 2015a, Meng et al., 2012), myocardial infarction (Ma et al., 2006, Gorog et al., 2021), and during perioperative periods (Candilio et al., 2015, Li et al., 2010). While the mechanisms underlying these effects are not completely understood, limb RIC has been shown to improve metabolic efficiency by

preserving mitochondrial respiration and attenuating ATP depletion (Thaveau et al., 2007, Mansour et al., 2012, Slagsvold et al., 2014) and reducing lactate production (Jensen et al., 2011, Bailey et al., 2012) during prolonged ischaemia. In addition, RIC has been shown to improve CBF (Meng et al., 2015a, Meng et al., 2012) and increase blood flow and oxygen delivery to skeletal muscles (Andreas et al., 2011b) by preserving endothelial function (Contractor et al., 2016, Manchurov et al., 2020, Kharbanda et al., 2001) and inducing arterial vasodilation (Kimura et al., 2007) during ischaemic stress. Based on these findings, limb RIC has gained interest as a novel therapy to improve muscle strength and exercise capacity in healthy individuals (De Groot et al., 2009, Patterson et al., 2015) and clinical populations (Hyngstrom et al., 2018, Durand et al., 2019). Considering the overlap between some of the mechanisms underlying RIC and PSF, it seems plausible to suggest RIC may help improve fatigue by reducing the mechanisms that promote it.

Hyngstrom et al. (2018) found a significant increase in maximal voluntary contraction (MVC) and in the magnitude of vastus lateralis muscle activation (measured using EMG) in the paretic leg of stroke patients after a single dose of RIC to the ipsilateral leg (5 x 5 min cuff inflation to 225 mmHg). Repeated RIC was associated with significant improvements in walking speed (10-metre walking test) and paretic muscle knee extensor strength and fatigability in chronic stroke survivors (patients in the RIC group maintained submaximal isometric contraction significantly longer compared to the sham group) (discussed in chapter one) (Durand et al., 2019).

The effect of RIC on muscle function and exercise tolerance has also been investigated in other neurological conditions, such as multiple sclerosis (MS) (Chotiyarnwong et al., 2020). A clinical study by Chotiyarnwong et al. (2020) investigated the effect of a single dose of RIC (3 x 5 min cuff inflations to 30 mmHg above systolic BP separated by 5-min reperfusion) or sham (cuff inflated to 30 mm Hg below diastolic BP) on distance walked (% improvement) in the 6MWT in 75 patients with MS. Secondary outcomes included gait speed (distance walked 6MWT, meters/time walked, seconds), the Borg rate of perceived exertion (RPE) scale, tolerability of RIC (Numerical rating scale of 0-10), and adverse events. The investigators found that despite a slightly higher discomfort rating in the RIC group (median rating (IQR) = 4 (3-6) vs 1 (0-2.5)), no SAEs occurred due to RIC and it was associated with a statistically significant improvement in the distance walked during the 6MWT (5.7% versus 1.9% in sham, P=0.012). These findings demonstrate how a single dose of RIC has the potential to increase

exercise tolerance in people with MS, another condition where fatigue is prevalent. These improvements may be even greater with repeated doses of conditioning. This was explored in our study.

A study of 17 healthy males by Crisafulli et al. (2011) found that bilateral lower limb RIC (3 x 5 min cycles of cuff inflation to 50 mmHg above SBP) before an incremental maximal cycle test significantly increased total exercise time (~40 sec), total work and increased maximal power output by 4%. One explanation for these findings was lowered sensitivity to fatigue signals after RIC which allowed the participants to exercise longer (Crisafulli et al., 2011). This is based on the theory that exercise capacity is regulated by a central ‘’complex intelligent system’’, which terminates exercise whilst maintaining skeletal muscle recruitment reserve (Noakes, 2012) and metabolic homeostasis (St Clair Gibson and Noakes, 2004, Tucker et al., 2006). This system explains why exercise can be terminated because of fatigue. According to this model, the CNS regulates exercise via the detection of peripheral fatigue (via cortical projection of muscle afferents) and alterations in intramuscular metabolic milieu. This leads to the inhibition of central neural output to the working muscle and limits the development of peripheral fatigue to an individuals critical threshold (Amann et al., 2006). Therefore, a certain level of muscular-functional reserve is preserved, which can be recruited if the inhibitory influence of muscle afferents (sensory neurons) is reduced (Amann et al., 2006). Crisafulli et al. (2011) suggested that RIC before the exercise test may have increased this threshold, at which the ‘’intelligent system’’ ends the exercise test, by desensitizing afferent groups. This in turn increased neural drive and the number of motor units recruited (De Oliveira Cruz et al., 2015), leading to an increase in force output. Increased neural drive after RIC has been suggested in another recent study in healthy volunteers by Surkar et al. (2020) who found that 8 sessions of upper limb RIC (5 x 5 min cuff inflations to 20 mmHg above resting SBP) combined with strength training of the contralateral arm (using a wall mounted pulley system with stackable weights), led to significantly greater improvements in wrist extensor muscle strength (measured using the one repetition maximum test, 1RM) in the RIC group (9.38 ± 1.01 lbs) compared to the sham group (6.3 ± 1.08 lbs, P=0.035). The researchers hypothesized that the strength gains may partially be the result of neural adaptations to strength training, with a significantly greater percentage change in EMG amplitude observed in the wrist extensor muscle in the RIC group compared to sham (P=0.023). These findings are in line with the study of chronic stroke patients by Hyngstrom et al. (2018).

Several studies of healthy volunteers and athletes have reported improvements in muscle strength after RIC (Patterson et al., 2015, Crisafulli et al., 2011, De Groot et al., 2009) which may help reduce symptoms of PSF by improving skeletal muscle fatigue resistance (Sundstrup et al., 2016), thus improving exercise capacity. A study of 13 healthy men by Barbosa et al. (2015b) investigated the effect of a single dose of lower limb RIC on both legs (3 x 5 min cycles of cuff inflation to 200 mmHg) on muscle fatigue (calculated as the rate of contraction and relaxation, ΔForce/ΔTime) and time to task failure (TTF) during a handgrip exercise. Compared to controls (cuff inflated to 10 mm Hg), RIC significantly reduced the slowing of contraction and relaxation (i.e., delayed the development of fatigue) throughout the handgrip exercise ($P < 0.05$ vs control) and increased TTF by 11.2% (95% confidence interval: 0.7–21.7%, $P<0.05$ vs control). These findings demonstrate how RIC can reduce the fatigability of the forearm muscles after leg RIC, supporting the systemic effects of conditioning.

Not all the studies of RIC on physical function have been positive (Williams et al., 2021, Tocco et al., 2015, Marocolo et al., 2015, Tanaka et al., 2021) and the overall effect is still ambiguous. This may be due to a number of factors (e.g., RIC protocol, participant population), but the data available suggests a potential beneficial effect in stroke patients thus far, and warrants investigation as a potential therapy for PSF.

3.2 Methodology

Details of recruitment, randomisation, intervention protocols, inclusion/exclusion criteria, and baseline and follow-up assessments are detailed in Chapter 2. We included a selection of secondary outcome measures in an attempt to measure fatigue and potential confounders for fatigue as well as physical function. All researchers undertaking assessments were trained in their application prior to study initiation.

3.2.1 Patient completed clinical outcome measures

3.2.1.1 Fatigue severity scale

The fatigue severity scale (FSS) is a 9-item self-report questionnaire used to measure the severity of fatigue symptoms, primarily focusing on the motor aspects of fatigue and its impact on the individual's daily function (Krupp et al., 1989). Each item is scored on a 7-point Likert scale ranging from 1 (strongly disagree) to 7 (strongly agree) (Rosti-Otajärvi et al., 2017).

Participants are asked to circle the number which best fits each statement based on how accurately it reflects their fatigue within the last week (Krupp et al., 1989). A shorter 7-item version (FSS-7) removes the first and second questions that focus on a cause (exercise) and a consequence (motivation) of fatigue and has been shown to have better psychometric properties and is more sensitive to detecting changes in fatigue over time (Lerdal et al., 2005, Lerdal and Kottorp, 2011). It is also one of the most common fatigue scores used in fatigue studies in stroke. For this reason, the FSS-7 was used in this study. The mean score of the seven items is used as the FSS-7 score. The cut-off score for debilitating fatigue is ≥ 4 (Tang et al., 2010, Lerdal et al., 2011), as less than 5% of healthy controls obtain this score or lower, compared to 60-90% of fatigued individuals (Krupp et al., 1989, Schwartz et al., 1993). The FSS is a reliable tool that has been validated in stroke populations (Choi-Kwon and Kim, 2011, Lerdal et al., 2009). The MCID (an index of responsiveness) for global changes in the FSS range from 0.45-1.2 points (Pouchot et al., 2008, Nordin et al., 2016, Rooney et al., 2019). In this study, the FSS-7 was completed at baseline, 6-weeks, 3-months, and 6-months.

3.2.1.2 The patient health questionnaire 9

The patient health questionnaire-9 (PHQ-9) is a self-administered, 9-item depression screening tool that assesses the 9 Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) depression symptom criteria (Appendix 11) (Williams et al., 2005). The PHQ-9 is a validated post-stroke depression screening tool (de Man-van Ginkel et al., 2012, Williams et al., 2005, Gilbody et al., 2007, Karamchandani et al., 2015), and has been reported to have a high sensitivity (Meader et al., 2014). The questionnaire collects information on how often the subject has been bothered by different depressive symptoms over the last two weeks including absence of pleasure/interest, feeling down/hopeless, problems sleeping, fatigue, changes in eating habits, feeling bad about oneself, trouble concentrating and thoughts of self-harm (Kroenke et al., 2001, Strong et al., 2021). Scores on the PHQ-9 range from 0 (no depression) to 27 (all symptoms occurring daily) (Kroenke et al., 2001), with a score of ≥15 usually indicating major depression (Kroenke et al., 2001) and was used as an exclusion criteria in this study (participants excluded if PHQ-9 >14). It is a rapid and effective tool that has been recommended for use in stroke due to its simplicity and strong psychometric properties (Miller et al., 2010, Williams et al., 2005). The MCID in the PHQ-9 has been reported as 5 points in older primary care patients (Lowe et al., 2004). In this study, the PHQ-9 was administered at baseline, 6-weeks, 3-months, and 6-months.

3.2.1.3 The General Anxiety Disorder Assessment-7

The General Anxiety Disorder Assessment (GAD-7) is a 7-item, self-report questionnaire designed to measure or assess the severity of generalised anxiety disorder (GAD) (Appendix 12) (Spitzer et al., 2006). It asks subjects how often, in the last 2-weeks, they have been bothered by the following symptoms: feeling nervous, anxious or on edge, not being able to stop or control worrying, worrying too much about different things, having trouble relaxing, being so restless that it is hard to sit still, becoming easily annoyed or irritable and feeling afraid as if something awful might happen (Spitzer et al., 2006). Patients are asked to select one of the following response options: 'not at all', 'several days', 'more than half the days' or 'nearly every day' which are scored as 0, 1, 2, and 3, respectively. GAD-7 scores range from 0-21, with the cut-off points for mild, moderate, and severe levels of anxiety being 5, 10 and 15, respectively. A cut-off score of ≥10 has been used to identify patients with clinical anxiety (Kellett et al., 2014, Bai et al., 2021). The GAD-7 has been validated for use in primary care (Sapra et al., 2020, Ruiz et al., 2011) and has been used in stroke studies to measure the impact of stroke on daily life (Kellett et al., 2014). It is a brief (2-3 minute completion time) and easy to administer scale with good internal consistency and test-retest reliability (Eccles et al., 2017, Spitzer et al., 2006). It also has good construct (degree to which the instrument is measuring the construct it claims to measure, e.g., anxiety) and convergent (how closely the scale is related to other measures of the same construct) validity (Löwe et al., 2008) and is sensitive to change (Kertz et al., 2012, Eccles et al., 2017). The estimated MCID of the total GAD-7 score is 4 points (Toussaint et al., 2020). In this study, the GAD-7 was administered at baseline, 6-weeks, 3-months and 6-months.

3.2.1.4 The European Quality of Life -5 Dimensions and Visual Analogue Scale

The European Quality of Life -5 Dimensions (EQ5D-5L) is a widely used instrument that is used to measure health status and QoL (Devlin and Brooks, 2017) (Appendix 13). The questionnaire is translated in several languages and has shown validity and reliability in stroke populations (Pinto et al., 2011, Hunger et al., 2012). The EQ5D-5L consists of 5 dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has one question with 5 levels ranging from 'no problems' (level 1) to 'severe problems' (level 5) (Herdman et al., 2011). Responses to the five dimensions can be converted to a single utility

score (EQ-Index score) using preference-based (typically country specific) weights (Devlin et al., 2017). Utility weights range between 0 (death) and 1 (full health) (Devlin et al., 2017). These values are derived from population studies using hypothetical EQ5D health profiles to find out the importance of different health states to different people (Stolk et al., 2019). A study that sampled members of the English general public found that problems with pain/discomfort and anxiety/depression carried the greatest weight (Devlin et al., 2017). The EQ5D-5L questionnaire also includes a Visual Analogue Scale (EQ-VAS), by which participants can rate their overall health on a scale of 0 (worst imaginable health) to 100 (best imaginable health). The instrument has been shown to have excellent psychometric properties and can be applied to a broad range of populations and settings (Feng et al., 2021). A study by Chen et al. (2016) of 65 stroke patients undergoing rehabilitation estimated the MCID of the EQ5D-5L and EQ-VAS as 0.10 and 8.61-10.82, respectively. The researchers also found that the EQ-index score was more responsive to change in HRQoL than the EQ-VAS (Chen et al., 2016). The EQ5D-5L and EQ-VAS was administered at baseline, 6-weeks, 3-months and 6-months.

3.2.1.5 Modified Rankin Scale

The Modified Rankin Scale (mRS) is a single item rating scale used to measure global disability post-stroke (Appendix 14) (Wilson et al., 2002, Dewilde et al., 2017). It is an ordinal scale ranging from 0 (no symptoms) to 6 (death) designed to categorise level of functional independence with reference to level of independence pre-stroke (Zeltzer, 2008). A score of 0-3 on the mRS indicates mild-moderate disability, and a score of 4-5 suggests severe disability (Zeltzer, 2008). It is the most widely used functional outcome measure in stroke trials (Dijkland et al., 2018, Broderick et al., 2017, Quinn et al., 2009, Nobels-Janssen et al., 2021). The mRS only takes around five minutes to complete and is a valid and reliable measure of post-stroke recovery (Saver, 2011). Studies have identified a single-point change on the mRS as clinically meaningful (Harrison et al., 2013, Askew et al., 2020). Smaller changes are likely to be clinically meaningful, so some studies introduced ordinal shift of mRS (Saver and Gornbein, 2009, Ganesh et al., 2020, Ganesh et al., 2018). However, our study applied the mRS and randomisation was stratified by baseline mRS scores. The mRS completed at baseline, 6-weeks, 3-months, and 6-months.

3.2.1.6 Barthel Index

The Barthel Index (BI) is a 10-item scale used to measure a subject's level of independence with activities of daily living (ADLs) (Harrison et al., 2013) (Appendix 15). The 10 items with a total score of 0-100 (5-point increments) include: feeding, bathing, grooming, dressing, bowel control, bladder control, toilet use, bed-chair transfer, walking on level surfaces and travelling up and down stairs (Sha et al., 2021). A higher score on the BI indicates greater independence (Harrison et al., 2013). The BI is the second most commonly used functional outcome measure in stroke trials after the mRS (Quinn et al., 2009, Quinn et al., 2011). It is a valid prognostic tool and a reliable predictor of recovery and the duration of rehabilitation required following stroke (Cohen and Marino, 2000, Duffy et al., 2013). Statistical modelling has suggested a BI score of 95/100 is the optimal descriptor of an excellent outcome in an acute stroke trial, while a score of 75/100 or lower is the best cut-off point for defining a poor trial outcome (Uyttenboogaart et al., 2005). The BI was completed at baseline and at the 6-week follow-up.

3.2.1.7 The Montreal Cognitive Assessment

The Montreal Cognitive Assessment (MoCA) is a brief, 30-point cognitive screening tool sensitive in detecting cognitive impairment in acute stroke (Dong et al., 2010, Blackburn et al., 2013) and stroke rehabilitation (Pendlebury et al., 2010) (Appendix 16). It can be performed in <10 minutes and assesses several cognitive domains including: visuospatial skills, executive functions, short-term memory, attention, language, concentration, calculation and orientation (Chiti and Pantoni, 2014). It was originally designed to identify cognitive deterioration in dementia (Nasreddine et al., 2005), however it has since been proven to be a valid and clinically feasible cognitive screening tool in several illnesses, including stroke (Burton and Tyson, 2015). The optimal cut-off point for detecting mild or greater cognitive impairment in mild stroke patients is <25 points out of 30 (Jaywant et al., 2019). The MCID of the MOCA in stroke has been estimated to be between 1.22 and 2.15 points (Wu et al., 2019). The MoCA was completed at baseline and 6-week follow-up.

3.2.2 Six-minute walk test

The six-minute walk test (6MWT) is a common clinical instrument for assessing functional exercise capacity in patients with cardiopulmonary conditions (Miyamoto et al., 2000, Cahalin et al., 1996, Casanova et al., 2011), as well as patients with stroke (Kosak and Smith, 2005, Dunn et al., 2015). It is a safe and easy test in which the distance an individual can walk within 6-minutes is measured (Hamidzadeh and Zeltzer, 2011, Lipkin et al., 1986). It requires a stopwatch and a flat corridor at least 30-metres in length (Figure 6). Participants are advised to wear comfortable footwear and are encouraged to use their usual walking aid (e.g., stick, walker) and to set their own pace during the test (Crapo et al., 2002). The primary outcome measure of the 6MWT is distance walked, calculated by the clinician or researcher as the total number of lengths plus the excess distance walked measured with a measuring tape or trundle wheel (Salvi et al., 2020). The 6MWT is a valid and reliable measure of functional exercise capacity in stroke populations (Eng et al., 2004, Fulk et al., 2008, Patterson et al., 2007). A study by Fulk and He. (2018) estimated the MCID of the 6MWT in people with stroke to be between 44 metres and 71 metres (Fulk and He, 2018). The 6MWT was completed at baseline and at 6-week follow-up.

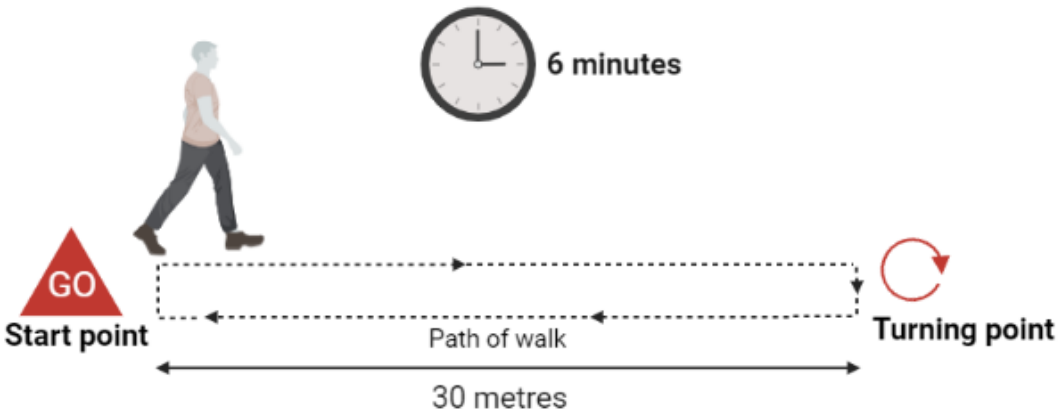


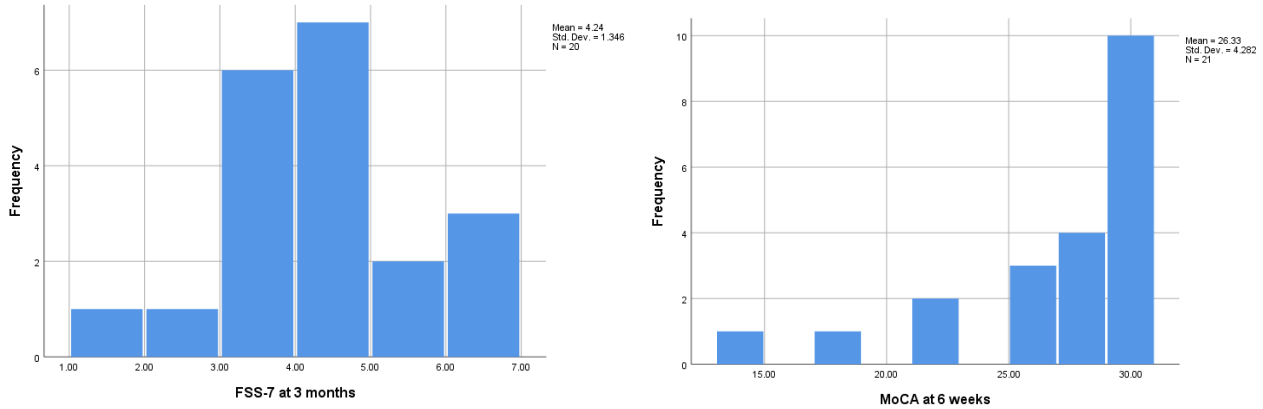
Figure 6. Six-minute walk test. *Figure created by author using Biorender (biorender.com).*

For all study measures attempts were made to minimize variation in the order in which assessments were undertaken (questionnaire-based assessments completed before the 6MWT) as it was possible that this may affect performance in the assessments due to fatigue. Further, the time of day that assessments were completed e.g., morning or afternoon, were matched for baseline and 6 week follow up as much as feasibly possible.

3.3 Statistical analysis

All data was analyzed using IBM SPSS Statistics version 26 and GraphPad Prism (version 9). An exploration of any potential differences between the treatment and sham groups in relation to the patient reported outcome measures and 6MWT was completed using a one-way analysis of covariance (ANCOVA). The participant's baseline score and mRS score were included as covariates for all outcome measures. As mentioned previously, randomisation was stratified by mRS. It was therefore necessary to account for baseline mRS scores in the trial analysis (Parzen et al., 1998, Kahan and Morris, 2012). Baseline scores of the measures were included to account for any random variation between the groups on the measure at the beginning of the study which may have continued to follow-up timepoints. For the analysis of the FSS-7, the participant's PHQ-9 score was included as an additional covariate. As discussed in chapter one, PSF and depression often co-exist (Wu et al., 2014). We controlled for baseline depression scores to allow greater accuracy in fatigue analysis. The ANCOVA model was also used to calculate the adjusted within group mean differences in outcome measures. All assumptions for ANCOVA were tested and satisfied before completion of the analysis. Outcome measures that did not meet these assumptions were reported accordingly (median and interquartile range, IQR) and analysed using a non-parametric Mann-Whitney U Test to test for differences between the groups. As these analyses were exploratory, the findings for both tests were reported. The assumptions for ANCOVA include:

- *Normality* – assumptions of normality for all outcome measures were assessed visually using histograms and quantile-quantile plots (Q-Q plots). These were reviewed by 2 researchers and a third when clarity on distribution was not clear. Figure 7 shows an example of a histogram that shows normality and one that shows non-normality (negative skew).



(A) (B)

Figure 7. Example of histograms showing data that is normally (A) and not normally (B) distributed.

- *Homogeneity of variances* – the variance of the residuals must be equal for both groups of the independent variable (IV). This assumption was tested using Levene's test of equality of variances. This assumption was met if the p-value was >0.05.

- *Outliers* – there must be no significant outliers in any group of the independent variable (IV).

- *Linearity* - there must be a linear relationship between the covariate (e.g., FSS-7 score at baseline) and the dependant variable (DV) (e.g., FSS-7 score at 6-weeks), for each level of the IV (treatment, sham). To test this assumption, we created a grouped scatter plot and added a line of best fit for each group (Figure 8).

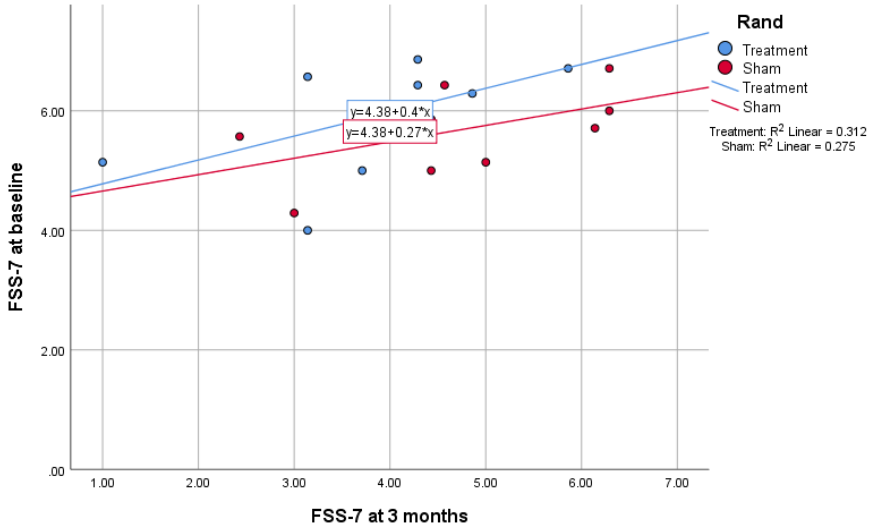


Figure 8. Example of a grouped scatter plot used to test whether there is a linear relationship between the covariate (baseline FSS-7) and dependant variable (FSS-7 at 3-months).

- *Homogeneity of regression slopes* – there must be no interaction between the covariates and the IV (treatment, sham). This assumption was tested graphically by inspecting the scatterplots obtained when testing the linearity assumption above. The assumption was met if the two regression lines (corresponding to the two groups) were parallel, i.e., similar in their slopes.

(Johnson, 1993, Elashoff, 1969)

The 95% confidence intervals (CI) of the adjusted mean differences and standardised effect sizes were calculated to evaluate any relationships present. Effect sizes from the ANCOVA analyses were calculated by converting partial Eta squared (η_p^2) to Cohen's *d* (Cohen, 1977). Partial Eta squared was converted to the more popular Cohen's d using the formula $d = 2\sqrt{\eta_p^2}/(1 - \eta_p^2)$ (Cohen, 1977). Cohen's d was also calculated from the Mann-Whitney U tests (Lenhard and Lenhard, 2016). These were calculated to provide a standardised metric for the effect shown which can be compared across measures and tests. A commonly used interpretation of Cohen d's effect size is to refer to the effect size as small ($d \leq 0.2$), medium (d=0.21-0.79) or large ($d \geq 0.8$) (Cohen, 1977). Pearson r correlation analysis was performed to explore correlations between outcome measures. Pearson r correlation varies between -1 (perfect negative correlation) and 1 (perfect positive correlation) (Liu et al.,

2019). A correlation of 0.1-0.3, 0.3-0.5 and 0.5-1.0 represent small, moderate and strong correlations respectively (Cohen, 1977).

Statistician input to the study was present throughout the development of the protocol all the way to analysis and write up. Analyses were planned prior to study commencement as part of the study protocol development. The original statistician inputting time into the study suggested non-parametric testing and assessments due to the anticipated low numbers of recruited participants that was expected. Due to factors outside of the control of the research team the statistician inputting into the study changed. Once we had the final number of participants recruited advice on using parametric and non-paramedic statistical tests depending on the normality of the data distribution was chosen under the supervision of a different statistician in the same department.

3.4 Results

3.4.1 Exploratory analysis of patient completed outcome measures

Exploratory analysis was performed on the secondary patient completed outcome measures at 6-weeks, 3-months, and 6-months. All ANCOVA assumptions were tested before completion of the analysis. At 6-weeks, the PHQ-9 and GAD-7 sufficiently met the assumptions for parametric testing. However, the FSS-7 and mRS did not meet the assumption of homogeneity of variances, as assessed by Levene's test (P =.024 and P =.002 respectively) and the BI, EQ5D-5L, EQ-VAS and MOCA were not normally distributed. Outcome measures that were not normally distributed were also analysed using a non-parametric Mann-Whitney U test and scores are reported with medians and IQRs instead of means and SDs. As these analyses are exploratory, we reported the findings of the parametric and non-parametric tests alongside each other for completeness (see *Table 10*). The adjusted and unadjusted mean change in scores from baseline to 6-weeks are shown in *Table 11*. At 3-months, the FSS-7, PHQ-9 and EQ-VAS sufficiently met the assumptions for parametric testing, the results of which are in *Table 12*. However, the mRS, GAD-7 and EQ5D-5L were not normally distributed so were also analysed using a Mann Whitney U test (*Table 12*). The adjusted and unadjusted mean changes from baseline to 3 months are shown in *Table 13.* At 6-months, the FSS-7, PHQ-9 and EQ-VAS sufficiently met the assumptions for parametric testing and were analysed using an ANCOVA, while GAD-7, EQ5D-5L and mRS did not and underwent analysis using a Mann-Whitney U

test, *Table 14*. The adjusted and unadjusted mean change in scores from baseline to 6-months are reported in *Table 15.*

3.4.1.1 Fatigue

At 6-weeks, the median difference in FSS-7 score between the treatment and sham group was not statistically significant (median difference -1.29, *P*= 0.27) (*Table 10*). Scores for fatigue (FSS-7) appeared to improve in both groups over the course of the study (Figure 9). However, participants in the treatment group appeared to experience greater reductions in median FSS-7 scores at 6-weeks (-2.57, IQR 2.71) compared to sham (-1.00, IQR 2.02) (*Table 11*). These potential beneficial reductions in FSS-7 appeared to persist at 3-month (RIC adjusted change -2.10, 95% CI -2.98 to -1.22 vs Sham -1.05, 95% CI -1.84 to – 0.26) *(Table 13)* and 6-month (RIC adjusted change -2.34, 95% CI -3.16 to -1.52 vs Sham -1.48, 95% CI -2.22 to – 0.74) follow-up *(Table 15)*. Standardised treatment effect sizes at 6-week, 3 month and 6 month follow up were 0.51, 0.41 and 0.33 respectively *(Tables 10, 12, 14)*. Differences in FSS-7 between groups at 3- and 6-month follow-up were also not statistically significant (*Tables 12 and 14)*, however all appeared to be clinically relevant in magnitude when considering the MCID for FSS-7 (0.45 to 1.2) (Nordin et al., 2016, Rooney et al., 2019).

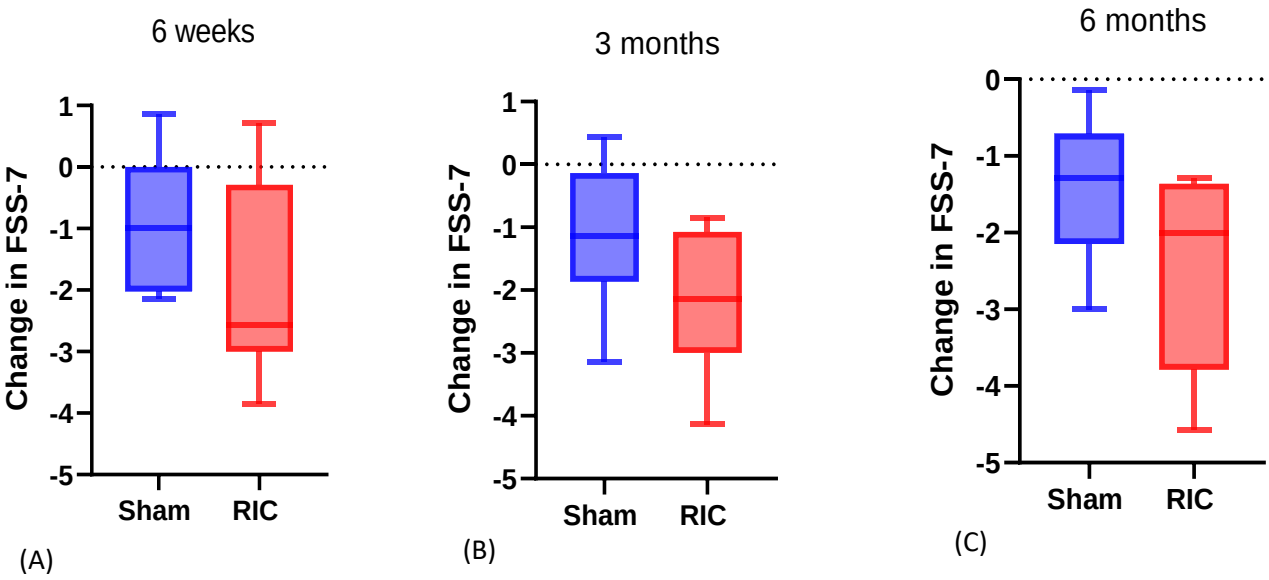


Figure 9. Change in fatigue scores (FSS-7) in the treatment and sham group at 6-weeks (a), 3-months (b) and 6-months (c).

3.4.1.2 Quality of life

Outcomes from the quality-of-life assessment were variable. Overall, there did not appear to be any consistent effects of treatment on EQ5D-5L utility scores, which generally deteriorated insignificantly (and at magnitudes that were insignificant) across all time points *(Tables 10-15).* On the contrary, EQ-VAS scores generally improved at all time points and across both groups, with the only real difference between groups appearing at 6 months follow up where the improvement in EQ-VAS amongst the RIC group appeared greater than the Sham group (adjusted improvement 10.68 95% CI -0.14 to 21.50 vs 3.08 95% CI -6.70 to 12.86 respectively). However, the between group difference at 6-months was not statistically significant (adjusted group difference 7.59 95% CI -7.06 to 22.25, *P*= 0.29) *(Table 14).*

3.4.1.3 Functional independence and activities of daily living

Of the 22 participants who completed the study, 17 (77.3%) were independent (mRS 0-2) and 5 (22.7%) were dependent (mRS 3-4) at baseline. At 6-week, 3-month and 6-month follow-up there did not appear to be any apparent meaningful differences in mRS or BI score between the two groups *(Tables 10-15).*

3.4.1.4 Mood

For mood, there does not appear to be a treatment effect i.e., no differences between the two groups either clinically from the adjusted differences or statistically *(Tables 10-15).* However, both measures for depression and anxiety (PHQ-9 and GAD-7) do appear to improve over the course of follow-up in both groups *(Tables 11, 13 and 15).* There appears to be a greater improvement in adjusted PHQ-9 scores in the sham group compared to the treatment group at 6-weeks (sham adjusted change -2.19, 95% CI -4.48 to 0.09 vs RIC adjusted change -1.81, 95% CI -4.09 to 0.48) and 3-months (sham adjusted change -4.86, 95% CI -7.23 to -2.49 vs RIC adjusted change -2.84, 95% CI -5.46 to -0.21), however this effect disappears at 6-months (RIC adjusted change -3.58, 95% CI -5.86 to -1.30 vs sham adjusted change -2.16, 95% CI -4.23 to -0.10) *(Tables 11, 13 and 15).* There were no real trends in change in GAD-7 between groups across time points (*Tables 11, 13 and 15*).

3.4.1.5 Cognition

For the MoCA at 6-weeks, there was a medium effect size from the non-parametric testing (effect size 0.51) (*Table 10).* However, the median difference between the treatment and sham group was not statistically significant (median difference 2.00, *P*= 0.28) (*Table 10).* When looking at the change in MoCA scores from baseline to 6-weeks, there was a slightly greater improvement in the treatment group, (1.00, IQR 1.50) compared to sham (0.00, IQR 3.00) *(Table 11),* however this difference appears neither clinically nor statistically important.

Table 10. Analysis of within group and between group differences in patient completed outcome measures at 6-week follow-up using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference at 6 weeks between treatment and sham group (95% CI)	P-value	Effect size
FSS-7	5.65 (0.67)	4.70 (1.22)	5.71 (0.95)	3.95 (1.86)	-0.71 (-2.01 to 0.60)	0.27	0.15
FSS-7 (Median, IQR) *	5.71 (0.86)	4.86 (2.43)	6.00 (1.57)	3.57 (2.71)	-1.29[1]	0.27	0.51
PHQ-9	9.91 (5.74)	7.45 (6.04)	8.09 (4.68)	6.55 (3.75)	0.39 (-2.88 to 3.65)	0.81	0.02
GAD-7	8.36 (7.50)	6.73 (5.55)	6.45 (6.59)	5.18 (4.02)	-0.42 (-2.62 to 1.79)	0.70	0.06
MRS	2.09 (1.14)	2.09 (1.14)	1.82 (0.98)	1.64 (1.03)	-0.19 (-0.45 to 0.08)	0.16	0.22
MRS (Median, IQR) *	2.00 (2.00)	2.00 (2.00)	2.00 (1.00)	2.00 (1.00)	0.00	0.44	0.36
EQ5D-5L	0.81 (0.20)	0.80 (0.23)	0.84 (0.19)	0.79 (0.27)	-0.05 (-0.14 to 0.04)	0.26	0.45
EQ5D-5L (Median; IQR)*	0.89 (0.11)	0.89 (0.09)	0.94 (0.30)	0.94 (0.34)	0.05[1]	0.48	0.33
EQ-VAS	60.00 (18.84)	67.91 (16.80)	62.27 (19.54)	68.36 (20.43)	-2.09 (-15.23 to 11.05)	0.74	0.02
EQ-VAS (Median, IQR) *	70.00 (35.00)	75.00 (20.00)	70.00 (35.00)	75.00 (30.00)	0.00[1]	0.70	0.18

BI	87.73 (24.73)	88.64 (23.57)	93.64 (13.62)	93.64 (16.29)	-0.88 (-4.46 to 2.71)	0.61	0.03
BI (Median, IQR) *	100.00 (10.00)	100.00 (10.00)	100.00 (5.00)	100.00 (5.00)	0.00[1]	0.85	0.09
MoCA	25.18 (3.74)	25.82 (4.51)	25.60 (4.45)	26.90 (4.18)	0.46 (-1.37 to 2.29)	0.60	0.03
MoCA (Median, IQR) *	25.00 (7.00)	27.00 (4.00)	27.00 (7.75)	29.00 (5.25)	2.00[1]	0.28	0.51

RIC = Remote Ischaemic Conditioning; SD = Standard deviation; IQR = Interquartile range; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = General Anxiety Disorder-7; MRS = Modified Rankin Scale; EQ5D = EuroQol-5 Dimension; EQ-5D VAS = EuroQol-5 Dimension Visual Analogue Scale; BI = Barthel Index; MOCA = Montreal Cognitive Assessment. A One-way ANCOVA was conducted. *Mann-Whitney U- test was applied in comparisons. [1]Unadjusted median change included.

Table 11. Adjusted and unadjusted within group mean change in scores in the patient completed outcome measures from baseline to 6-weeks calculated using a one-way ANCOVA.

	Sham group	**Sham group**	**RIC Group**	**RIC group**
Outcome measure	**Mean change from baseline to 6-weeks (95% CI)**	**Adjusted mean change (95% CI)**	**Mean change from baseline to 6-weeks (95% CI)**	**Adjusted mean change (95% CI)**
FSS-7	-0.95 (-1.61 to -0.29)	-1.00 (-1.90 to -0.11)	-1.77 (-2.80 to -0.73)	-1.71 (-2.61 to -0.81)
FSS-7 median change (IQR)[#]	-1.00 (2.02)	NA	-2.57 (2.71)	NA
PHQ-9	-2.45 (-5.46 to 0.56)	-2.19 (-4.48 to 0.09)	-1.55 (-3.38 to 0.29)	-1.81 (-4.09 to 0.48)
GAD-7	-1.64 (-4.08 to 0.81)	-1.25 (-2.79 to 0.30)	-1.27 (3.68 to 1.13)	-1.66 (-3.21 to -0.12)
mRS	0.00 (0.00 to 00.00)	0.00 (-0.18 to 0.19)	-0.18 (-0.45 to 0.09)	-0.18 (-0.37 to 0.00)
mRS (median change, IQR)[#]	0.00 (0.00)	NA	0.00 (0.00)	NA
EQ5D-5L	-0.02 (-0.07 to 0.03)	-0.01 (-0.08 to 0.05)	-0.06 (-0.14 to 0.03)	-0.06 (-0.13 to 0.00)
EQ5D-5L (median change, IQR)[#]	0.00 (0.07)	NA	-0.04 (0.26)	NA
EQ-VAS	7.91 (-1.33 to 17.15)	8.05 (-1.21 to 17.30)	6.09 (-6.69 to 18.87)	5.96 (-3.30 to 15.21)
EQ-VAS (median change, IQR)[#]	2.00 (20.00)	NA	0.00 (25.00)	NA
BI	1.00 (-1.26 to 3.26)	0.95 (-1.79 to 3.69)	0.00 (-3.00 to 3.00)	0.05 (-2.57 to 2.66)
BI (median change, IQR) [#]	0.00 (0.00)	NA	0.00 (0.00)	NA
MoCA	0.64 (-1.07 to 2.34)	0.73 (-0.52 to 1.99)	1.30 (0.29 to 2.31)	1.19 (-0.12 to 2.51)
MoCA (median change, IQR)[#]	0.00 (3.00)	NA	1.00 (1.50)	NA

RIC = Remote Ischaemic Conditioning; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = Generalised Anxiety Disorder-7; mRS = Modified Rankin Scale; EQ5D-5L = EuroQol-5 Dimension; EQ-VAS = EuroQol-5 Dimension Visual Analogue Scale; BI = Barthel Index; MOCA = Montreal Cognitive Assessment. All outcome measures were adjusted for baseline score and mRS. FSS-7 was also adjusted for baseline PHQ-9 score as discussed in section 3.3 of methodology. [#] Median change included as they did not meet normality assumptions.

Table 12. Analysis of within group and between group differences in patient completed outcome measures at 3-month follow-up using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 3m follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 3m follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group (95% CI)	P-value	Effect size
FSS-7	5.65 (0.67)	4.61 (1.28)	5.71 (0.95)	3.78 (1.35)	-1.05 (-2.26 to 0.16)	0.08	0.41
PHQ-9	9.91 (5.74)	4.91 (3.05)	8.09 (4.68)	6.56 (5.43)	2.02 (-1.53 to 5.57)	0.25	0.18
GAD-7	8.36 (7.50)	5.73 (5.14)	6.45 (6.59)	5.11 (4.48)	0.04 (-2.56 to 2.65)	0.97	0.00
GAD (Median, IQR) *	9.00 (15.00)	8.00 (9.00)	3.00 (10.00)	5.00 (5.50)	-3.00[2]	0.77	0.14
mRS	2.09 (1.14)	2.09 (1.14)	1.82 (0.98)	1.89 (1.05)	0.00 (0.00 to 0.00)	NA[3]	0.00
mRS (Median, IQR)	2.00 (2.00)	2.00 (2.00)	2.00 (1.00)	2.00 (1.50)	NA	0.71	0.17
EQ5D-5L	0.81 (0.20)	0.77 (0.17)	0.84 (0.19)	0.78 (0.26)	0.01 (-0.11 to 0.13)	0.85	0.00
EQ5D-5L (Median, IQR) *	0.89 (0.11)	0.81 (0.34)	0.94 (0.30)	0.83 (0.44)	0.02[2]	0.55	0.29
EQ-VAS	60.00 (18.84)	56.50 (18.11)	62.27 (19.54)	66.22 (22.33)	2.09 (-11.05 to 15.23)	0.74	0.13

RIC=Remote ischaemic conditioning; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = Generalised Anxiety Disorder-7; mRS = modified Rankin Scale; EQ5D-5L = EuroQol-5 Dimension; EQ-VAS = EuroQol-5-dimension Visual Analogue Scale. A One-way ANCOVA was conducted. *A Mann-Whitney U test was applied in comparisons. [2]Unadjusted median change included. [3]No P-value was available from the mRS ANCOVA. We believe this is due to lack of variability in the outcome measure.

Table 13. Adjusted and unadjusted within group mean change in scores in the patient completed outcome measures from baseline to 3-months calculated using a one-way ANCOVA.

	Sham group	**Sham group**	**RIC Group**	**RIC group**
Outcome measure	**Mean change (95% CI)**	**Adjusted mean change (95% CI)**	**Mean change (95% CI)**	**Adjusted mean change (95% CI)**
FSS-7	-1.04 (-1.77 to -0.31)	-1.05 (-1.84 to -0.26)	-2.11 (-2.99 to -1.24)	-2.10 (-2.98 to -1.22)
PHQ-9	-5.00 (-8.49 to -1.51)	-4.86 (-7.23 to -2.49)	-2.67 (-5.16 to -0.18)	-2.84 (-5.46 to -0.21)
GAD-7	-2.64 (-5.92 to 0.64)	-2.57 (-4.31 to -0.83)	-2.44 (-4.97 to 0.08)	-2.53 (-4.45 to -0.60)
GAD7 (median change, IQR)[#]	-1.00 (6.00)	NA	-2.00 (5.50)	NA
mRS	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)	0.00 (0.00 to 0.00)
mRS (median change, IQR)[#]	0.00 (0.00)	NA	0.00 (0.00)	NA
EQ5D-5L	-0.04 (-0.12 to 0.04)	-0.05 (-0.12 to 0.03)	-0.04 (-0.12 to 0.05)	-0.03 (-0.12 to 0.05)
EQ5D-5L (median change, IQR)[#]	-0.02 (0.08)	NA	-0.08 (0.15)	NA
EQ-VAS	0.00 (-21.03 to 21.03)	0.49 (-17.51 to 18.47)	2.33 (-17.02 to 21.69)	1.74 (-18.17 to 21. 65)

RIC = Remote Ischaemic Conditioning; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = Generalised Anxiety Disorder-7; mRS = Modified Rankin Scale; EQ5D-5L = EuroQol-5 Dimension; EQ-VAS = EuroQol-5 Dimension Visual Analogue Scale. All outcome measures were adjusted for baseline score and mRS. FSS-7 was also adjusted for baseline PHQ-9 score as discussed in section 3.3 of methodology. [#] Median change included as they did not meet normality assumptions.

Table 14. Analysis of within group and between group differences in patient completed outcome measures at 6-month follow-up using a one-way ANCOVA

Outcome measure	Sham baseline (mean, SD)	Sham 6m follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6m follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group (95% CI)	P-value	Effect size
FSS-7	5.65 (0.67)	4.23 (0.89)	5.71 (0.95)	3.48 (1.61)	-0.86 (-1.99 to 0.27)	0.12	0.33
PHQ-9	9.91 (5.74)	7.64 (4.63)	8.09 (4.68)	5.78 (4.47)	-1.42 (-4.50 to 1.67)	0.35	0.12
GAD-7	8.36 (7.50)	4.55 (4.12)	6.45 (6.59)	3.78 (4.44)	-0.46 (-3.93 to 3.02)	0.79	0.01
GAD-7 (Median, IQR) *	9.00 (15.00)	4.00 (9.00)	3.00 (10.00)	2.00 (3.50)	-2.00[4]	0.82	0.12
mRS	2.09 (1.14)	2.27 (1.10)	1.82 (0.98)	1.67 (1.11)	-0.42 (-0.82 to -0.01)	**0.05**[#5]	0.49
mRS (Median, IQR) *	2.00 (2.00)	2.00 (2.00)	2.00 (1.00)	1.00 (1.50)	-1.00[4]	0.20	0.62
EQ5D-5L	0.81 (0.20)	0.77 (0.25)	0.84 (0.19)	0.82 (0.23)	0.04 (-0.09 to 0.17)	0.54	0.05
EQ5D-5L (Median, IQR) *	0.89 (0.11)	0.92 (0.38)	0.94 (0.30)	0.88 (0.38)	-0.04[4]	0.55	0.29
EQ-VAS	60.00 (18.84)	61.36 (21.80)	62.27 (19.54)	71.67 (15.41)	7.59 (-7.06 to 22.25)	0.29	0.15

*** RIC = Remote Ischaemic Conditioning; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = General Anxiety Disorder-7; MRS = Modified Rankin Scale; EQ5D-5L = EuroQol-5 Dimension; EQ-VAS = EuroQol-5 Dimension Visual Analogue Scale.**
A One-way ANCOVA was conducted. *Mann-Whitney U- test was applied in comparisons. [#]Significant at the 0.05 significance level. [4]Unadjusted median change included. [5]Even though this looks significant it is not normally distributed so we have taken the non-parametric analysis as the true value i.e., not statistically different.

Table 15. *Adjusted and unadjusted within group mean change in scores in the patient completed outcome measures from baseline to 6-months calculating using a one-way ANCOVA*

	Sham group	**Sham group**	**RIC Group**	**RIC group**
Outcome measure	**Mean change (95% CI)**	**Adjusted mean change (95% CI)**	**Mean change (95% CI)**	**Adjusted mean change (95% CI)**
FSS-7	-1.42 (-2.04 to -0.79)	-1.48 (-2.22 to -0.74)	-2.41 (-3.40 to -1.43)	-2.34 (-3.16 to -1.52)
PHQ-9	-2.27 (-5.04 to 0.50)	-2.16 (-4.23 to -0.10)	-3.44 (-5.41 to -1.48)	-3.58 (-5.86 to -1.30)
GAD-7	-3.82 (-7.15 to -0.49)	-3.60 (-5.92 to -1.27)	-3.78 (-8.90 to 1.35)	-4.05 (-6.62 to -1.48)
GAD-7 (median, IQR)[#]	-2.00 (9.00)	NA	-1.00 (9.00)	NA
mRS	0.18 (-0.09 to 0.45)	0.19 (-0.06 to 0.44)	-0.13 (-0.42 to 0.17)	-0.13 (-0.43 to 0.16)
mRS median change (IQR)	0.00 (0.00)	NA	0.00 (0.00)	NA
EQ5D-5L	-0.04 (-0.15 to 0.06)	-0.04 (-0.13 to 0.05)	0.00 (-0.06 to 0.07)	-0.00 (-0.10 to 0.10)
EQ5D-5L median change (IQR)	0.00 (0.09)	NA	-0.01 (0.08)	NA
EQ-VAS	1.36 (-18.05 to 20.78)	3.08 (-6.70 to 12.86)	12.78 (2.23 to 23.32)	10.68 (-0.14 to 21.50)

*** RIC = Remote Ischaemic Conditioning; FSS-7 = Fatigue Severity Scale-7; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = Generalised Anxiety Disorder-7; mRS = Modified Rankin Scale; EQ5D-5L = EuroQol-5 Dimension; EQ-VAS = EuroQol-5 Dimension Visual Analogue Scale.**

3.4.2 Exploratory analysis of the six-minute walk test

Out of the 22 participants who completed the 6-week intervention 19 participants (86.4%) (10 treatment, 9 sham) completed the 6MWT at baseline and at 6-week follow-up. The 6MWT data sufficiently met assumptions for analysis using ANCOVA. For the 6MWT at 6-weeks, there appears to be a small to medium beneficial effect size (effect size 0.42) in favour of the RIC group albeit this is not statistically significant (*Table 16*, Figure 10). The adjusted parametric analysis suggested a between group difference of 43.81 m (95% CI = -5.96 to 93.57, *P*= 0.08) *(Table 16)*. Pearson r correlation analysis shows fatigue severity (FSS-7) is negatively correlated with distance walked during the 6MWT at baseline (R = -0.23, *P*= 0.32) and at 6-weeks (R = -0.22, *P* = 0.37) but this was not statistically significant (Figure 11).

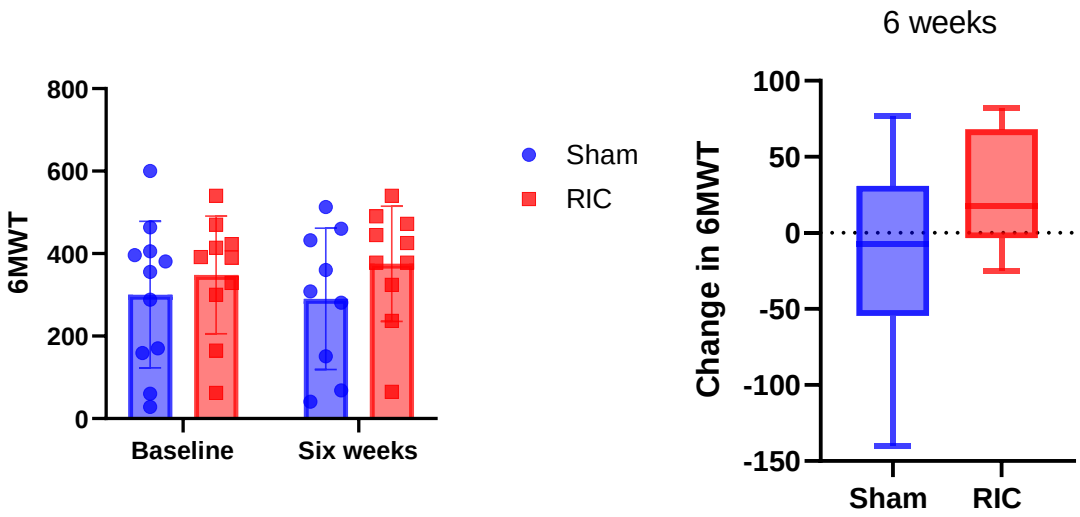


Figure 10. Change in distance walked during the 6MWT (metres) in the treatment and sham group at 6-weeks.

Table 16. Analysis of within group and between group differences in 6MWT at 6-week follow-up using one-way ANCOVA

Outcome measure	Sham baseline (mean, SD)	Sham 6w follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-w follow-up	Adjusted mean difference between the treatment and sham group (95% CI)	P-value	Effect size
6MWT (meters)	300.65 (177.45)	290.41 (171.28)	348.46 (142.75)	375.48 (139.48)	43.81 (-5.96 to 93.57)	0.08	0.42

A One-way ANCOVA was conducted.

Table 17. Adjusted and unadjusted within group mean change in 6MWT scores from baseline to 6-weeks calculated using a one-way ANCOVA.

	Sham group	Sham group	RIC group	RIC group
Outcome measure	Mean change (95% CI)	Adjusted mean change (95% CI)	Mean change (95% CI)	Adjusted mean change (95% CI)
6MWT (meters)	-13.10 (-62.70 to 36.50)	-15.04 (-50.95 to 20.86)	27.02 (-0.18 to 54.21)	28.76 (-5.28 to 62.80)

6MWT = Six-minute walk test, RIC = remote ischaemic conditioning.

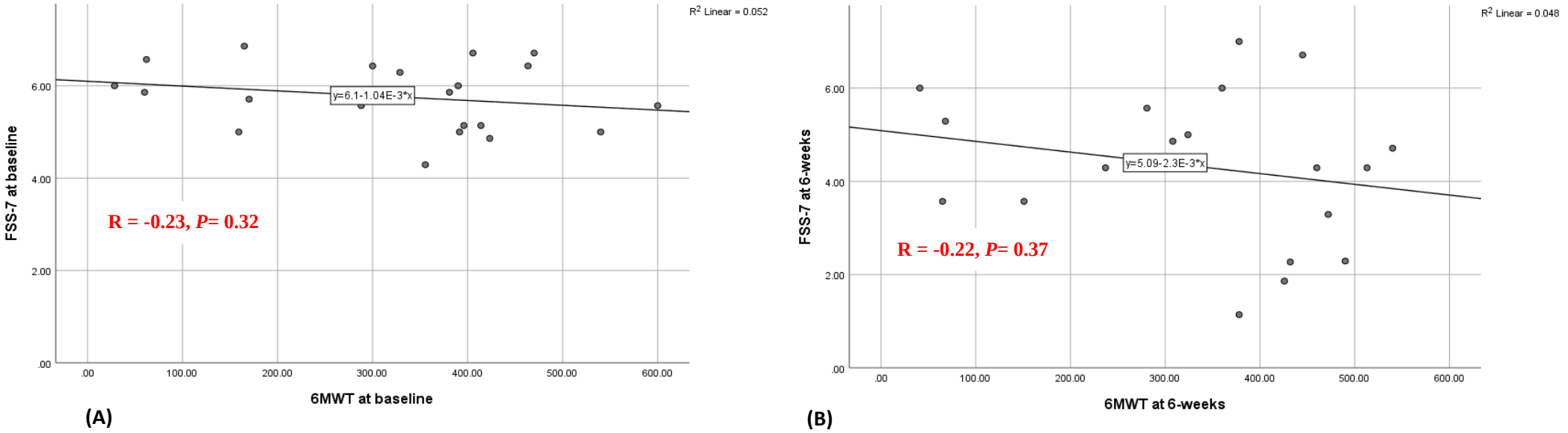


Figure 11. Simple scatter of FSS-7 score by distance walked on the 6MWT at baseline (a) and 6-week follow-up (b)

3.5 Discussion

3.5.1 Fatigue

The data suggests an improvement in fatigue across all timepoints following the 6-week intervention. This improvement appears to be meaningful as it is of a similar magnitude to the MCID reported in previous studies (Nordin et al., 2016, Rooney et al., 2019, Pouchot et al., 2008). However, one needs to recognise that these effect signals are not statistically powered and thus one cannot say for certain that RIC results in improvements in FSS. Despite this, at first glance, the improvement in fatigue scores does not appear to be due to bias because of unbalanced changes in mRS or BI, or because of changes in mood since there was no apparent or statistically significant difference in these scores between the two groups at any timepoint. The improvement in FSS-7 scores does seem to mirror improvements in walking distances and speed evidenced in the changes in 6MWT at 6-weeks. One may have expected that differences in baseline function (mRS) to account for differences in walking distance and speed at follow up however the median mRS at baseline in both groups appeared similar (2.0) and the final changes in 6MWT between both groups also adjusted for differences in baseline mRS individually.

Interestingly and importantly improvements in fatigue scores appeared to persist beyond termination of the intervention when measured at 3 months and 6 months follow-up. This suggests that if RIC is causing biological changes to tissues, blood flow, immune function or metabolism, it may doing so by 'switching on' mechanisms that lead to biological effects beyond the triggering response (the RIC itself). Such effects may hypothetically be those such as switching on particular genes that encode cellular responses to mitochondria or immune cells, or for example altering the metabolic activity of the mitochondria that last for the lifespan of the mitochondria (mitochondrial DNA and protein half-life is approximately 8-30 days depending on the cell type e.g., skeletal muscle cells, epithelial cells, neurons) (Kowald et al., 2014, Henriksson and Reitman, 2022, Hickson and Rosenkoetter, 1981, Korr et al., 1998).

Measuring changes to gene profiles (metabolic and immune), markers of mitochondrial function and immune function alongside our clinical measures would help investigate these hypotheses. Alternatively, it may provide a short-lived change to physical fatigue and function that then makes it easier for patients with PSF to then increase physical activity. Measuring step counts or measures of other measures of physical activity would help understand if there

was an upward inflection of such markers immediately after the RIC intervention ended that persisted at follow up. We attempted to undertake such measures in this study but found the practicalities of such measures difficult to perform reliably. Persistent improvements in fatigue at 3 and 6 months did not appear to be due to disproportionate improvements in mood or cognition. In fact, the magnitude of fatigue improvement appeared to increase at 3 months follow-up despite a trend towards better depression scores in the sham group.

The FSS is a sensitive tool for detecting changes in fatigue. A study by Valko et al. (2008) sought to validate the FSS by administering the questionnaire 454 healthy subjects and to people diagnosed with disorders commonly associated with fatigue such as MS (n=188), stroke (n=235) and sleep disorders (n=429). They found the FSS had high internal consistency (Cronbach $\alpha = 0.93$) and high test-retest reliability. The authors also highlighted how healthy subjects scored significantly lower than patients ($P<0.01$). From this, Valko et al. (2008) concluded that the FSS can effectively differentiate healthy individuals from patients and is therefore a sensitive measure of fatigue. Taking this point further, Flachenecker et al. (2002) found significant differences between FSS scores of MS patients with and without fatigue ($P<0.0001$), demonstrating its effectiveness in discriminating between people with and without fatigue in diseased populations. The FSS-7 is a simple, easy to administer and reliable measure of fatigue. However, scores may be influenced by external factors (e.g., pain, social stressors) and the participant's opinion on what the words mean and their ability to understand the questions and to follow instructions (Finsterer and Mahjoub, 2014, Lenaert et al., 2018, Gawron, 2016).

3.5.2 Six-minute walk test

The 6MWT is a valid measure of walking capacity and balance in stroke (Kosak and Smith, 2005, Dunn et al., 2015, Regan et al., 2020, Fulk et al., 2008). Our data suggests an improvement in distance walked during the 6MWT in the treatment group compared to the sham, in the realm of that considered clinically meaningful, reflected by improved walking distances in the treatment group but a reduction in the sham at 6-weeks. A minimum improvement of 44 meters is considered MCID in stroke (Fulk and He, 2018), in MS it ranges from 9.1 and 21.6 meters (Chotiyarnwong et al., 2020) and in adults with pathology (e.g., chronic obstructive pulmonary disease, lung cancer) it ranges between 14.0 and 30.5 meters

(Bohannon and Crouch, 2017) which suggests the between group difference of 43.81 meters in our study *(Table 16)* could be clinically meaningful. The MCID for 6MWT in stroke patients may depend on baseline gait speed. If gait speed in patients is >0.40 m/s then the MCID is 71m (Fulk and He, 2018, Cheng et al., 2020). In the study on the effect of RIC on walking distances in people with MS baseline gait speed was ~0.8m/s (Chotiyarnwong et al., 2020), thus the change may be just below that thought to be clinically meaningful. Indeed, the finding itself is still interesting and encouraging to see and it fits with the changes in fatigue. As previously mentioned, due to space limitations the 6MWT was sometimes administered on a 9m corridor. This may have negatively impacted distance covered during the test and underestimated the change in distance between the treatment and sham arms (Ng et al., 2011), however shorter walkway distances are more feasible to implement than the 30-m walkway in research and hospital settings (Cheng et al., 2020). Also, we made sure the lengths participants walked were the same at baseline and at follow-up, so it was the 'change' that really mattered.

Fatigue scores were inversely correlated with walking distances. It may be that improvements in 6MWT were due to slightly higher functional levels in the RIC group compared to sham, however there were no differences in baseline median mRS between the two. The improvements in the 6MWT at 6-weeks may well be due to changes in fatigue scores, mediated by mechanisms such as improved blood flow (Jeffries et al., 2018a, Meng et al., 2012, Andreas et al., 2011b), reduced inflammation (Meng et al., 2015a, Konstantinov et al., 2004), or improved mitochondrial energy metabolism (Slagsvold et al., 2014), observed after RIC. Further, qualitative feedback from participants in the treatment group (discussed in chapter 6) included improved sleep, improved concentration, and reduced breathlessness after the RIC treatment. These factors may have contributed to the improvement in distance walked in the 6MWT. As mentioned previously, a study in people with MS found significant improvements in distance walked after a single episode of RIC (Chotiyarnwong et al., 2020). Participants randomized to a single cycle of RIC exhibited greater improvements in 6MWT compared to sham (13.5m vs 6.6m respectively). In our study, the difference within the two groups in our study is much greater (RIC group 28.76m vs sham -15.04m) *(Table 17)*. This may be due to repeated RIC treatments. However, it may also be due to methodological limitations in our study limiting the performance in the sham group. Standardisation of the 6MWT protocol was not always possible due to COVID-19 restrictions and researcher availability, and participant convenience, thus meaning the test was not always completed at the same times of the day and at the same location which may introduce some bias and variation. Skeletal muscle circadian

rhythms, blood pressure, body temperature, energy metabolism, hormone levels, mood, and motivation all vary depending on the time of day and affect physical activity performance (Mirizio et al., 2020, Postolache et al., 2020). Several studies have reported that physical activity performance peaks in the early afternoon as a result of synchronization between metabolic, physiological and psychological rhythms (Bellastella et al., 2019, Sabzevari Rad et al., 2020, Ayala et al., 2021). This should be considered in future studies.

3.5.3 Functional independence and activities of daily living

There did not appear to be any meaningful changes in functional score, either measured with the mRS or BI between groups at any timepoint. This is not particularly surprising. While on the one hand improvements in fatigue may theoretically encourage improvements in functional activities, the mRS is a blunt instrument that in its simplest form may not be sensitive enough to detect subtle changes to people's activities. The change in one's function would need to be of significant magnitude for a change of score to be detected. The BI concentrates on the assistance one needs with differing daily activities. Most of the participants in this study were largely independent at baseline, thus improvements in fatigue were unlikely to alter scores based on this i.e. it has a ceiling effect (Sarker et al., 2012), the phenomenon by which a score does not change from maximum despite clinical change (Schepers et al., 2006b). Both scores do not capture well any changes in cognitive resilience or social activity participation that one may expect could improve with lower levels of fatigue. Another validated measure of ADLs in the stroke population is the Nottingham Extended ADL (NEADL) scale (Gladman et al., 1993). The NEADL includes questions about 21 activities within four categories including mobility, kitchen activities, domestic activities and leisure activities (Gladman et al., 1993). Unlike the BI which simply asks participants what they have the capability to do, the NEADL asks participants what they have actually done in the last few weeks (Harrison et al., 2013). It is a responsive scale of ADLs in the stroke population (Wu et al., 2011a), and is less susceptible to ceiling effects (Sarker et al., 2012). The NEADL may be a more appropriate test of ADLs in future trials.

3.5.4 Mood

There were no apparent meaningful differences in mood scores (for depression nor anxiety) between the two groups at either time point of the study. Overall, small improvements in

depression and anxiety symptom scores occurred in both groups, below levels that are considered clinically meaningful (Toussaint et al., 2020, Lowe et al., 2004). One reason for the slight improvement in mood could be participation in the study itself. Many individuals experience social isolation and loneliness after stroke (Yang et al., 2021, Liu et al., 2021). Indeed, one theme that emerged from the qualitative interviews was that participants enjoyed the social aspect of the study (e.g., talking with the researchers). Emotional disturbances such as depression and anxiety are common after stroke and negatively influence functional outcomes and QoL (Kim, 2016). However, for many stroke survivors' depression symptoms improve over time (Towfighi et al., 2017, Duncan et al., 2005). A study by Zhang et al. (2012) examined the incidence of post-stroke depression (diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders) at different time points within 1-year post-stroke. The incidence of post-stroke depression was highest in the acute phase and at 3-months (8.4% and 11.3%, respectively), but reduced to 5.2% at 1-year (Zhang et al., 2012). Therefore, the improvement in mood could be the result of the natural time course of depression and anxiety post-stroke.

3.5.5 Cognition

Cognitive screening with the MoCA did not demonstrate any evident treatment effect on cognition at 6-weeks. While scores in the RIC group were marginally better than the sham group the effect size was small, and it was not statistically significant. Cerebral small vessel disease (CSVD) is the most common, progressive vascular disease that affects small veins, arterioles and capillaries supplying the white matter and deep brain structures (Chojdak-Łukasiewicz et al., 2021). It is responsible for 15-25% of all ischaemic strokes (Petty et al., 2000) and is a leading cause of vascular cognitive impairment (VCI) after stroke (Zanon Zotin et al., 2021). Studies have examined the protective effect of RIC in patients with CSVD and have shown promising results (Mi et al., 2016). A study by Wang et al. (2017) examined the effects of chronic bilateral upper limb RIC (5 x 5 min cuff inflation to 200 mmHg) twice daily for 1-year in patients with cerebral SVD and mild cognitive impairment (MCI). Compared to baseline, there was a significant reduction in the volume of white matter hyperintensities (WMH) (characteristic of pathological alterations in cerebral small vessels) in the RIC group at 1-year (9.10 ± 7.42 versus 6.46 ± 6.05 cm^3; P=0.020) measured using brain MRI. However, there was no significant difference in WMH volume in the sham group (8.99±6.81 versus 8.07±6.56 cm^3; P=0.085). When the two groups were compared, the volume of WMH change in the RIC group was significantly reduced compared to sham at 1-year (−2.632 versus −0.935

cm^3; *P*=0.049). Cognitive function was assessed using the Mini-Mental State Exam (MMSE) and MoCA. Although no significant difference in the overall scores on these cognitive tests were observed, significant improvements on visuospatial and executive function favouring the RIC group were reported after individual item analysis (mean change = 0.639 for the RIC group and 0.191 for the sham group (intervention−placebo difference: 0.449 points; 95% confidence interval, 0.036–0.933; *P*=0.048) (Wang et al., 2017). These findings demonstrate how long-term repeated RIC may benefit patients with cerebral SVD by reducing the volume of WMH and slowing cognitive decline. A study of 86 patients presenting with first ever AIS by Xiaofang et al. (2019) investigated the effects of upper limb RIC (5 x 5 min cycles of cuff inflation to 200 mmHg) for 6-months on cognitive impairment. Compared to controls (standard treatment), patients in the RIC group had significantly higher MoCA scores compared to controls (25.02 ± 1.97 vs 23.82 ± 2.61, *P*=0.018) at 6-months. Further analysis revealed significantly higher scores on the ‘’attention’’ domain in the RIC group compared to controls (5.31 ± 0.91 vs 4.62 ± 0.41, *P*= 0.031), as well as the ‘’visuospatial and executive functioning’’ domain (4.23 ± 0.73 vs 3.63 ± 1.01, *P*= 0.023). These findings were coupled with improvements in CBF (measured using transcranial doppler at the MCA) and endothelial function (indicated by reduced ICAM-1 and endothelin-1, ET-1 in serum) in the RIC group compared to controls (Xiaofang et al., 2019). We did not see much difference in MoCA scores between the two groups in this study. This may be because the time period between the baseline and follow-up MOCA test was too short (6-weeks), or the dose and duration of RIC was not sufficient to see changes in cognitive function. If RIC had influenced the cognitive domain of fatigue, then we may have anticipated that attention and concentration may have improved (Boksem et al., 2005, Faber et al., 2012, Johansson and Rönnbäck, 2012). Therefore, a more in-depth investigation of these aspects of fatigue may be warranted. It may be that the MoCA is not sensitive enough to detect changes in attention. An alternative test that looks at attention tasks (e.g., auditory or visual reaction time task) may be better (De Doncker et al., 2021a, Cumming et al., 2012).

3.5.6 Quality of life

One may hypothesise improvements in QoL if levels of fatigue and walking distance improve with RIC, however no obvious and consistent between group differences in EQ-5D-5L scores were observed in this study. While EQ5D-5L scores trended in a deteriorating manner, the VAS appeared to improve in both groups. It may be that the EQ5D-5L is not a sensitive enough

tool to observe improvements in fatigue. Problems with pain and anxiety/depression carry the greatest weight on the EQ-5D-5L questionnaire (Devlin et al., 2017) which may have clouded any improvements in fatigue. Deteriorations in this score were not of a magnitude that was clinically meaningful (Chen et al., 2016). Another popular self- QoL measure is the 36-item Short Form Health Survey (SF-36) (Ware and Gandek, 1998). The SF-36 includes 8 domains of global health status including: (1) physical functioning, (2) physical role limitations, (3) pain, (4) general health, (5) *energy/vitality*, (6) social functioning, (7) emotional problems, and (8) mental health (Lins and Carvalho, 2016). The SF-36 is a valid and reliable HRQoL tool that has been tested in a variety of populations including stroke (Hobart et al., 2002, Anderson et al., 1996, Cameron et al., 2021). A study by Unalan et al. (2008) administered the SF-36 to 70 stroke patients six months post-stroke to examine the effect of stroke on QoL. In this study, the researchers found that general health and vitality were the most common domains on the SF-36 that deteriorated QoL (Unalan et al., 2008). These findings suggest the SF-36 could be a more sensitive tool for measuring QoL in stroke patients with PSF in future studies.

3.5.7 Potential mechanisms of effect

A potential mechanism driving the reduction in fatigue is preserved mitochondrial respiration, reduced ROS production, and reduced ATP depletion in the brain (Lv et al., 2020), heart (An et al., 2017, Leung et al., 2014) and skeletal muscle (Addison et al., 2003) observed after RIC. As discussed in Chapter one, a postulated mechanism underlying the development of fatigue after stroke is physical deconditioning (i.e., physical and/or cognitive decline in function) due to inactivity (Billinger et al., 2014). Skeletal muscle deconditioning after stroke is associated with increased muscle weakness, reduced muscle mass (atrophy), hypertrophy of slow-twitch muscle fibres, and atrophy of fast-twitch muscle fibres (section 1.3.2.2 Chapter 1) (English et al., 2010, Sions et al., 2012). Individuals may also experience profound cardiovascular deconditioning after stroke (reduced cardiac output, exercise intolerance) (Ivey et al., 2005), associated with loss of muscle strength and reduced peak oxygen consumption (VO_{2peak}) (Macko et al., 2005, Pang et al., 2005b). Inactivity and ageing are associated with a decline in mitochondrial content (e.g., succinate dehydrogenase enzyme) and reduced muscle oxidative capacity in all types of skeletal muscle fibres (St-Jean-Pelletier et al., 2017, Marzetti et al., 2013). The number and volume density of mitochondria in skeletal muscle are important to sustain steady-level muscle work (Chance et al., 1985). A study by Distefano et al. (2018) of 39 healthy volunteers (young active, older active, older sedentary) found lower ATP synthesis

capacity in the older sedentary group (measured using ^{31}Phosphorus magnetic resonance spectroscopy) following muscle contraction (isometric contractions of the quadriceps) and concluded this was likely due to reduced electron transport system (ETS) respiratory capacity (assessed *ex vivo* in muscle biopsy specimens) (Distefano et al., 2018). These findings suggest that reduced physical activity and cardiorespiratory fitness after stroke (i.e., physical deconditioning) are likely associated with reductions in mitochondrial energetics in skeletal muscle (St-Jean-Pelletier et al., 2017), which may contribute to the development of PSF. However, studies have demonstrated how RIC can protect skeletal muscle mitochondrial function (Mansour et al., 2012). Using an experimental mouse model, Mansour et al. (2012) found that hind limb RIC (3 x 10 min ischaemia separated by 10 min-reperfusion) followed by 3 hours of ischaemia by aortic cross-clamping, protected rat skeletal muscle (gastrocnemius muscle) mitochondrial function by restoring mitochondrial maximal oxidative capacity (e.g., by improving mitochondrial respiratory chain function and reducing the proapoptotic *Bax* gene) after I/R injury (Mansour et al., 2012). This likely enhanced skeletal muscle ATP production, potentially explaining the beneficial effects of RIC on exercise performance (Lisbôa et al., 2017, Jean-St-Michel et al., 2011), including muscle force (Patterson et al., 2015, Hyngstrom et al., 2018) and endurance (Tanaka et al., 2016, Kido et al., 2015). These findings suggest inhibition of the mitochondrial apoptotic pathway plays a key role in enhanced muscle survival and function after RIC (Hatoko et al., 2002, Gao et al., 2017). This has also been demonstrated in the brain (Lv et al., 2020). Using a mouse model of MCA occlusion, Lv et al. (2020) found that bilateral RIC (3 x 3 min cycles of ischaemia separated by 5-min reperfusion) performed using aneurysm clips to occlude the femoral arteries, exerted significant neuroprotective effects. These effects included reduced neurological impairment after cerebral I/R injury (measured using the modified neurological severity score, mNSS), increased glucose metabolism, reduced infarct size (measured using PET/CT), suppressed apoptosis and reduced nuclear translocation of apoptosis-inducing factor (AIS) and endonuclease G (EndoG) (mitochondrial enzyme involved in apoptosis) from mitochondria, and improved mitochondrial-derived vesicle (MDV) formation. As mentioned in Chapter one, a clinical study by Slagsvold et al. (2014) found that a single dose of upper limb RIC (3 x 5 min cuff inflation to 200 mmHg) before undergoing CABG surgery preserved mitochondrial respiration. These findings suggest that RIC may help improve some of the effects of physical deconditioning on mitochondria after stroke and improve fatigue.

Another potential mechanism driving the reduction in fatigue is the effect of RIC on the vasculature (Nyquist and Georgakis, 2019, Maxwell et al., 2019). Single doses of RIC have been shown to protect conduit vessels (large arteries that transport blood to the systemic circulation, e.g., brachial and radial artery) from endothelial I/R injury in healthy volunteers (Loukogeorgakis et al., 2005, Kharbanda et al., 2001) and clinical populations (Manchurov et al., 2020). Clinical studies have also looked at the effects of repeated RIC on endothelial function and have shown promising results (Jones et al., 2014, Luca et al., 2013). For example, Jones et al. (2014) looked at the effect of repeated unilateral upper limb RIC (4 x 5 min cycles of cuff inflation to 220 mmHg) for 7 days on brachial artery endothelial function (measured using flow-mediated dilation, FMD) in 13 healthy male volunteers. In this study, FMD (measured at baseline, post-RIC [day8] and 8 days after treatment [day 15]) significantly increased (the brachial artery vasodilated) after repeated RIC and remained significantly elevated 8 days post-intervention in the preconditioned arm (5.0 ± 2.2%, 6.1 ± 2.2% and 6.6 ± 2.3%) and contralateral arm (i.e., control arm) (5.4 ± 2.2%, 6.0 ± 2.2.% and 7.5 ± 2.2%, P= 0.03). In this study, the investigators also assessed bilateral forearm microcirculation (cutaneous vascular conductance, CVC) and found that CVC significantly increased (indicative of improved skin microcirculation) by 0.03±0.03 mV/mm Hg during the 7-day RIC treatment period (P=0.001) (Jones et al., 2014). Increased levels of endothelial progenitor cells (key players in regeneration of endothelial lining and maintenance of vascular integrity) observed after ischaemic conditioning (Kimura et al., 2007) may facilitate increased endothelial function after RIC. Several experimental and clinical studies have demonstrated increased blood flow to the central (Meng et al., 2012, Kitagawa et al., 2018, Khan et al., 2018, Qin et al., 2020) and peripheral (Andreas et al., 2011b, Jeffries et al., 2018a) nervous system after RIC. This is likely mediated by improved endothelial function and vasoactive active substances (NO, adenosine) released into the circulation in response to the conditioning stimulus (Rassaf et al., 2014). Adequate supply of blood to skeletal muscles is essential as it provides oxygen and nutrients required for aerobic ATP synthesis and muscle contraction (Wan et al., 2017). It is also important for the removal of lactate (fatigue-inducing metabolite) from the blood and active muscles and is associated with improved perception of recovery (reduced pain/discomfort) after exercise (Sañudo et al., 2020). Physical deconditioning after stroke is associated with diminished peripheral blood flow dynamics (Ivey et al., 2005), with significantly greater reductions in blood flow and tissue mass observed in the stroke affected limb (Ivey et al., 2004). Low blood flow and muscle oxygenation leads to a reduction in muscle force (i.e., muscle fatigue), decreased coordination, increased perceived exertion (perceived effort of a task) and

altered EMG signals (Rashedi and Nussbaum, 2015). Blood flow is also important for fatigue recovery after exercise (Martin et al., 2006). A study by Broxterman et al. (2015) examined the effect of blood flow occlusion (blood pressure cuff inflated ≥275 mmHg) during hand grip exercises in six healthy male subjects and demonstrated quicker physical and neuromuscular (EMG) markers of fatigue in conditions of reduced limb perfusion. These findings demonstrate how reductions in oxygen delivery during and after exercise play a key role in the development of central and peripheral fatigue. As mentioned in previous chapters, Meng et al. (2012) found significantly improved CBF (measured using SPECT) after bilateral upper limb RIC (5 x 5 min cycles) for 300 days in stroke patients with intracranial arterial stenosis. Furthermore, Jeffries et al. (2018a) found significantly improved local muscle oxidative capacity (measured using near-infrared spectroscopy placed on the gastrocnemius medialis muscle) and microcirculation (assessed during recovery from submaximal isometric plantar flexion exercises at 40 and 60% of MVC) after bilateral lower limb RIC (4 x 5 min cuff inflation to 220 mmHg) compared to sham (cuff inflated to 20 mmHg). In their study, Jeffries et al. (2018a) found that after 7 consecutive days of RIC, resting muscle oxygen consumption ($m\dot{V}O_2$) reduced by 16.4% (indicating reduced oxygen demand at rest) (pre 0.39 ± 0.16%.s^{-1} vs. post 0.33 ± 0.14%.s^{-1}; $P < 0.05$). After 72 hours (late phase of protection), $m\dot{V}O_2$ recovery rate was increased by 13% (rate constant pre 2.89 ± 0.47 min^{-1} vs. post 3.32 ± 0.69 min^{-1}; $P < 0.05$) with no change in the sham group. The findings demonstrate how repeated episodes of RIC enhance microvascular muscle blood flow and reoxygenation in the muscle after exercise. These findings are in line with enhanced vascular and mitochondrial function after repeated RIC (Jones et al., 2014, Kimura et al., 2007, Epps et al., 2016).

Inflammation has been linked to fatigue in various clinical populations. For example, a study of 15 MS patients by Heesen et al. (2006) found significantly higher levels of proinflammatory cytokines (e.g., TNFα: 478.9 v 228.2 pg/ml, P= 0.01) in MS patients with fatigue (measured using the fatigue severity scale). A cross-sectional study of 70 chronic stroke patients by Gyawali et al. (2020) explored the relationship between PSF (measured using the fatigue assessment scale, FAS) and the proinflammatory cytokines IL-6 and C-reactive protein (CRP). The investigators found a significant relationship between the level of fatigue reported on the FAS and serum levels of IL-6 and CRP (measured using ELISA assay kits). Although this relationship was no longer statistically significant once cardiovascular covariables were introduced into a linear regression model (Gyawali et al., 2020). Improvements in fatigue observed in our study may be caused by downregulation of systemic inflammatory processes

and enhanced antioxidant activity observed after RIC (Pearce et al., 2021). Experimental studies of RIC have shown how RIC is associated with reduced inflammatory cell infiltration (macrophages, neutrophils) and oxidative stress in the context of myocardial infarction (Wei et al., 2011), and stroke (Wei et al., 2012, Meng et al., 2015a). The downregulation of pro-inflammatory gene expression has also been shown in human leukocytes after RIC (Konstantinov et al., 2004) (section 1.8.4 Chapter 1).

3.6 Limitations

Although this study provides a potential simple, non-invasive intervention to improve fatigue symptoms after stroke, the study has several limitations. Firstly, the study was a single-center pilot study which was not powered to detect significant differences in fatigue between the RIC and sham group. Secondly, most of the stroke survivors recruited were largely independent with daily activities, thus the generalizability to those more impaired following strokes is questionable. Although every effort was made to ensure participants were not aware of treatment allocation, it is possible that some of the control participants guessed that they were in the sham group, introducing bias from poor blinding. We did not ask participants whether they thought they were in the treatment or sham group which is something we should consider in future larger trials. When participants were asked what they expected the treatment to be like during their qualitative interview (discussed in chapter 6) they said they expected it feel like getting a blood pressure check. Therefore, they could have compared the two feelings. They could have also done their own research about the treatment. For example, one participant in the home-RIC group expressed disappointment when they were told to inflate the cuff to 20 mmHg, suggesting they had done their own research before the trial started. However, previous studies have found that most participants do not know they are in the control group despite the low cuff inflation pressures (Vanezis et al., 2018). For example, England et al. (2019) found that 56 out of 60 participants (93%) did not know whether they were in the treatment or sham group. However, despite this, we attempted to counteract this by using a second assessor blinded to treatment allocation as much as feasibly possible and included outcome measures that would be difficult for participants to control e.g., cardiopulmonary exercise testing and ^{31}P-MRS (discussed in following chapters).

The study may suffer from ‘small-study effects’ which is a term for the phenomenon that smaller studies often report larger treatment effects than larger studies (Schwab et al., 2021).

Some reasons why smaller studies have larger effect sizes include: confirmation bias, selection bias in treatment groups, reporting bias, outcome switching, clinical heterogeneity between participants and chance (Schwarzer, 2015, Schwab et al., 2021). Although every effort was made to avoid these issues, due to the covid-19 pandemic we chose not to approach people who were elderly and clinically vulnerable. Participants in our sample were therefore younger, fitter and more independent. We were also unable to blind the researcher who delivered the RIC intervention, potentially introducing bias. The same researcher also completed some of the follow-up assessments at 6 weeks, 3 months and 6 months due to COVID-19 pandemic restrictions.

It is difficult to know for certain how effectively the home-RIC group completed RIC. For example, we may have underestimated the effect if the cuff was not inflated tight enough. There is also the chance that participants in the home-sham group read about the treatment and decided to do it properly, which again would dampen down any treatment effects. However, this was not borne out of the qualitive interviews which is reassuring.

Outcome measures that aimed to measure physical activity (activity watches, GPAQ) were not practical thus we lack data on how this treatment may impact day to day levels of activity. Finally, follow-up data beyond 6-months is yet unavailable but will be collected and presented later.

3.7 Conclusion and future directions

RIC appears to help reduce fatigue and improve walking distances in people with stroke. However, the study is small and exploratory. Outcome measure for a larger trial could be made optimal by, for example, using NEADL for assessment of ADLs and SF-36 to measure QoL. Determination of sample size and characteristics for a definitive multi-centre study is required. An evaluation of potential mechanisms of effect is also needed as this will help to consolidate the creditability of the intervention itself, as well as help identify useful biomarkers of effect and potentially improve patient selection as well as enhance our understanding of the treatment in people with PSF.

CHAPTER 4. CARDIOPULMONARY EXERCISE TESTING (CPET) IN POST-STROKE FATIGUE AND THE EFFECTS OF REMOTE ISCHAEMIC CONDITIONING ON CPET MEASURES

4.1. Introduction

The aim of this chapter was to explore whether PSF severity is related to cardiopulmonary function by objectively measuring peak oxygen consumption (VO_{2peak}), ventilatory anaerobic threshold (VAT) and minute ventilation/carbon dioxide production (VE/VCO_2) slope during a symptom-limited graded maximal exercise test – *cardiopulmonary exercise testing (CPET)*. We also wanted to explore whether RIC affected these measures and potentially reveal clues as to potential mechanisms underlying any effect of RIC on PSF.

We discussed in previous chapters how physical deconditioning and reduced CRF (Billinger et al., 2012a, Lewis et al., 2011), and skeletal muscle mitochondrial dysfunction (Klinedinst et al., 2019) may contribute to the development of PSF. We also briefly mentioned how CRF is usually assessed by measuring a person's oxygen consumption at peak exercise (VO_{2peak}) (van de Port et al., 2015). Factors associated with lower VO_{2peak} in stroke include hypertension, low level of physical activity, increasing age, female sex, smoking and being overweight (Boss et al., 2017). Consequences of low CRF include reduced ability to perform ADLs (e.g., walking, dressing, bathing), loss of independence, activity intolerance, *fatigue*, and reduced mood and QoL (Gordon et al., 2004, McCain et al., 2019, Saunders et al., 2020). Low CRF is also associated with increased risk of cardiovascular disease (CVD) (Kennedy et al., 2018), stroke (Kurl et al., 2003, Prestgaard et al., 2018), and mortality (Blair et al., 1995, Clausen et al., 2018, Mandsager et al., 2018). Pathophysiological mechanisms of insufficient exercise and low CRF include reduced insulin sensitivity in muscle (Alibegovic et al., 2009), endothelial dysfunction (Bowden Davies et al., 2021), muscle atrophy (Trappe et al., 2007), reduced skeletal muscle mitochondrial function and oxidative capacity (Kenny et al., 2017).

As discussed in previous chapters, studies have demonstrated the beneficial effects of RIC on exercise performance including muscle strength and endurance (De Oliveira Cruz et al., 2015, Cheng et al., 2021, De Groot et al., 2009). The exact mechanisms are not fully understood but may involve the same factors that protect organs from I/R injury (e.g., improved organ

perfusion, reduced inflammation, reduced mitochondrial dysfunction) (Slagsvold et al., 2014, Meng et al., 2012, Jeffries et al., 2018a, Konstantinov et al., 2004).

Barbosa et al. (2015a) found that a single dose of bilateral lower limb RIC (3 x 5 min cycles of cuff inflation to 200 mmHg) reduced the slowing of contraction and relaxation (i.e., delayed fatigue onset) and increased time to task failure during a handgrip exercise in healthy volunteers. In this study the investigators found that RIC increased forearm deoxygenation (measured using near-infrared spectroscopy, NIRS) during peak exercise. This was also demonstrated in a study of eleven divers by Kjeld et al. (2014) who found that forearm RIC (4 x 5 min episodes of cuff inflation 40 mmHg above SBP) reduced forearm oxygen saturation (measured using NIRS) from 65% ± 7% to 19% ± 7% (mean ± SD; $P < 0.001$). They also found that RIC improved a static breath hold by ~17% and increased the distance covered during underwater swimming by ~8% (Kjeld et al., 2014). These findings suggest RIC can increase oxygen extraction from the muscle during peak exercise or ischaemia and can prepare the body for physical activity (DeLorey et al., 2003). Kido et al. (2015) compared deoxygenation of the vastus lateralis muscle (measured using NIRS) at equal timepoints during submaximal cycling exercise in 15 healthy male subjects and found that RIC (3 x 5 min bilateral leg occlusion at >300 mmHg) speeds up deoxygenation at the onset of exercise, so alters the kinetics rather than the magnitude of deoxygenation (Kido et al., 2015). Accelerated oxygen extraction may result from mitochondrial activation in skeletal muscle after RIC. Thus, delayed fatigue development in the study by Barbosa et al. (2015a) could be a result of improved metabolic efficiency and may involve humoral factors described in chapter one (*Table 3*) (Shimizu et al., 2009, Steensrud et al., 2010, Mubagwa et al., 1996a, Hess et al., 2015).

It may also be related to the influence of RIC on cardiorespiratory parameters. For example, De Oliveira Cruz et al. (2015) found that bilateral lower limb RIC (4 x 5 min cuff inflation to 220 mmHg) resulted in a 2.9% increase in VO_{2peak} during an incremental cycling test in trained cyclists compared to controls (cuff only inflated to 20 mmHg). It is believed this was achieved through an increase amplitude of the slow component of VO_2 kinetics driven by additional motor unit recruitment towards the end of the exercise test (De Oliveira Cruz et al., 2015).

Numerous other studies have demonstrated how RIC improves oxygen uptake kinetics and VO_{2peak} in healthy volunteers and athletes (Wiggins et al., 2018, Paradis-Deschênes et al., 2020, De Groot et al., 2009, Lindsay et al., 2017b). RIC has also been shown to improve ventilatory

anaerobic thresholds, the threshold from which respiration and energy production switches from aerobic to anaerobic pathways (Mezzani, 2017c, Wasserman and McIlroy, 1964). Indeed, improvement in the participant's anaerobic capacity, defined as the maximal amount of ATP resynthesized via anaerobic metabolism during short-duration maximal intensity exercise (Green and Dawson, 1993), and reduced lactate accumulation (Bailey et al., 2012), have been suggested as potential mechanisms underlying the effects of RIC on sports performance (Crisafulli et al., 2011). However, some studies have found no benefits on anaerobic performance after RIC and have suggested RIC may have greater benefit on aerobic performance (e.g., VO_{2peak}) (Lalonde and Curnier, 2015). Other aerobic capacity variables include respiratory exchange ratio (RER), ventilation and heart rate (HR) (Taylor et al., 2015) which will be discussed in following sections.

4.1.1 Measuring CRF in stroke

Measuring CRF in people with stroke can be challenging due to muscle weakness, spasticity, deficits in balance and gait, and fatigue (Michael et al., 2005, Balaban and Tok, 2014, MacKay-Lyons and Makrides, 2002). The current method of measuring CRF in the stroke population is cardiopulmonary exercise testing (CPET) which involves a graded maximal exercise test on a recumbent stepper, treadmill, or cycle ergometer (Macko et al., 2001, Billinger et al., 2008, Stoller et al., 2014, van de Port et al., 2015, Wittink et al., 2017). CPET is a physiological investigation that provides a global assessment of cardiovascular, metabolic and ventilatory responses to exercise (Taylor et al., 2015). It is a powerful diagnostic and prognostic tool that offers clinicians and researchers a wealth of information beyond that obtainable from field walking tests commonly used to evaluate exercise capacity, such as the 6MWT or incremental shuttle walk test (ISWT) (Holland et al., 2014). CPET joins ventilatory flow measurements and respired gas analysis (oxygen consumption and carbon dioxide production) to routine physiological and performance parameters measured during incremental exercise testing (e.g., work rate, heart rate, blood pressure) (Mezzani, 2017b). CPET generates information (e.g.,VO_{2peak}, VAT) that is useful for the assessment of disease severity, functional capacity and all-cause mortality (Stevens et al., 2018). CPET to measure CRF has been utilized in several studies in people with acute (Chen et al., 2010, Tang et al., 2006) and chronic (Billinger et al., 2012b) stroke. However, the studies are heterogenous in terms of protocols used (bicycle, treadmill), stroke severity, and duration and are therefore difficult to compare (Smith et al., 2012, van de Port et al., 2015). CPET is most commonly conducted on a cycle ergometer and is a safe test, even in populations with underlying high-risk cardiac diagnoses; including

chronic obstructive pulmonary disease (COPD), heart failure, pulmonary hypertension, hypertrophic cardiomyopathy and aortic stenosis (Skalski et al., 2012). CPET using bicycle ergometry is also safe in people early after stroke (Yates et al., 2004, Johnson et al., 2020), and can be used to determine risk of CVD and to prescribe exercise programmes in stroke rehabilitation (Mustafa and Aytür, 2021).

A few studies have looked at the association between PSF and VO_{2peak} (Michael et al., 2006, Tseng et al., 2010, Duncan et al., 2012) however none have reported any significant associations. Given what we know about physical deconditioning after stroke we cannot rule out the possibility of an association between CRF and PSF (Billinger et al., 2014, Lewis et al., 2011, De Doncker et al., 2018). One study of stroke inpatients on a subacute rehabilitation ward looked at the association between PSF severity (FSS) and several cardiorespiratory variables including VO_{2peak} (measured at peak exercise) and VO_2 at the ventilatory threshold, respiratory exchange ratio (RER) and oxygen uptake efficiency slope (OUES) (Oyake et al., 2021). The investigators found no significant associations between FSS score and these cardiorespiratory variables but did however find that fatigue severity was significantly correlated with longer *time constant* VO_2 (oxygen consumption) at the onset of a submaximal exercise test (rho = 0.530; *P*= 0.009). These findings suggest PSF severity is associated with delayed increases in VO_2 at the onset of exercise, but not with VO_2 at peak exercise. It may be that PSF is associated with other measures of CRF such as VAT or VE/VCO_2 slope that is independent of exercise intensity and patient effort (Belardinelli et al., 1995, Mezzani et al., 2009) and may be a more appropriate measure of CRF in individuals with reduced exercise capacity such as people with stroke (Boyne et al., 2017a). There is lack of data on the association between PSF and ventilatory efficiency (VE/VCO2 slope) or VAT, so we wanted to explore this. CPET measures may also help explain any effect that RIC has on PSF.

4.2 Aims and objectives

1. To undertake CPET using a bicycle ergometer in patients with debilitating PSF to measure cardiorespiratory parameters including peak oxygen consumption (VO_{2Peak}), ventilatory anaerobic threshold (VAT), minute ventilation/carbon dioxide production (VE/VCO_2) slope, and respiratory exchange ratio (RER).

2. To assess whether PSF severity is associated with CPET parameters.

3. Investigate whether the effects of RIC can be explained in part by changes in CPET parameters.

4.3 Hypotheses

1. PSF severity is associated with VO_{2peak}, VAT, VE/VCO_2 slope and RER.

2. RIC increases VO_{2Peak} and VAT, and lowers VE/VCO2 and RER, changes that reflect improved CRF.

4.4 Methodology

4.4.1 Design

To investigate these hypotheses CPET was undertaken at baseline and at 6-weeks following the RIC/sham intervention. CPET-derived variables include: peak oxygen consumption (VO_{2Peak}), ventilatory anaerobic threshold (VAT), minute ventilation/carbon dioxide production (VE/VCO_2) slope, and respiratory exchange ratio (RER) (Chambers and Wisely, 2019).

4.4.2 CPET outcome measures

4.4.2.1 Peak oxygen consumption

Peak oxygen consumption (VO_{2Peak}) is a metabolic rate defined as the highest oxygen uptake (VO_2) attained on an incremental exercise test. It reflects the body's maximal capacity to generate energy through aerobic metabolism and is normally reported in absolute terms (litres/min) or indexed to body mass (ml/kg/min) (Nichols et al., 2015, Levett et al., 2018). VO_{2Peak} may not be the maximum VO_2 achievable for that individual (VO_{2max}), however VO_{2max} is usually only achieved by young, physically fit individuals (Chambers and Wisely, 2019). Elderly individuals who are physically deconditioned and/or have comorbidities such as arthritis rarely reach their maximum, therefore $\underline{VO_{2Peak}}$ is a more appropriate measure of exercise capacity in clinical populations (Chambers and Wisely, 2019). During incremental exercise, VO_2 increases linearly in proportion to work rate (Chambers and Wisely, 2019). A

persons VO_{2Peak} is considered 'abnormally low' if it is <75% of their predicted VO_{2max} (Nichols et al., 2015). It is important to evaluate whether a participant has given maximal effort during a test (discussed below), as low VO_{2Peak} may be due to poor participant effort (Nichols et al., 2015). A cardiovascular limitation caused by reduced cardiac output, arterial oxygen content or muscle oxygen extraction may also explain why a subject has an abnormally low VO_{2Peak} (Nichols et al., 2015). Other factors that may influence VO_{2Peak} include age, sex, disease severity, muscle mass, deconditioning, test familiarity and protocol design (Nichols et al., 2015, Balady et al., 2010a). A mean VO_{2Peak} of 14.6 mL/kg/min has been reported in reviews of CRF in stroke (Boyne et al., 2017b). VO_{2peak} in people with stroke is 26-87% of that of healthy age-and gender-matched individuals (Smith et al., 2012). The MCID in VO_{2peak} has been reported to be between +2.0 and 2.5 ml/kg/min in patients with stroke (Brazzelli et al., 2011, Marsden et al., 2013) and heart failure (Edelmann et al., 2011, Mueller et al., 2021).

4.4.2.2 Ventilatory anaerobic threshold

During incremental exercise, VO_2 and expired carbon dioxide (VCO_2) increase linearly until oxidative metabolism can no longer sustain the required workload (Nichols et al., 2015). At this point, anaerobic metabolism is activated leading to an increase in blood lactate concentration and progressive metabolic acidosis (Mezzani, 2017a). During an incremental exercise test the ventilatory anaerobic threshold (VAT) is defined as the VO_2 above which pulmonary ventilation (VE) increases disproportionately relative to increase in VO_2 and lactate first begins to increase systematically (Wasserman and McIlroy, 1964). The VAT is also known as the 'lactate threshold'(Ghosh, 2004, Walsh and Banister, 1988) and is a useful measure of submaximal, sustainable exercise capacity and is reported in ml/kg/min (Levett et al., 2018). Lactic acid is buffered by intra- and extracellular bicarbonate, generating further CO_2 that is ventilated to maintain PH balance (Nichols et al., 2015, Levett et al., 2018). VCO_2 increases disproportionally compared to VO_2 causing an inflection in the VCO_2/VO_2 response (Figure 12) (Levett et al., 2018, Nichols et al., 2015).

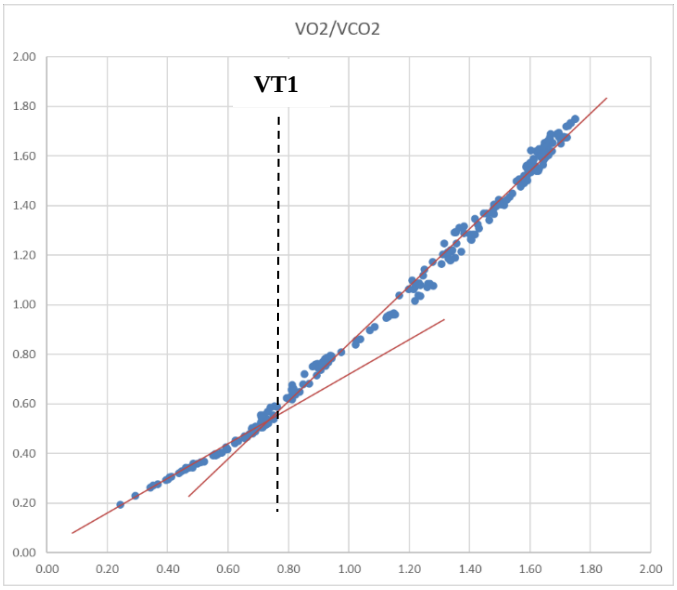


Figure 12. Volume of oxygen uptake (VO_2) as a function of exhaled carbon dioxide (VCO_2) during an incremental exercise test. *The point in time where VCO_2 increases disproportionally compared to VO_2 is the ventilatory anaerobic threshold. This has been identified using the V-slope method (red lines).*

This inflection point has been labelled VAT (also known as VT1) and it occurs in most healthy subjects and patients between 40-60% of VO_{2Peak} (Wasserman, 2012). A ventilatory anaerobic threshold <40% of VO_{2Peak} is commonly observed in people with cardiac disease or those with significant physical deconditioning (Mezzani et al., 2009). The most widely used method of determining the ventilatory anaerobic threshold is the V-slope method (Beaver et al., 2016, Wasserman, 2012) which is achieved by plotting VO_2 as a function of VCO_2 (Nichols et al., 2015) (Figure 12). It requires visual determination and is therefore a subjective measurement which should be assessed in a blinded manner (Nishijima et al., 2019). It involves drawing a line of best fit through the plots from the beginning of exercise to the point at which the linear relationship between VCO_2 and VO_2 is lost. A second line of best fit is then drawn from the end of the exercise test through to the deflection point (Nichols et al., 2015). The point at which these two lines cross is the ventilatory anaerobic threshold (VAT) (Nichols et al., 2015).

4.4.2.3 VE/VCO_2 slope

The minute ventilation/carbon dioxide production (VE/VCO_2) slope is an index of ventilatory efficiency and quantifies the ventilatory rate required to eliminate 1 litre of CO_2 (Nichols et al., 2015, Levett et al., 2018). The threshold for a normal VE/VCO_2 relationship is <30, without

adjustment for age and sex (Balady et al., 2010a). The VE/VCO_2 slope will steepen when excess ventilation caused by hyperactive peripheral chemoreceptors or increased ratio of physiological dead space over tidal volume (V_D/V_T) (i.e., wasted ventilation) causes the partial pressure of arterial carbon dioxide ($PaCO_2$) to drop (Mezzani, 2017a, Nichols et al., 2015). Another postulated mechanism of increased VE/VCO_2 is skeletal muscle ergoflex overactivation in patients with chronic heart failure (Kaczmarek et al., 2004, Wensel et al., 2004). Other postulated mechanisms of increased VE/VCO_2 slope (i.e., reduced ventilatory efficiency) include pulmonary vasoconstriction, reduced oxidative metabolism and increased anaerobic glycolysis (Prado et al., 2016). A higher-than-normal VE/VCO_2 slope is commonly observed in patients with respiratory or cardiac disease, and increasing values associated with progression of disease severity and age (Wasserman, 2012, Shen et al., 2015, Sullivan et al., 1988, Johnson and Robert, 2000). For example, VE/VCO_2 values >60 can be seen in patients with advanced pulmonary hypertension (Arena et al., 2010). Derivation of the VE/VCO_2 slope appears to be uninfluenced by exercise type (Maeder et al., 2008) or aggressiveness of the CPET protocol (Agostoni et al., 2005), and the relationship demonstrates high test-retest reliability (Barron et al., 2014, Bensimhon et al., 2008). Exercise training (Guazzi et al., 2004), cardiac resynchronisation therapy (Malfatto et al., 2005), and heart transplantation (Carter et al., 2006), are all treatments that have been shown to effectively lower VE/VCO_2 slope.

4.4.2.4 Respiratory exchange ratio

Respiratory exchange ratio (RER) is the ratio between VCO_2 and VO_2 (CO_2 production/O_2 uptake) (Mezzani, 2017a). As discussed, during increasing exercise intensity bicarbonate buffering of lactic acid results in increased CO_2 production, which increases the RER numerator at a faster rate than the denominator (Mezzani, 2017a). Therefore, a RER higher than 1.00 is indicative of significant anaerobic glycolysis above VT1 and implies significant exercise-induced body stress (Mezzani, 2017a). However, a peak RER lower than 1.00 reflects submaximal cardiovascular effort (Mezzani, 2017a). A peak RER > 1.10 along with a peak HR within 10 beats min^{-1} can be used to support the assessment of $VO2_{peak}$ in the absence of a plateau in the VO_2 response (Howley et al., 1995). This physiological response to exercise is consistent across healthy individuals and clinical populations, making peak RER an objective measure of maximal effort attainment in cardiopulmonary exercise testing. It helps researchers obtain a reliable and clinically meaningful assessment of an individual's peak aerobic capacity.

4.4.2.5 Work rate

CPET usually evaluates CRF by means of a personalised ramp exercise protocol (Agostoni et al., 2005). Ramp protocols using a cycle ergometer start the participant on zero or low resistance and increase the work rate (WR) evenly every minute (Myers and Bellin, 2000). In clinical settings, a popular ramp protocol is 10-watts per min (10-w/min) which increases the WR by 1 watt (w) every 6 seconds (Albouaini et al., 2007, Mezzani, 2017b). Ramp protocols can be individualised depending on the sample population, patient capabilities and purpose of the test (Myers and Bellin, 2000). Ramp protocol workloads should be calculated on the subject's estimated exercise capacity, in order to elicit volitional exhaustion (participant's expressed inability to continue exercising) within 8-12 min (regardless of baseline fitness level) (Fletcher et al., 2013).

4.4.2.6 The Borg Rating of Perceived Exertion

The Borg Rating of Perceived Exertion (RPE) (Appendix 18) is a scale used to quantify the subjective feeling of exercise intensity and fatigue during an exercise test (Noble, 1982, Borg, 1982). It measures an individual's self-rated physical exertion with scores ranging from 6 (no exertion) to 20 (maximum exertion) (Compagnat et al., 2018). The Borg RPE provides a measure of how hard someone feels their body is working during exercise based on the physical sensations that the person experiences, such as sweating, increased heart rate, increased breathing rate and muscle fatigue (Centers for Disease Control and Prevention, 2020). People with stroke can accurately rate the RPE scale, regardless of the severity of their motor impairments (Hampton et al., 2014, Sage et al., 2013, Wallace et al., 2010). It is well-studied in both healthy subjects and cardiac patients and correlates with an individual's heart rate and VO_2, therefore is a reliable indicator of how hard someone is working (Whaley et al., 1997a, Whaley et al., 1997b). An RPE >17 is suggestive of maximal exercise and values >15 to 16 suggest that the first ventilatory anaerobic threshold has been surpassed (Balady et al., 2010a).

4.4.3 CPET protocol

Participants were advised to attend the specialised exercise laboratory (Collegiate Hall, Sheffield Hallam University) in a euhydrated state, having not taken part in strenuous exercise within the previous 24 hours. Participants were also asked to avoid caffeine and alcohol on the

day of testing. The exercise lab room temperature was identical at baseline and follow-up (20-22°C). Any contraindications to maximal exercise testing were established using the guidelines of Taylor, Nichols and Ingle et al (2015) before referral of the participant (*Table* 18). Absence of any contraindications for exercise testing were reconfirmed at the CPET appointment by the test administrator (lab technician), by querying the past medical history, and any current symptoms with the participant. Medication history was taken to identify drugs that may interfere with the exercise response (e.g., Beta blockers) (Myers et al., 2009). The researcher measured the participant's height and weight, and their resting blood pressure was measured using an automated blood pressure machine (OMRON M2). Resting arterial oxygen saturation (SPO_2) and resting heart rate (HR) was measured using an SPO_2 oxygen saturation monitor. The lab technician input the participant's gender, age, weight and height into the CPET computer software package (MetaSoft® Studio). Based on this information the software calculated predicted normal values for that individual (Chambers et al. 2019). The researcher then compared the predicted values with work achieved at the end of the test. It allows the researcher to know if the participant achieved a maximal effort. To collect respiratory data a facemask (Hans Rudolph 7450 V2 series reusable mask) was attached around the participant's mouth and nose and secured with V2 headgear and strap locking clips (see Figure 13). The mask includes a valve and a system of tubing which feeds breath-by-breath data to a metabolic gas analyser (Cortex Metalyser 3B). Due to the nature of CPET including increased levels of ventilation it was classed as high-risk for infection and cross contamination during the covid-19 pandemic (Association for respiratory technology and physiology, 2022). Strict guidelines and risk assessments were followed to ensure participant safety and to enable recruitment to continue (Appendix 17). CPET consists of four main stages: rest phase (3 min), unloaded exercise (3 min), ramp phase (8-10 min), and recovery (~5 min) (Glaab and Taube, 2022).

Table 18. Absolute and relative contraindications for cardiopulmonary exercise testing

Absolute contraindication	Relative contraindication
Acute myocardial infarction (3–5 Days)	Left main coronary stenosis or its equivalent Moderate stenotic valvular heart disease
Unstable Angina	Severe untreated arterial hypertension at rest (>200 mm Hg systolic, >120 mm Hg diastolic)
Uncontrolled arrhythmias causing symptoms or haemodynamic compromise	Uncontrolled arrhythmias causing symptoms or haemodynamic compromise
Syncope	Tachyarrhythmias or bradyarrhythmias
Active endocarditis	High-degree atrioventricular block
Acute myocarditis or pericarditis	Hypertrophic cardiomyopathy
Symptomatic severe aortic stenosis	Significant pulmonary hypertension
Uncontrolled heart failure	Advanced or complicated pregnancy
Acute pulmonary embolus or pulmonary infarction	Electrolyte abnormalities
Thrombosis of lower extremities	Orthopaedic impairment that compromises exercise performance
Suspected dissecting aneurysm	
Uncontrolled asthma	
Pulmonary oedema	
Ambient desaturation at rest <85%	
Respiratory failure	
Acute non-cardiopulmonary disorder that may affect exercise performance or be aggravated by exercise	
Mental impairment leading to inability to cooperate	

Source: Taylor et al. (2015)

Figure 13. Cardiopulmonary exercise testing using the cycle ergometer. *Picture taken by the author of this thesis. Permission obtained from the participant to use this image.*

Rest phase

Before the participant was asked to begin cycling there was a 3-minute rest phase. Resting breath-by-breath metabolic gas-exchange data was collected whilst the participant was seated on a static exercise bike. The participant's heart rate was continuously monitored throughout the CPET using a Polar Heart rate monitor (chest strap).

Unloaded exercise

The unloaded exercise phase involved 3 minutes of resistance-free pedalling. This was to allow participants, particularly those who were functionally limited, to familiarise themselves with pedalling (Levett et al., 2018). It also allowed the participant's HR, BP, VO_2, VCO_2, and VE to stabilise before the ramp phase (Levett et al., 2018). The participant was encouraged to adopt a comfortable pedalling cadence, between 55 and 65 revolutions per minute (rpm).

Ramp phase

The participant progresses into the ramp phase without receiving any cues from the researcher. The workload increased at 10 w per minute until the participant reached volitional exhaustion or until they experienced any of the following symptoms:

- Palpitations
- Dizziness or faintness
- Symptoms of myocardial ischaemia
- Sudden pallor
- Mental confusion
- Signs of respiratory failure
- Loss of coordination

(American Thoracic society, 2003)

The test was also stopped if the participant was unable to maintain the cadence or if the research technician noticed there was a plateau of O_2 uptake despite increases in workload. Regardless of baseline fitness level, the aim of the ramp protocol is to elicit volitional exhaustion after 8-12 minutes (Taylor et al., 2015). Breath-by-breath expired gases were monitored continuously and VO_2 were averaged over 15 seconds. VO_{2peak} was calculated from the highest consecutive 15-second period of expired gas fractions (Martin-Rincon and Calbet, 2020, Schwaiblmair et

al., 2012, Maxwell et al., 2021). A RPE on the 6-20 Borg scale (Appendix 18) was recorded at each minute throughout the test, and at the end of peak exercise capacity. Every minute the scale was held in front of the participant, and they were asked to point to the number they felt best reflected the way they were feeling. If participants were unable to do this, the researcher pointed at the numbers and asked participants to nod when they were happy.

Recovery

Participants were asked to conduct a cool down on the bike until they felt recovered (~5 mins). The load was removed and the participant was encouraged to pedal to prevent venous pooling in the legs (Levett et al., 2018). Participants were monitored and were not permitted to leave the testing facility until their BP and HR had returned to near baseline values. The study visit was approximately 1-hour. The CPET was considered a peak test if the participant achieved two of the following criteria outlined in *Table 19.* (Nichols et al., 2015).

Table 19. Criteria for maximal effort during cardiopulmonary exercise testing.

Maximal effort criteria
• **A plateau in VO_2 (or failure to increase by 150 $mLmin^{-1}$) with increased workload.**
• **Failure of HR to increase with further increases in exercise intensity (achieving ≥85% of age-predicted maximal HR is an indicator of patient effort).**
• **RER (VCO_2/VO_2) at peak exercise >1.10**
• **An RPE >17 on the 6–20 scale.**
***VO_2 = oxygen consumption; HR = heart rate; RER = respiratory exchange ratio; RPE = rate of perceived exertion.**

4.4.4 Statistical analysis

All data was analyzed using SPSS Statistics version 26 and GraphPad Prism (version 9). Pearson r correlation analysis was performed to explore correlations between CPET parameters and fatigue severity (FSS-7) and 6MWT. An exploration of any potential differences between the treatment and sham groups in relation to the CPET outcomes was completed using a one-way ANCOVA. Like with the patient completed clinical outcome measures (see section 3.3 *Statistical analysis*), the participant's baseline scores and mRS were included as co-variates for all CPET outcome measures. The same ANCOVA model was used to calculate the adjusted within group mean change in outcome measures using the same covariates as above. All assumptions for ANCOVA (see section 3.1.2.2 *Statistical analysis)* were tested and satisfied before completion of the analysis. CPET outcome measures that did not meet these assumptions were also analysed using a non-parametric Mann-Whitney U Test and reported accordingly (median and IQR). As these analyses were exploratory, the findings for both tests were reported for completeness. The 95% confidence intervals (CI) of the adjusted mean differences and standardised effect sizes were calculated to evaluate any relationships present. Details of how effect sizes were calculated are provided in section 3.3 *Statistical analysis*.

4.5 Results

In total, out of the 22 participants who completed the 6-week RIC/sham intervention, 17 participants (77.3%) (9 treatment, 8 sham) completed cardiopulmonary exercise testing at baseline and at 6-week follow-up. Demographic and clinical characteristics are summarised in *Table 20*. Some participants were unable to complete a baseline or follow-up CPET (or both) due to stroke-related impairments (spasticity, weakness) (n=2) which meant they were unable to get on the bike, illness on the day of testing (breathlessness, cough) (n=2), and protocol amendments which restricted access to research sites (n=1).

Table 20. Participant characteristics of participants who completed cardiopulmonary exercise testing.

Characteristic	All	Treatment	Sham
n	17	9	8
Age, years (mean; SD)	60.3 (10.8)	57.8 (9.8)	63.1 (11.7)
Sex	12 M: 5 F	6 M: 3 F	6 M: 2 F
Height, cm (mean; SD)	173.7 (7.5)	174.6 (7.4)	172.6 (7.9)
Weight, kg (mean; SD)			
Pre-intervention	88.3 (19.7)	90.9 (23.2)	85.3 (16.1)
Post-intervention	88.3 (18.9)	91.5 (21.4)	85.2 (16.9)
BMI (mean; SD)			
Pre-intervention	29.6 (8.3)	30.2 (9.6)	28.9 (7.1)
Post-intervention	29.7 (8.0)	30.4 (9.0)	28.9 (7.5)

Exploratory analysis was performed on the CPET outcome measures at 6-weeks. All ANCOVA assumptions were tested before completion of the analysis. At 6-weeks, all CPET outcome measures except peak RPE sufficiently met the assumptions for parametric testing. Peak RPE data was also analysed using a non-parametric Mann-Whitney U test and scores are reported with medians and IQRs instead of means and SDs. As these analyses are exploratory, we reported the findings of the parametric and non-parametric test alongside each other for completeness.

4.5.1 VO_{2Peak}

Pearson's correlation demonstrated a strong significant positive correlation between VO_{2peak} and 6MWT (R=0.66, *P*=0.01) and a small but statistically insignificant negative correlation between VO_{2peak} and FSS-7 (R=-0.28, *P*=0.28) (Figures 14 and 15). The adjusted mean difference in VO_{2peak} between the treatment and sham group at 6-weeks was not statistically significant (-0.24 95% CI -4.05 to 3.58, *P*= 0.90) (*Table 21*). VO_{2peak} seemed to improve slightly in both groups at 6-weeks (RIC adjusted change 0.48 ml/kg/min CI -2.11 to 3.07 vs

sham 0.71 ml/kg/min -2.04 to 3.46) with no effect of RIC over sham (*Table 22*, Figure 16). The overall small improvements in VO_{2peak} were not of a magnitude that is considered clinically meaningful (Brazzelli et al., 2011, Marsden et al., 2013).

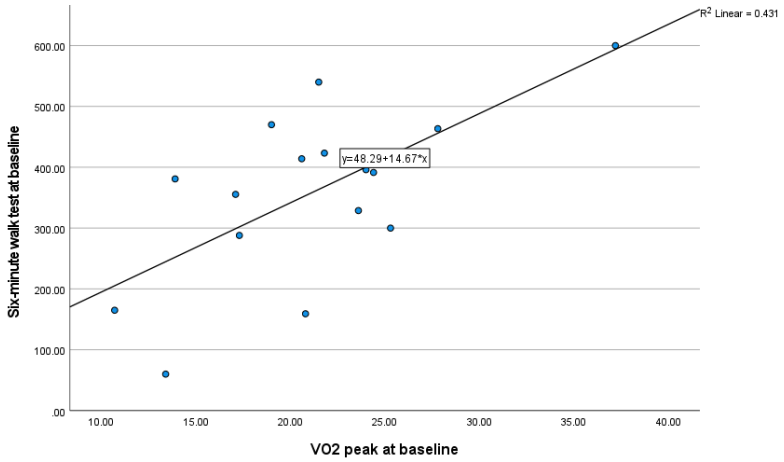


Figure 14. Relationship between VO_{2peak} and distance walked during the six-minute walk test (6MWT) at baseline.

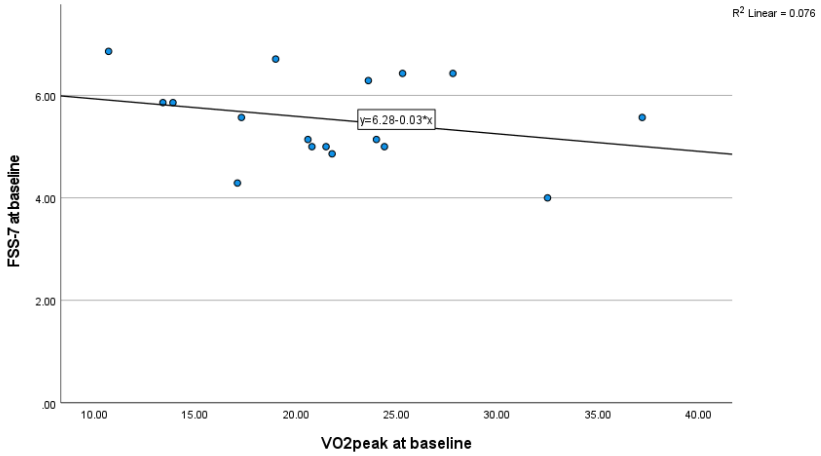


Figure 15. Relationship between VO_{2peak} and fatigue severity (FSS-7) at baseline.

Table 21. Analysis of within group and between group differences in VO_{2Peak} at 6-week follow-up using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group at 6-weeks (95% CI)	P-value	Effect size
VO_{2Peak} (ml/kg/min)	21.44 (8.05)	21.88 (8.97)	22.16 (5.79)	22.88 (6.92)	-0.24 (-4.05 to 3.58)	0.90	0.06

***RIC = remote ischaemic conditioning; CI = confidence interval; VO_{2Peak} = peak oxygen consumption.**

Table 22. Adjusted and unadjusted within group mean change in VO_{2Peak} from baseline to 6-weeks.

Outcome measure	Sham group Mean change (95% CI)	Sham group Adjusted mean change (95% CI)	RIC group Mean change (95% CI)	RIC group Adjusted mean change (95% CI)
VO_{2Peak} (ml/kg/min)	0.44 (-1.97 to 2.85)	0.71 (-2.04 to 3.46)	0.72 (-2.38 to 3.83)	0.48 (-2.11 to 3.07)

***RIC = remote ischaemic conditioning; CI = confidence interval; VO_{2Peak} = peak oxygen consumption.**

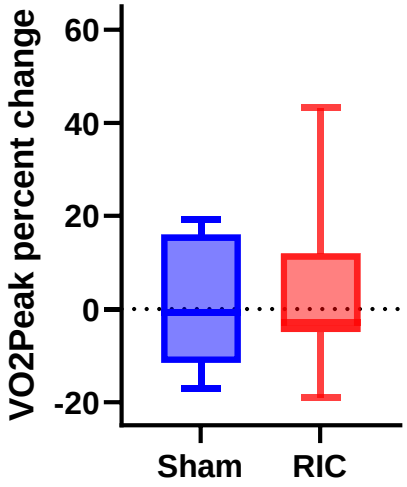


Figure 16. Percentage change in peak oxygen consumption (VO_{2peak}) in the treatment (red) and sham (blue) group at 6-weeks.

4.5.2 VAT and Time to VAT

In a similar manner to VO_{2peak}, VAT was also positively correlated with 6MWT albeit less strongly (moderate strength) than VO_{2peak} (R= 0.26, *P*=0.32) (Figure 17) and negatively correlated with FSS-7 (R= -0.23, *P*= 0.39) (Figure 18). At 6 weeks, the adjusted between group difference in VAT between the two groups was not statistically significant (-0.14 mL/O_2/kg^{-1}min^{-1}, 95% CI -2.31 to 2.02, *P*= 0.89) (*Table 23*). The VAT increased in the RIC group (0.21 mL/O2/Kg/min, 95% CI -1.36 to 1.79) while it fell in the sham group (-0.08, 95% CI -1.75 to 1.59) (*Table 24*, Figure 19). The time taken to reach the VAT increased in both groups by 26.08 and 35.91 seconds in the RIC and sham groups respectively (Table 24). However, the adjusted between group difference in time to VAT at 6-weeks was not statistically significant (-19.08 seconds, 95% CI -141.93 to 103.77, *P*= 0.74) (*Table 23*).

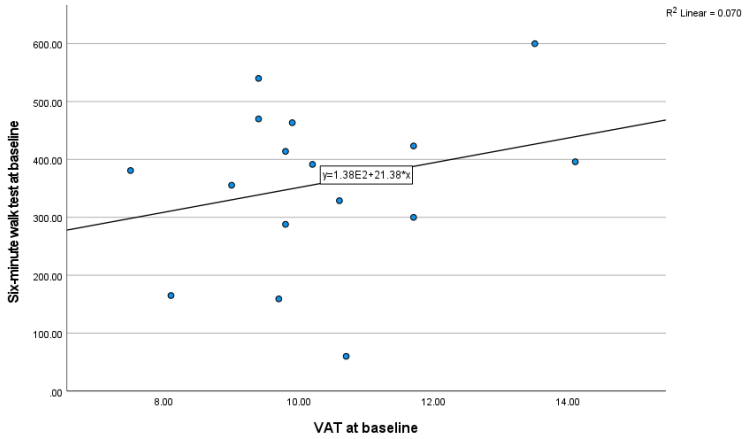


Figure 17. Relationship between ventilatory anaerobic threshold (VAT) and distance walked during the six-minute walk test at baseline.

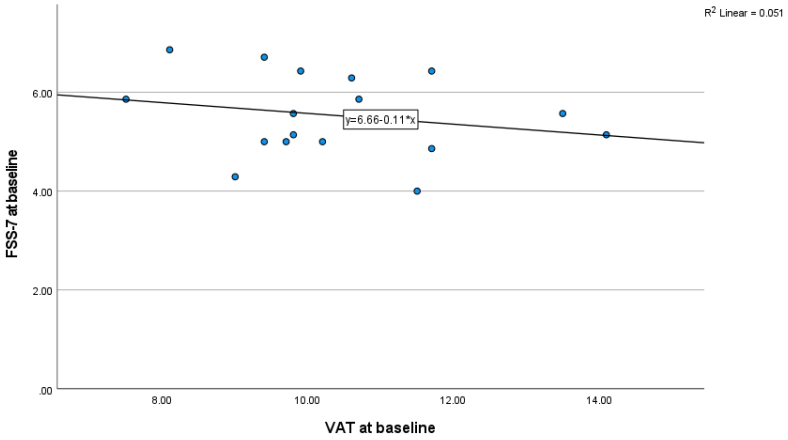


Figure 18. Relationship between ventilatory anaerobic threshold (VAT) and fatigue severity (FSS-7) at baseline.

Table 23. Analysis of within group and between group differences in ventilatory anaerobic threshold (VAT) and time to VAT at 6-week follow-up using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group at 6-weeks (95% CI)	P-value	Effect size
VAT ($mL/O_2/kg^{-1}min^{-1}$)	10.53 (2.23)	10.29 (1.96)	10.27 (1.23)	10.26 (2.61)	-0.14 (-2.31 to 2.02)	0.89	0.09
Time to VAT (sec)	170.38 (103.02)	192.63 (124.52)	176.67 (93.47)	182.22 (100.10)	-19.08 (-141.93 to 103.77)	0.74	0.19

***RIC = remote ischaemic conditioning; CI = confidence interval; VAT = ventilatory anaerobic threshold.**

Table 24. Adjusted and unadjusted within group mean change in ventilatory anaerobic threshold (VAT) and time to VAT from baseline to 6-weeks.

Outcome measure	Sham group Mean change (95% CI)	Sham group Adjusted mean change (95% CI)	RIC group Mean change (95% CI)	RIC group Adjusted mean change (95% CI)
VAT ($mL/O_2/kg^{-1}min^{-1}$)	-0.24 (-2.19 to 1.71)	-0.08 (-1.75 to 1.59)	0.36 (-1.15 to 1.86)	0.21 (-1.36 to 1.79)
Time to VAT (s)	34.50 (-69.67 138.67)	35.91 (-62.34 to 134.15)	27.33 (-83.02 to 137.69)	26.08 (-66.43 to 118.60)

***RIC = remote ischaemic conditioning; CI = confidence interval; VAT = ventilatory anaerobic threshold.**

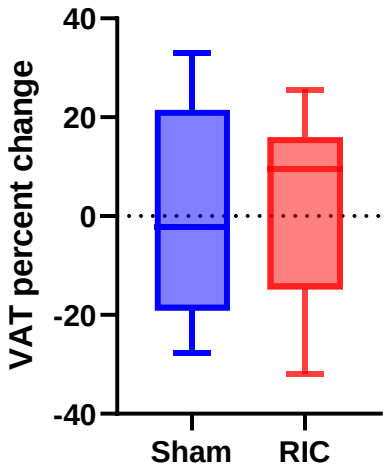


Figure 19. Percentage change in ventilatory anaerobic threshold (VAT) in the treatment (red) and sham (blue) group at 6-weeks.

4.5.3 VE/VCO₂

The VE/VCO_2 slope did not appear to be correlated with 6MWT (R = 0.01, *P*= 0.98) (Figure 20) however displayed a small but non-significant positive correlation with fatigue severity (R= 0.28, *P*=0.27) (Figure 21). The adjusted between group difference in VE/VCO_2 slope between the two groups at 6-weeks was not statistically significant (-2.79, 95% CI -5.90 to 0.32, *P*= 0.08) (*Table 25*). However, participants in the RIC group experienced a trend towards a reduced VE/VCO_2 slope (adjusted mean change -1.00, 95% CI -3.09 to 1.09) while there was an increased trend in the sham group (adjusted mean change 1.91, 95% CI -0.31 to 4.13) suggesting an improvement in ventilatory efficiency in the RIC group (*Table 26*, Figure 22).

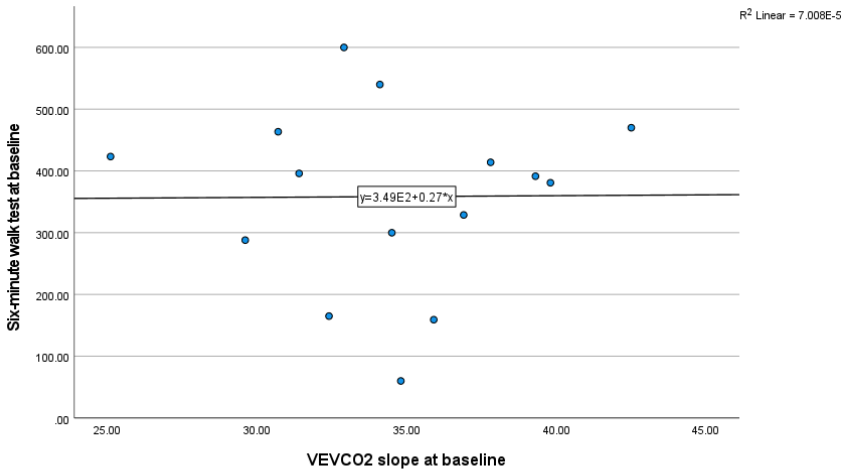


Figure 20. Relationship between VE/VCO_2 slope and distance walked during the six-minute walk test at baseline.

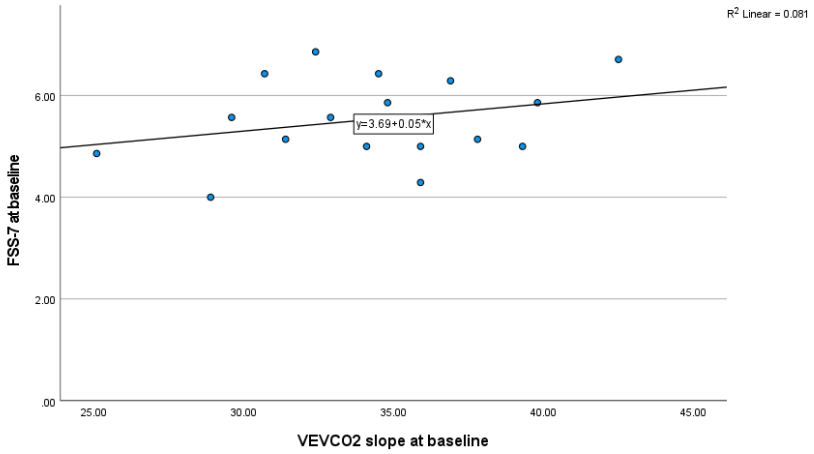


Figure 21. Relationship between VE/VCO_2 slope and fatigue severity (FSS-7) at baseline.

Table 25. Analysis of within group and between group differences in the VE/VCO2 slope at 6-weeks using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group at 6-weeks (95% CI)	P-value	Effect size
VE/VCO 2	33.86 (3.37)	35.98 (4.49)	34.61 (5.34)	33.36 (5.00)	-2.79 (-5.90 to 0.32)	0.08	1.07

***RIC = remote ischaemic conditioning; CI = confidence interval; VE/VCO2 slope = minute ventilation/carbon dioxide production.**

Table 26. Adjusted and unadjusted within group mean change in VE/VCO2 slope from baseline to 6-weeks.

Outcome measure	Sham group Mean change (95% CI)	Sham group Adjusted mean change (95% CI)	RIC group Mean change (95% CI)	RIC group Adjusted mean change (95% CI)
VE/VCO2	2.20 (-0.65 to 5.05)	1.91 (-0.31 to 4.13)	-1.26 (-3.31 to 0.80)	-1.00 (-3.09 to 1.09)

***RIC = remote ischaemic conditioning; CI = confidence interval; VE/VCO2 slope = minute ventilation/carbon dioxide production.**

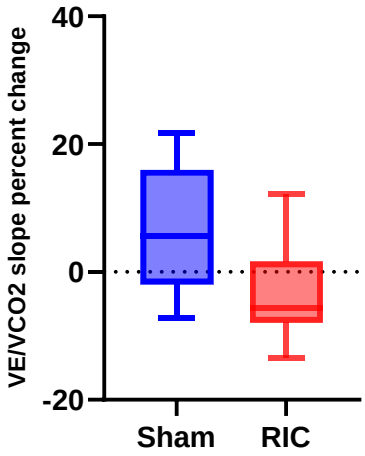


Figure 22. Percentage change in minute ventilation/carbon dioxide production (VE/VCO2) slope in the treatment (red) and sham (blue) group at 6-weeks.

4.5.4 Peak RER

The RER at peak VO_2 looked to exhibit a small negative correlation with 6MWT (R= -0.23, *P*= 0.41) (Figure 23) and small positive correlation with fatigue severity (R= 0.12, *P*=0.66) (Figure 24), both correlations were not statistically significant. The adjusted between group difference between the two groups at 6-weeks was not statistically significant (-0.01, 95% CI -0.07 to 0.06, *P*= 0.86) (*Table 27*). There were no real discernable differences when comparing the adjusted mean changes between both groups at 6-weeks (RIC adjusted change -0.01, 95% CI -0.06 to 0.04 vs sham -0.00, 95% CI -0.05 to 0.05) (*Table 28*, Figure 25). At baseline, only 2 participants (13%) reached an RER >1.10, demonstrating these participants achieved maximal effort. At 6-weeks, five participants (31%) reached an RER >1.10, indicating more participants applied maximal effort at 6-week follow-up.

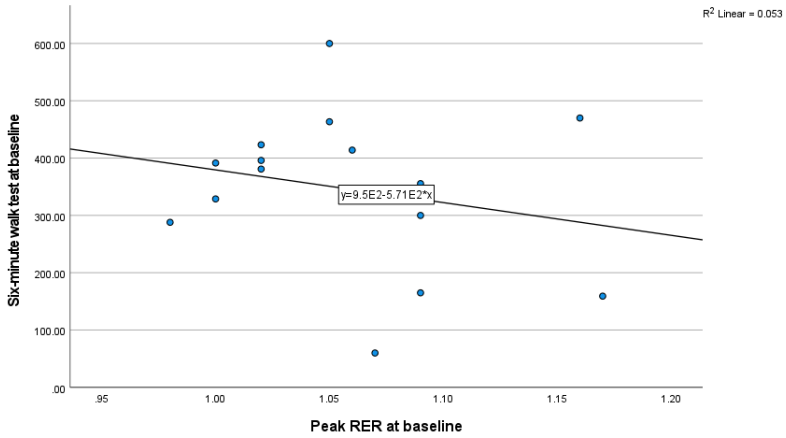


Figure 23. Relationship between peak respiratory exchange ratio (RER) and distance walked during the six-minute walk test at baseline.

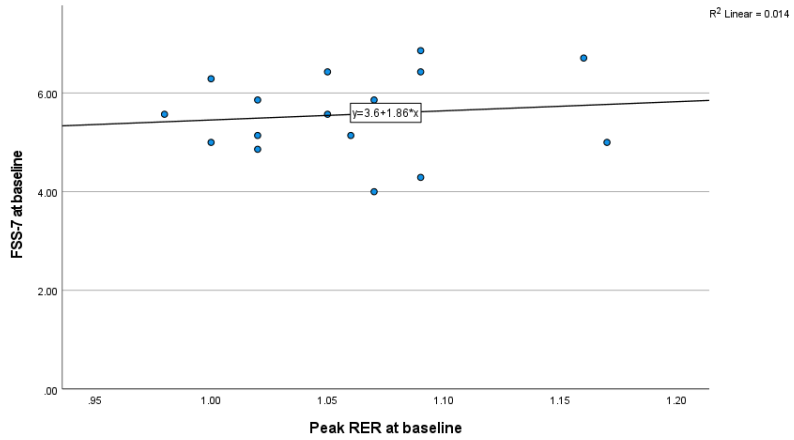


Figure 24. Relationship between peak respiratory exchange ratio (RER) and fatigue severity (FSS-7) at baseline.

Table 27. Analysis of within group and between group differences in peak respiratory exchange ratio (RER) at 6-weeks using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group at 6-weeks (95% CI)	P-value	Effect size
Peak RER	1.06 (0.06)	1.05 (0.07)	1.06 (0.05)	1.05 (0.06)	-0.01 (-0.07 to 0.06)	0.86	0.11

***RIC = remote ischaemic conditioning; SD = standard deviation; Peak RER = peak respiratory exchange ratio.**

Table 28. Adjusted and unadjusted within group mean change in peak respiratory exchange ratio (RER) from baseline to 6-weeks.

Outcome measure	Sham group Mean change (95% CI)	Sham group Adjusted mean change (95% CI)	RIC group Mean change (95% CI)	RIC group Adjusted mean change (95% CI)
Peak RER	-0.01 (-0.06 to 0.05)	-0.00 (-0.05 to 0.05)	-0.01 (-0.06 to 0.05)	-0.01 (-0.06 to 0.04)

***RIC = remote ischaemic conditioning; CI = confidence interval; Peak RER = peak respiratory exchange ratio.**

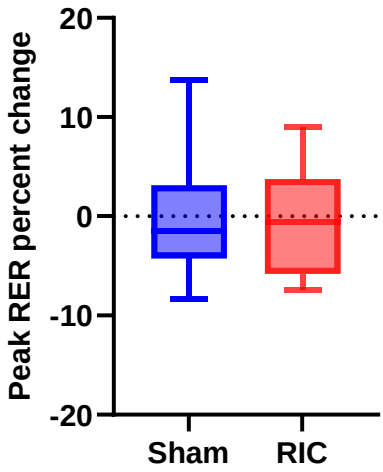


Figure 25. Percentage change in peak respiratory exchange ratio (RER) in the treatment (red) and sham (blue) group at 6-weeks.

4.5.5 Test effort

The adjusted between group difference in HR, WR, and test duration between the two groups at 6-weeks was not statistically significant (*Table 29*). However, HR, WR, and test duration data suggest that the sham group have increased the effort put into the CPET test at 6-week follow-up with increased HR (sham adjusted change 5.71 bpm CI -6.64 to 18.05 vs RIC -4.93 CI -20.64 to 10.78) WR (sham adjusted change 2.13 w CI -7.43 to 11.69 vs RIC -1.78 w CI -10.78 to 7.21) and test duration (sham adjusted change 36.34 s CI -21.65 to 94.33 vs RIC -23.75 s CI -78.32 to 30.83) compared to the RIC group (*Table 30*). Despite this we have not seen a great increase in VO_{2peak} in the sham compared to the RIC group (*Tables 20 and 21)*. This may be due to changes in efficiency of ventilation and energy production (*Tables 23-26*). The perceived effort inputted by both groups appears to be equal however (RPE) (*Table 29*). At baseline, 11 out of 17 (64.7%) participants (6 treatment, 5 sham) met the criteria for maximal effort and 13 out of 17 (76.5%) participants (8 treatment, 5 sham) met the criteria at 6-weeks (*Table 19*).

Table 29. Analysis of within group and between group differences in measures of cardiopulmonary exercise test effort (heart rate, work rate, test duration and rate of perceived exertion) at 6-weeks using a one-way ANCOVA.

Outcome measure	Sham baseline (mean, SD)	Sham 6-week follow-up (mean, SD)	RIC baseline (mean, SD)	RIC 6-week follow-up (mean, SD)	Adjusted mean difference between the treatment and sham group at 6-weeks (95% CI)	P-value	Effect size
Peak HR (beats/min)	138.00 (41.39)	145.63 (31.53)	150.20 (21.89)	144.00 (16.45)	-10.64 (-30.87 to 9.60)	0.27	0.79
Peak WR (w)	128.88 (62.51)	132.75 (52.76)	151.56 (48.63)	148.22 (41.64)	-3.92 (-17.22 to 9.39)	0.54	0.35
Test duration (sec)	657.25 (374.63)	693.50 (310.81)	797.78 (288.52)	765.22 (262.11)	-48.96 (-136.68 to 38.77)	0.25	0.67
Peak RPE	18.86 (1.07)	18.57 (1.90)	18.78 (1.39)	19.25 (0.71)	0.38 (-0.96 to 1.73)	0.54	0.38
Peak RPE (median, IQR)*	19.00 (2.00)	19.00 (3.00)	19.00 (2.00)	19.00 (1.00)	0.00[6]	0.78	0.15

***RIC = remote ischaemic conditioning; SD = standard deviation; CI = confidence interval; HR = heart rate; WR = work rate; RPE = rate of perceived exertion; IQR = interquartile range. [6]Unadjusted median difference included.**

Table 30. Adjusted and unadjusted within group mean change in peak HR, peal WR, test duration and peak RPE from baseline to 6-weeks

Outcome measure	Sham group Mean change (95% CI)	Sham group Adjusted mean change (95% CI)	RIC group Mean change (95% CI)	RIC group Adjusted mean change (95% CI)
Peak HR (beats/min)	7.63 (-9.69 to 24.94)	5.71 (-6.64 to 18.05)	-8.00 (-24.50 to 8.50)	-4.93 (-20.64 to 10.78)
Peak WR (w)	3.88 (-7.13 to 14.88)	2.13 (-7.43 to 11.69)	-3.33 (-16.94 to 10.27)	-1.78 (-10.78 to 7.21)
Test duration (sec)	46.25 (-13.81 to 106.31)	36.34 (-21.65 to 94.33)	-32.56 (-118.07 to 52.96)	-23.75 (-78.32 to 30.83)
Peak RPE	-0.29 (-1.95 to 1.38)	-0.01 (-0.97 to 0.96)	0.63 (-0.37 to 1.62)	0.38 (-0.52 to 1.28)
Peak RPE median change (median; IQR)	0.00 (0.00)	NA	0.50 (1.00)	NA

***RIC = remote ischaemic conditioning; SD = standard deviation; CI = confidence interval; HR = heart rate; WR = work rate; RPE = rate of perceived exertion; IQR = interquartile range.**

4.6 Discussion

The data produced from this study are invaluable in documenting CPET outcome measures in patients with PSF as well as allowing insights into how RIC may be affecting individuals with fatigue.

4.6.1 Associations between CPET parameters and fatigue severity

In line with previous studies, we did not find a statistically significant association between fatigue severity (FSS-7) and VO_{2peak} (Oyake et al., 2021, Duncan et al., 2012, Tseng et al., 2010, Michael et al., 2006). Although associations between fatigue severity and other CPET parameters were weak and statistically insignificant, the correlations were consistent and theoretically plausible. Lower VO_{2peak} and fitness was associated with higher levels of fatigue (Figure 15) thus suggesting that poor CRF is associated with PSF (Billinger et al., 2014, Lewis et al., 2011, De Doncker et al., 2018).

Duncan et al. (2012) performed a systematic review of observational studies that have reported the association between PSF and cardiorespiratory variables (e.g., VO_{2peak}). Only three studies fulfilled the inclusion criteria and no significant associations between PSF, and any measures of physical fitness were found. One study that met the inclusion criteria was a study by Michael et al. (2006) who investigated the relationship between fatigue (measured using the fatigue severity scale paired with a visual analogue scale) and economy of gait, VO_{2peak} (measured by open circuit spirometry during a graded treadmill test) and ambulatory activity (steps per 24h) in 53 chronic stroke patients. The investigators found no significant differences between fatigued and non-fatigued groups on all three outcome measures. Later the same investigators investigated the association between fatigue severity (fatigue severity scale) and daily step count, economy of gait and VO_{2peak} in 79 chronic stroke patients. Again, no significant associations between fatigue and all measures of physical activity/fitness were found (Michael and Macko, 2007). A larger study by Shaughnessy et al. (2006) found no association between PSF and self-reported exercise behaviour in 312 stroke patients (mean months since stroke 60.2 months). However, these studies were small (Michael et al., 2006, Michael and Macko, 2007) or only measured fatigue by only asking a single question ‘does fatigue influence daily activities’ which required participants to rate on a 5-point Likert scale (Shaughnessy et al., 2006).

Oyake et al. (2021) found that fatigue severity (measured using the FSS) was associated with *time constant* VO_2 kinetics (reflects the ability of the body to adapt from rest to a new steady state of submaximal exercise) but not VO_{2peak} in patients with subacute stroke (Oyake et al., 2021). The investigators highlighted how compared to VO_{2peak}, time constant VO_2 has been shown to be more sensitive to changes in levels of physical activity (Phillips et al., 1995, Hamasaki et al., 2018, Fukuoka et al., 2002) and time constant VO_2 at the onset if submaximal exercise has also been shown to provide objective information on CRF in people with stroke (Tomczak et al., 2008, Manns et al., 2010). Considering PSF is associated with low levels of physical activity after stroke (Thilarajah et al., 2018) it may be more appropriate to assess the relationship between PSF and cardiorespiratory variables that can be measured at submaximal exercise intensities (e.g., VAT, VE/VCO_2 slope).

We found that lower VATs appeared to be associated with greater levels of fatigue – lower VAT may mean earlier switch to anaerobic production of energy and earlier build-up of acids e.g., lactic acid and earlier and greater development of fatigue during physical activity (Ghosh, 2004). Greater VE/VCO_2 slope and RER are associated with disease and lower levels of fitness (Shen et al., 2015, Nakade et al., 2018) and were both positively correlated with fatigue severity although these associations were small. An individual's VAT may be a more specific measure of aerobic capacity post-stroke than VO_{2peak}. A study of 59 chronic stroke patients by Boyne et al. (2017a) investigated the influence of motor function (comfortable gait speed [CGS], lower extremity Fugl-Meyer [LEFM]) on aerobic capacity measures including VO_{2peak} and VAT. The investigators found that the inter-rater reliability of VAT was high (inter-class correlation: 0.93, 95% CI: 0.89 to 0.96) and that VAT was less distorted by post-stroke motor function than VO_{2peak} (Boyne et al., 2017a). These results suggest VAT which can be obtained at submaximal exercise may provide a more specific assessment of CRF in the stroke population.

The mean VO_{2peak} in the treatment and sham group in our study is higher than several studies investigating CRF in stroke (Mustafa and Aytür, 2021, Eng et al., 2004, Chen et al., 2010, Yates et al., 2004, Teixeira da Cunha Filho et al., 2001, Han et al., 2021), demonstrating higher fitness levels in our patient sample. Reviews on studies investigating CRF in stroke have found that VO_{2peak} ranged from 8 to 22 mL/kg/min (Smith et al., 2012) . This is 26% to 87% of normative values (Smith et al., 2012), and indicates how aerobic deconditioning after stroke is a primary cause of disability that severely limits performance of ADLs (Billinger 2012; Ivey

et al. 2005; Billinger 2014). A limitation of assessing maximal exercise responses in stroke is that untrained patients with stroke often do not reach the limits of their cardiopulmonary system during CPET. This was demonstrated in a study by Marzolini et al. (2012) whereby 98 chronic stroke patients underwent CPET at baseline and after 6 months of exercise training (90-min exercise classes involving aerobic and resistance training once per week). At baseline, only 68.4% of CPETs provided sufficient information (subject either reached $VO2_{max,}$ VAT or a clinically relevant abnormality occurred) for the researchers to develop a safe and effective exercise prescription. However, this was increased to 84.7% after 6-months of exercise training (Marzolini et al., 2012). Higher mean VO_{2peak} in our study may be related to time since stroke and the fact that all participants were in the chronic stage of recovery (11 months to 5-years post-stroke). A study of 63 patients with chronic stroke (average post-stroke interval 5.5 ± 4.9 years) by Pang et al. (2005a) found that mean VO_{2peak} measured using a cycle ergometer was 22 ± 4.8 ml/kg/min which is similar to that observed our study. In studies of patients in the sub-acute phase after stroke, VO_{2peak} values range between 12 and 18 mL/kg/min, which is lower than 60% of healthy age-and gender-matched individuals (MacKay-Lyons and Makrides, 2002, Baert et al., 2012, Chen et al., 2010). A study by Boss et al. (2017) of patients with recent minor ischaemic stroke or TIA supports the relationship between VO_{2peak} and stroke severity. In their study, mean VO_{2peak} was 22 mL/kg/min (SD=6) (Boss et al., 2017). Although this is poor compared to healthy controls (Smith et al., 2012, Aspenes et al., 2011), it demonstrates how VO_{2peak} is higher in people with mild stroke and better neurological function. Similar VO_{2peak} in our study to Boss et al. (2017) suggests mild stroke severity in our participant sample, which is reflected by almost 80% of participants scoring as independent (score 0-2) on the mRS at baseline.

4.6.2 Associations between CPET parameters AND 6MWT

VO_{2peak} was significantly and strongly correlated with 6MWT, a reliable marker of physical function in stroke (Kosak and Smith, 2005, Dunn et al., 2015, Fulk et al., 2008). This association has been well documented previously (Kelly et al., 2003, Dunn et al., 2019, Patterson et al., 2007, Carvalho et al., 2008) and suggest VO_{2peak} may be a valid biomarker for physical fitness after stroke. A study by Dunn et al. (2019) explored whether the 6MWT or incremental shuttle walk test (SWT) (participant asked to walk up and down a 10 metre course at different speeds until exhaustion) are appropriate indicators of CRF in patients with stroke.

Twenty-three stroke patients within one-year post-stroke performed a graded exercise test on a cycle ergometer, the 6MWT, and SWT and VO_{2peak} and peak HR were recorded. A portable open-circuit spirometry system was used to collect breath-by-breath data and measure VO_{2peak} throughout the three exercise tests. The researchers found no significant differences in mean VO_{2peak} among the three exercise tests (min-max: 17.08-18.09 mL $kg^{-1}min^{-1}$, $P>0.05$). However, peak HR was significantly lower during the 6MWT. There was a strong relationship between VO_{2peak} and performance measures within each test (cycle ergometer VO_{2peak} and workload: $r = 0.77$, 6MWT VO_{2peak} and 6MWT distance: $r=0.73$, SWT VO_{2peak} and shuttles: $r = 0.73$). Although our study did not measure VO_{2peak} during the 6MWT, there was an association between CPET VO_{2peak} and distance walked during the 6MWT. These findings suggest the 6MWT and the SWT may be clinically useful as proxy measures of CRF when CPET using a cycle or treadmill ergometer is not possible. VAT was also positively correlated with waking distances, both associations reflect increased functional abilities in those with increased levels of fitness. Fitting this theme, the RER was inversely associated with 6MWT. However, there was no apparent association between 6MWT and VE/CO_2 slope.

4.6.3 Effect of RIC on CPET parameters

4.6.3.1 VO_{2peak}

VO_{2peak} improved insignificantly in both groups suggesting a small increase in CRF, however this was not a clinically meaningful difference (Brazzelli et al., 2011, Marsden et al., 2013). We may not have seen an improvement in VO_{2peak} in the treatment group compared to the sham group because effort level may not have been as good as the sham group at 6-week follow-up suggested by a reduction in peak WR, HR and test duration in the treatment group *(Tables 29 and 30).* Although this was not supported when we compared the number of participants meeting test effort criteria as laid out by Nichols et al. (2015) (*Table 19*) (8 participants in the treatment group met the criteria for maximal effort at 6-weeks vs 5 participants in the sham). Thus, it may be related to the fact participants in our study were relatively fit which is why studies on the effects of RIC on CRF and exercise performance in healthy volunteers and athletes are so mixed (Caru et al., 2019) (*Table 4).*

Mean pre- and post- intervention weight was higher in the treatment group compared to the sham group (*Table 20)*. Studies have shown how increased weight is associated with lower VO_{2peak} (Horwich et al., 2009, Vargas et al., 2018). Despite this, mean VO_{2peak} was higher in the treatment group compared to the sham at baseline (22.16 mL/kg/min vs 21.44 mL/kg/min, respectively) and at 6-weeks (22.88 mL/kg/min vs 21.88 mL/kg/min). Participants in the treatment group could also exercise for longer compared to the sham group at baseline (test duration 797.78s vs 657.25s, respectively) and at 6-weeks (test duration 765.22s vs 693.50s) (*Table 29*). These findings demonstrate greater overall fitness and ability to tolerate higher exercise intensity in the treatment group compared to the sham at both time points.

A study of 19 individuals with CVD by Maxwell et al. (2021) investigated the effects of either repeated upper limb RIC for 8-weeks (4 x 5 min cuff inflation to 220 mmHg) or 8-weeks of RIC and exercise (three 50-min exercise sessions per week for 8-weeks on a cycle ergometer). Cardiorespiratory fitness (VO_{2peak}) was measured using a treadmill ergometer at baseline and at 8-weeks. The investigators found that there was a greater improvement in VO_{2peak} in the RIC + exercise group compared to RIC alone (0.1ml/kg/min (-1.0, 1.4) vs 2.8ml/kg/min (1.7, 3.9)), however this did not reach statistical significance. Thus, it may be that to produce a marked effect on VO_{2peak} one would need to pair RIC with CRF training.

There are several reasons why we may not be seeing significant differences. Firstly, the study is small and not powered to detect significant differences in the outcome measures. Secondly, the dose of RIC may not be high enough. In the study by Maxwell et al. (2021) the cuff was inflated to a higher pressure (220 mmHg) and for a longer period of time (8-weeks). Furthermore, VO_{2peak} may not be the most appropriate measure of CRF in patients with stroke as it requires maximal effort which may be difficult for some stroke survivors to achieve as we have seen in our study.

4.6.3.2 Ventilatory anaerobic threshold

The data shows a trend towards increased VAT in the RIC group compared to a reduced VAT in the sham group. Although the change was small, an increase in VAT indicates that the exercise intensity a participant can sustain without producing lactic acid increases (Wasserman et al., 1973). Lactate is produced by active muscles and is the end-product of anaerobic metabolism and is a determinant of exercise intensity (Pennington, 2015). The VAT reflects

the point lactate production by active muscles exceeds the ability of tissues to remove it from the blood and it cannot be volitionally influenced by the participant (Pennington, 2015). The VAT is independent of patient motivation and can be determined from submaximal exercise testing (Ghosh, 2004, Arena et al., 2007b). Many activities of daily living (ADLs) are performed below or near the VAT. Therefore, although improvements in VO_{2peak} may be important for post-stroke prognostic outcome (Swank et al., 2012, Ehlken et al., 2016, Vanhees et al., 1995), it may be more clinically relevant to delay lactate accumulation (Marcinik et al., 1991, Boyne et al., 2017a).

The trend towards an improvement in VAT observed in the treatment group compared to the sham may be the result of improved skeletal muscle oxidative metabolism (Andreas et al., 2011a) and increased skeletal muscle blood flow (Jeffries et al., 2018a) observed after RIC. Andreas et al. (2011a) found that a single dose of RIC administered to the lower limb (3 x 5 min) before 20-min ischaemia significantly improved maximal phosphocreatine (serves as a skeletal muscle energy store) and blood-oxygen-level-dependent (BOLD) signals during reperfusion (discussed in section 1.8.5.2). Similarly, Jeffries et al. (2018a) found that repeated doses of RIC for 7 days increased skeletal muscle oxidative capacity (measured using near infrared spectroscopy) and improved microvascular oxygenated blood flow to the lower limbs. Improved mitochondrial resilience to states of ischaemia or energy deficiency after RIC may also play a role in these effects (Ramachandra et al., 2020, Slagsvold et al., 2014) (discussed in Chapter 5).

Studies show how exercise training can increase the ventilatory anaerobic threshold. For example, Denis et al. (1982) found that 40 weeks of endurance training (bicycle ergometer exercise 1 hour per day three times weekly) in five healthy subjects significantly increased their ventilatory anaerobic threshold by 10%. VAT determination is not always possible (Balady et al., 2010a) particularly in people with heart failure (Myers et al., 2010) or subjects with periodic breathing patterns (Kaczmarek et al., 2019). Preclinical studies that have compared RIC and exercise in rat models of stroke have shown how RIC is equally, or superiorly effective in inducing neuroprotection in rats compared to exercise (Geng et al., 2021). Long-term RIC has been shown to mimic the effects of exercise and they may share common underlying mechanisms including: enhanced antioxidant activity, increased adenosine triphosphate-sensitive potassium (K_{ATP}) channels function, increased heat-shock proteins (HSP), production of autocoids (e.g., opioids, adenosine, bradykinin) and modulation of immune and

inflammatory responses (Zhao et al., 2018b). Long-term RIC may enrich the modes of exercise and benefit unhealthy individuals with severe diseases and those who are unable to exercise because of disabilities or history of cardiac or pulmonary diseases (Thompson et al., 2007). Compared to regular exercise, RIC is safer and more convenient and can be applied safely in the elderly without the concern of serious adverse events or injury (Meng et al., 2015a). Furthermore, RIC can be incorporated regularly into an individual's lifestyle and would free up additional time for work or leisure activities. As discussed previously, RIC can be applied safely in acute stroke patients where it can play protective roles immediately (Hougaard et al., 2014). This may include preventing PSF if found to be effective. Another potential commonality through which RIC is likened to exercise may be an increase in the ventilatory anaerobic threshold by improving efficiency of energy production (Denis et al., 1982, Gaskill et al., 2001) which we see a trend towards in this study. However, larger studies will need to be conducted to confirm whether this is a true effect.

4.6.3.3 VE/VCO_2 slope

The data shows a slight reduction in the VE/VCO_2 slope in the treatment group and an increase in the sham group at 6-weeks. The reduction in the VE/VCO_2 in the treatment group suggests a trend towards improved fitness levels and ventilatory efficiency in the treatment group compared to the sham (Lewis et al., 2008, Meyer et al., 1996). The response of V'_E relative to $V'CO_2$ during incremental exercise reflects an individual's ventilatory efficiency (Forster and Pan, 1988) and has prognostic value in several disease states (Arena et al., 2007c, Baba et al., 1996, Tang et al., 2017, Shen et al., 2015). Elevation of the VE/VCO_2 slope is common in cardiac and respiratory diseases and indicates decreased $PaCO_2$ set point (partial pressure of carbon dioxide in arterial blood) and/or increased dead space (inhaled air that does not take part in gas exchange) (Sun et al., 2001, Arena et al., 2010, Weatherald et al., 2021). It is well established that exercise training in cardiac patients improves VE/VCO_2 slope by 6-23% (Fu and Wang, 2011, Gademan et al., 2008, Tomita et al., 2003, Arena et al., 2007a, Prado et al., 2016). For example, Gademan et al. (2008) found that exercise training improved the VE/VCO_2 slope by 14% (VE/VCO_2 slope pre-exercise, 35.8 ± 3.9 vs post-training 31.0 ± 6.1 units) in patients with chronic heart failure. Similarly, Meyer et al. (1996) found that 3-weeks of exercise training in patients with severe chronic heart failure had a 14.6% decrease in their VE/VCO_2 slope demonstrating an improvement in ventilatory efficiency. Postulated mechanisms for

reduced ventilatory efficiency include reduced oxidative metabolism, increased anaerobic glycolysis and pulmonary vasoconstriction (Prado et al., 2016). The slight improvement in VE/VCO_2 slope in the treatment group compared to the sham may be the result of the vasodilatory effects of humoral mediators released in response to the conditioning stimulus (e.g., adenosine, bradykinin) (Liem et al., 2002, Sharma et al., 2015), increased tissue perfusion (Jeffries et al., 2018a, Meng et al., 2012) and preserved mitochondrial respiration (Slagsvold et al., 2014) after RIC. However, the study is small and needs to be repeated in larger populations of PSF. A study by Kim et al. (2019a) investigated the effects of upper limb RIC (4 x 5 min cuff inflation to 200 mmHg) or control (cuff inflated to 20 mmHg) on pulmonary arterial pressure and gas exchange during hypoxic (12.5% O_2) exercise (exercise protocol performed on supine bicycle ergometer) in 16 healthy adults. The investigators found a significant improvement in ventilatory efficiency (VE/VCO2), pulmonary arterial systolic pressure and mean pulmonary arterial pressure, after RIC.

The research and clinical focus has mostly been on the measurement of VO_{2peak} to assess CRF in clinical populations. However, VO_{2peak} relies heavily on maximal effort and motivation from the participant and does not capture all the characteristics associated with CRF (Ross et al., 2016). Unlike VO_{2peak}, the VE/VCO_2 slope (like the ventilatory anaerobic threshold) is independent of patient effort and can be derived from submaximal exercise (Belardinelli et al., 1995, Balady et al., 2010b). The VE/VCO_2 slope has been shown to be superior to the VO_{2peak} in several studies (Arena et al., 2004, Kleber et al., 2000, Chua et al., 1997). It more accurately quantifies the efficacy of pharmacological, surgical and lifestyle interventions (Balady et al., 2010a, Ross et al., 2016). This could be why we found a trend towards beneficial changes in VAT and VE/VCO_2 but not VO_{2peak} in the treatment group.

4.7 Strengths and limitations

Due to the heterogeneity of studies in terms of protocol used (e.g., treadmill, bicycle), stroke severity and duration, there is no consensus on the protocol that should be utilized for optimal VO_{2peak} measurement in people with stroke (Wittink et al., 2017, van de Port et al., 2015). Although bicycle ergometer CPET has been preferred in subacute and acute hemiplegic patients (Yates et al., 2004, Kelly et al., 2003, Tang et al., 2006, Chen et al., 2010), some studies suggest CPET using a treadmill rather than a cycle ergometer may be more appropriate and leads to greater VO_{2peak} measurement. For example, a study by Mustafa and Aytür et al. (2021)

compared CPET using a treadmill ($CPET_{tread}$) and cycle ($CPET_{bic}$) ergometer in 38 patients with stroke and 22 healthy controls. In stroke patients, mean VO_{2peak} measured by $CPET_{tread}$ was significantly higher than that measured by $CPET_{bic}$ (14.5 ± 3.7 ml/kg/min vs 12.6 ± 2.9 ml/kg/min respectively, P<0.001). This was also observed in healthy volunteers (23.5 ± 4.2 ml/kg/min in $CPET_{tread}$ vs 16.7 ± 4.0 ml/kg/min in $CPET_{bic}$, P<0.001). The investigators also analysed VO_{2peak} at the VAT and found it was significantly lower in $CPET_{bic}$ compared to $CPET_{tread}$ in the stroke population (9.6 ± 2.2 ml/kg/min vs 11.6 ± 2.9 ml/kg/min, respectively, P<0.000). A study of chronic stroke patients by Eng et al. (2004) found that the VO_{2peak} measured by cycle ergometer was higher than VO_{2peak} measured by treadmill (14.0 ml/kg/min vs 13.3 ml/kg/min, respectively), however the findings of two tests were not statistically compared so the investigators did not comment on which protocol would be the most suitable in stroke. Intensity of the treadmill test was increased more rapidly in study by Eng et al. (2004) compared to Mustafa and Aytür et al. (2021) (treadmill slope increased to 6% at the end of the first minute compared to the end of the sixth minute, respectively) which may have resulted in early fatigue and lower VO_{2peak} in the study by Eng et al. (2004).

Walking is a natural activity and more familiar than cycling (Albouaini et al., 2007). Local muscle fatigue during cycling may result in participants reaching VAT earlier and lower VO_{2peak} in people with stroke and those not accustomed to cycling (Mustafa and Aytür, 2021). However, the cycle ergometer is usually safer, cheaper, less noisy and occupies less space than a treadmill (Fletcher et al., 2013, Mezzani, 2017c). Cycling also eliminates gravity and is possible in a supine position (Dillon et al., 2020). Therefore, although VO_{2peak} values may be higher using a treadmill, cycle ergometry is more appropriate and accessible to determine VO_{2peak} in clinical and research settings.

A limitation of the cycle ergometer is discomfort of the bike. In our study, some participants thought the seat was uncomfortable and struggled to hold both handles due to stroke-specific muscle weakness on one side. This made it difficult for participants who were more physically impaired to balance themselves on the bike. Another limitation is fatigue of the quadriceps muscles which can cause premature termination of the test before reaching a true VO_{2peak} (Fletcher et al., 2013). Most participants in our study terminated the test early due to leg fatigue/loss of performance. Other reasons for test termination included breathlessness, issues with hemiparesis, and spasticity. The participant who stopped the test due to spasticity was excluded from analyses due to very premature test termination making the test invalid. Reasons

for test termination is in line with previous studies that have used CPET in people with stroke (Koseoglu et al., 2006, Tang et al., 2013). Participants with severe weakness found it difficult to maintain a consistent peddling cadence, particularly further along the test when the resistance was high. However, the researcher and technician used all available methods to help facilitate completion of the tests (e.g., mild verbal encouragement) and the test effort data shows that this worked well.

The VAT was determined by two individuals experienced in CPET and was checked by the study chief investigator and PhD researcher. Having two or three experienced, independent observers to perform the VAT calculation will increase confidence in VAT values (Balady et al., 2010a). Issues with inter- and intraobserver variability aside, determination of VAT by respiratory measurements and the V-slope method is safe, objective and accurate method to assess aerobic capacity (Matsumura et al., 1983).

One limitation of this study relates to the use of a facemask to collect respiratory parameters. Although an effort was made to ensure the facemask was tightly fitted around the participant's face, there is a chance of gas leakage from the side of the mask particularly at high exercise intensities (Radtke et al., 2019). This can lead to inaccurate respiratory measurements and dead space (V_D) (ventilated air that does not participate in gas exchange) (Radtke et al., 2019). The gold standard for measuring respiratory data in CPET is a bite-block mouthpiece combined with a nose clip (Radtke et al., 2019). However, the mouthpiece/nose-clip combination can be uncomfortable as it makes swallowing difficult, irritates the throat and causes dry-mouth (Bell et al., 2012) which can lead to altered breathing patterns during CPET (Amis et al., 1999). Other criticisms of the mouthpiece include jaw pain, sensation of choking and embarrassment due to saliva dripping from mouth (Radtke et al., 2019). In our study, a few participants thought the facemask was uncomfortable/suffocating and felt it hindered their performance. The facemask also made it difficult for participants who were more impaired to communicate their RPE score during the test. Participants were advised not to talk whilst wearing the mask and were asked to point at the RPE sheet every minute. This was difficult for participants who needed to balance themselves on the bike using one hand. However, the facemask allows swallowing as well as oral and nasal breathing (Kelly and Dawes, 2013) and is associated with higher peak power attainment during CPET in healthy volunteers (Bell et al., 2012). It is important that the participant is as comfortable as possible so they can exercise for longer

without needing to end the test prematurely. This will allow VO_{2peak} to be accurately assessed. A survey of 34 patients with chronic lung disease who had experience of using both a facemask and a mouthpiece during CPET found that 10 participants preferred a facemask, 12 preferred the mouthpiece and 12 had no preference (Radtke et al., 2019). These findings demonstrate how CPET using both pieces of equipment are acceptable. In a future, definite trial giving participants the option of either a facemask or mouthpiece and explaining the advantages and disadvantages of each might improve performance (i.e., more likely to reach maximal exertion) and accuracy of respiratory measurements (Radtke et al., 2019, Baran et al., 2001).

Another limitation of the study relates to the accuracy of the Polar chest strap HR monitor. On a few occasions during the exercise test the monitor failed to detect a HR and the strap had to be readjusted. For two participants, baseline HR data was unavailable. HR data is important for the assessment of maximal effort (Nichols et al., 2015) (*Table 19*). HR data was unavailable for one of the participants who we concluded did not meet the criteria for maximal effort at baseline. If we had their HR data, they might have met these criteria. However, in this instance it was difficult to know.

Inability to always ensure standardisation of the time-of-day participants had their baseline and follow-up CPETs was another limitation. An effort was made to arrange follow-up tests for a similar time to baseline CPETs. However, due to availability of the exercise lab and covid-19 restrictions this was not always possible. Studies have shown how RER is significantly influenced by diurnal factors (Decato et al., 2018).

4.8 Conclusion

VO_{2peak}, VAT, VE/VCO_2 slope and RER did not demonstrate statistically significant correlations with PSF but appear to be promising biomarkers. Despite data to suggest the effort imparted during the follow up test in the RIC group was marginally less, we still found trends towards slightly improved VO_{2peak} levels in both groups, but disproportionate improvements in VAT and VE/VCO_2 slopes in the RIC group. This could suggest that improvements in ventilation efficiency and how long tissues can respire aerobically during exercise are due to RIC, potentially due to effects exerted at a cellular level, in the mitochondria, or potentially

due to improved tissue (muscle) perfusion. Further investigation to validate such biomarkers and evaluate mechanisms involved in RIC and in PSF are warranted.

CHAPTER 5. PHOSPHORUS 31-MAGNETIC RESONANCE SPECTROSCOPY TO ASSESS SKELETAL MUSCLE BIOENERGETICS IN POST-STROKE FATIGUE

5.1 Introduction

The experiments reported in this chapter aimed to explore potential mechanisms underlying the effect of RIC on fatigue by non-invasively measuring phosphate metabolites involved in bioenergetics in the skeletal muscle of stroke patients using Phosphorous-31 Magnetic Resonance Spectroscopy (^{31}P-MRS). Our collaborators in Sheffield have recently developed a protocol that successfully investigated lower limb bioenergetics using ^{31}P-MRS in patients with motor neurone disease (MND) (Sassani et al., 2020). Our study employed this spectroscopic protocol and is the first of its kind to use ^{31}P-MRS *in vivo* to investigate indices of mitochondrial oxidative capacity in stroke patients with debilitating fatigue.

In previous chapters, we have shown that RIC may alleviate fatigue severity in patients with PSF. This is independent of confounding factors (such as mood, cognition, or function) and seems to be associated with improvements in walking distance/speed. While cardiopulmonary exercise testing did not demonstrate this to be due to clear beneficial trends in CRF (no change to $VO_{2peak)}$, the ventilatory anaerobic threshold (VAT) seemed to increase suggesting tissues and cells in the body can create energy by aerobic mechanisms for longer in the treatment group compared to the sham. These findings were purely exploratory, but are plausible as they may be consistent with those beneficial mechanisms postulated to be activated by RIC.

We elaborated on the effects of RIC on mitochondrial function in other populations preclinically (Wu et al., 2011b, Konstantinov et al., 2005, Moses et al., 2005, Leung et al., 2014, Mansour et al., 2012, Thaveau et al., 2007) and clinically (Slagsvold et al., 2014) in previous chapters. Evidence comes primarily from experimental models and conclusive data *in vivo* in patients is lacking. As mentioned in Chapter one, it has been proposed that fatigue following stroke may occur because of an imbalance between energy production and demand during exertion and underlying alterations in bioenergetics (Klinedinst et al 2019). Alterations

in the way cellular energy is created in response to RIC may help us understand why it may be a useful treatment in PSF and may also help explain why some studies have found improvements in exercise performance, muscle strength and fatigability after RIC (Durand et al., 2019, Barbosa et al., 2015a, De Groot et al., 2009, Jean-St-Michel et al., 2011, Hyngstrom et al., 2018).

ATP is comprised of adenosine (consists of adenine attached to a ribose sugar) bound to three phosphate groups (alpha, beta and gamma) held together by phosphoanhydride bonds that, when broken, can release free energy. The products of such bond breaking (i.e., hydrolysis reaction) are inorganic phosphate (Pi) and adenosine diphosphate (ADP). ATP is the main energy currency of the cell and its concentration is regulated to meet energy demand. In skeletal muscle, ATP synthesis is associated with the creatine kinase reaction, which requires phosphocreatine (PCr), acting as a store to quickly allow synthesis of ATP at times of rapidly increasing energy demand (e.g., during muscle contraction). Hence, it is important that PCr is rapidly resynthesized after physical activity (Hargreaves and Spriet, 2020). Increased PCr availability and appropriate ATP synthesis is important for exercise performance (Hargreaves and Spriet, 2020). During skeletal muscle contraction PCr and glycogen are broken down and there are marked increases in Pi, ADP and lactate which have been associated with skeletal muscle fatigue at various steps within the excitation-contraction pathway (Allen et al., 2008).

ATP can be produced aerobically (via mitochondrial oxidative phosphorylation) or anaerobically (exclusively via glycolysis) (Horscroft and Murray, 2014). Anaerobic glycolysis is less efficient and generates fewer ATP molecules per unit of substrate, in addition, it generates lactate which contributes to fatigue. Aerobic metabolism is required during endurance activities from running and cycling to walking for long periods (Hargreaves and Spriet, 2020).

The effects of RIC are thought to converge on the mitochondria and initiate a number of signalling pathways that may either directly or indirectly make the mitochondria more resilient to states of ischaemia or energy deficiency. Those most widely described in the literature are highlighted in *Table 31*.

Table 31. Proposed signalling pathways initiated by RIC that may directly or indirectly act on the mitochondria.

Signalling pathway	Function of the pathway
Survival activating factor enhancement (SAFE) pathway	• Pro-survival metabolic pathway activated by tumor necrosis factor-alpha (TNF-α) (pro-inflammatory mediator that also has protective properties). • SAFE pathway involves the activation of *signal transducer and activator of transcription 3 (STAT3)* in mitochondria. • STAT3 is a transcription factor and antioxidant defence protein which modulates apoptosis, inflammation and reduces oxidative stress. • STAT3 mitigates production of ROS from complex 1 of the mitochondrial membrane. • STAT3 also targets the inhibition of mitochondrial permeability transition pore (mPTP) opening. Opening of mPTP mediates cell death via dissipation of the mitochondrial membrane potential, mitochondrial swelling and increased ROS production. Thus, inhibition of mPTP opening after RIC promotes mitochondrial survival (discussed in Chapter one).
Reperfusion injury salvage kinase (RISK) pathway	• Pro-survival pathway recruited during reperfusion. • Involves the recruitment of pro-survival kinases (e.g., PI3K/Akt and Erk1/2) and exerts anti-apoptotic protective effects. • Protects against ischaemia/reperfusion injury via inhibition of mPTP opening.
PINK/Parkin mitophagy pathway	• During ischaemic stress, Parkin (amino acid) translocates to damaged mitochondria in a PINK1 (mitochondrial protein) dependant manner. • Parkin binds to damaged mitochondria to induce mitophagy. • Mitophagy is the selective degradation and removal of damaged mitochondria by autophagy. This is important for mitochondrial quality control and to maintain cellular homeostasis. • Mitophagy reduces production of ROS and leaves behind healthy mitochondria better equipped to resist ischaemic stress.

Uncoupling proteins (UCP)	• Proteins that ‘’short circuit’’ oxidative phosphorylation i.e., dissipate the proton gradient and reduce ATP synthesis. • Upregulation and downregulation of UCP after RIC may have protective effects. • Upregulation (*mild* mitochondrial uncoupling): reduces mitochondrial membrane potential and ROS production (antioxidant defences). Note: severe uncoupling can lead to energy deficits. • Downregulation (mitochondrial coupling): increases membrane potential, efficiency of substrate utilization and ATP synthesis.
Nitric oxide (NO)-cGMP/PKG pathway	• NO activates cyclic guanosine monophosphate (cGMP) which binds to and activates protein kinase G (PKG) (protein kinase that phosphorylates several biologically important targets and is implicated in the regulation of platelet function, cell division and smooth muscle relaxation). • The nitric oxide (NO)-cGMP/PKG pathway mediates vasodilation of smooth muscle cells, lowers blood pressure and has a cardioprotective effect. • PKG activation also induces opening of mitoK_{ATP} channels in the inner mitochondrial membrane which leads to inhibition of mPTP. This plays a role in the development of ischaemic tolerance by enhancing mitochondrial respiration, reducing ROS production, modulating Ca^{2+} production, and reducing ATP hydrolysis rate thus reducing ATP depletion during ischaemia.

Source: (Hadebe et al., 2018, Huang et al., 2011, Hausenloy et al., 2005, Park et al., 2018, Thompson et al., 2014, Heusch et al., 2010) Abbreviations: STAT3 = Signal Transducers and Activators of Transcription, SAFE = Survival activating factor enhancement pathway; ROS = reactive oxygen species, mPTP = mitochondrial permeability transition pore, phosphatidylino-sitol 4,5-bisphosphate 3-kinase (PI3K), UCP = Uncoupling proteins

5.1.1 Methods for measuring skeletal muscle mitochondrial function

Both *in vivo* (research done within a whole, living organism, e.g., a human) and *in vitro* (research performed outside a living organism, e.g., cells) methodologies exist for the assessment of mitochondrial bioenergetic function in skeletal muscle. An example of an *in vitro* method includes using a luminometer (bioluminescent approach) to measure light emission in isolated mitochondria from samples of human muscle to quantify maximal rates of ATP production (Wibom and Hultman, 1990, Lanza and Nair, 2009). An example of another *in vitro* method is high-resolution respirometry (polarographic approach) to measure oxygen consumption and ATP synthesis in small skeletal muscle needle biopsies (Lanza and Nair, 2009, Djafarzadeh and Jakob, 2017). *In vitro* analyses of isolated mitochondria provide an insight into the mechanisms of the organelles. However, they involve measuring the activity of mitochondria in artificial environments (e.g., in solution) and only look at small tissue samples (Harper et al., 2021). In addition, muscle biopsies can be invasive and require local anaesthesia (Bourgeois and Tarnopolsky, 2004, Debigare et al., 2003, Maltais et al., 1996). Other limitations include accessibility of muscle biopsy and the requirement of careful and extensive sample preparation to avoid damaging the sample (Acin-Perez et al., 2021). These limitations make these methods challenging and unfeasible on a large scale.

A technique used to measure mitochondrial function non-invasively *in vivo* includes near-infrared spectroscopy (NIRS) which measures mitochondrial respiratory capacity and corresponds to the high-resolution respirometry in skeletal muscle biopsies (Ryan et al., 2014). NIRS monitors tissue oxygenation by utilizing the oxygen-dependant absorption of near-infrared light by oxy- and deoxy-heme groups (i.e., haemoglobin, myoglobin, and mitochondrial cytochrome C) (Jöbsis, 1977, Lanza and Nair, 2010). NIRS assesses mitochondrial respiratory capacity by measuring the recovery kinetics of muscle oxygen consumption ($m\dot{V}O_2$) (Ryan et al., 2012, Ryan et al., 2013). However, a limitation of NIRS is the difficulty differentiating multiple heme-containing metabolites (Boushel et al., 2001, Lai et al., 2009).

5.1.2 31-Magnetic Resonance Spectroscopy

An *in vivo* technique that indirectly measures mitochondrial function is Phosphorus-31 Magnetic Resonance Spectroscopy (^{31}P-MRS). ^{31}P-MRS is a non-invasive technique used to assess tissue metabolism (Liu et al., 2017). It can detect phosphorus containing metabolites involved in ATP synthesis and utilization (Liu et al., 2017), and is the modality of choice to assess mitochondrial function in humans (Chance et al., 1981, Ernst et al., 1993, Liu et al., 2017). ^{31}P-MRS has been used to assess skeletal muscle energetics in healthy (Edwards et al., 2012) and clinical populations (e.g., motor neurone disease) (Sassani et al., 2020) and has been found to be reproducible (i.e., the ability of different researchers to obtain the same results from an experiment using ^{31}P-MRS) (Layec et al., 2009, McCully et al., 2009). It allows quantification of ATP, phosphocreatine (PCr), adenosine diphosphate (ADP) and inorganic phosphate (Pi) (Klemm et al., 1998). ^{31}P-MRS has been used evaluate phosphate metabolism in brain and muscle in clinical populations during rest and exercise (Sassani et al., 2020), and offers a unique window into tissue metabolism. We chose to focus on ATP, PCr and Pi because these are the most commonly investigated metabolites amongst studies of clinical populations (e.g., MND, Parkinson's disease) (Sassani et al., 2020, Penn et al., 1995, Hattingen et al., 2009) as well as being the most representative of bioenergetic status of a tissue.

5.2 Aims and hypotheses

Aims

1. Explore whether it was feasible to undertake the ^{31}P-MRS protocol developed in Sheffield in stroke patients with fatigue.

2. Investigate whether indices of mitochondrial oxidative capacity are related to the severity of PSF and physical measures such as 6MWT and maximal isometric voluntary contraction (MIVC) of the anterior tibialis muscle.

3. Investigate whether RIC can alter cellular energetics through its potential effect on mitochondrial function.

Hypotheses

1. Undertaking ^{31}P-MRS in patients with PSF is feasible.

2. ATP in skeletal muscle measured using ^{31}P-MRS is associated with severity of PSF, 6MWT and MVIC.

3. RIC improves mitochondrial function and thus results in increased levels of tissue ATP, increased levels of phosphocreatine and reduced levels of inorganic phosphate compared to sham on longitudinal assessments.

5.3 Methodology

5.3.1 Ethics

Ethical approval for this sub-study was granted on 04/09/2020 (Northwest – Preston REC 18/NW/0401).

5.3.2 Setting

All experiments took place at the Royal Hallamshire hospital, Sheffield and the University of Sheffield Academic Department of Radiology, Royal Hallamshire hospital.

5.3.3 Sample

Eight participants were invited to undergo the ^{31}P-MRS sub-study. They were invited to return after 6 weeks to assess longitudinal changes.

5.3.4 Exclusion criteria

In addition to the exclusion criteria listed and discussed in Chapter two, exclusion criteria for all subjects were:

- Pregnancy
- Respiratory failure impairing ability to lie flat
- MRI contraindications, e.g., cardiac pacemaker or other non-magnetic resonance compatible device such as a metallic foreign body or electronic implant or intracranial aneurysm clips (Appendix 20 and 21).

5.3.5 Study protocol

5.3.5.1 Clinical and muscle force data

Informed consent and demographic and clinical data were obtained during the participant's first study visit. ^{31}P-MRS was the final step in baseline data collection and was completed on a different day to clinical outcome measures, 6MWT and CPET. On the day of the scan, participants first completed an MRI safety questionnaire and request form (Appendix 19 and 20) to ensure they had no contraindications for MRI scanning.

Next, the participant's maximal voluntary isometric contraction (MVIC) of their tibialis anterior (stroke side and non-affected side) was measured using a fixed myometry (Quantitative Muscle Strength Assessment – QMA, Aeverl Medical, Gainessville, GA) and pulley system designed by researchers in Sheffield (Figure 26) (Sassani et al., 2020).

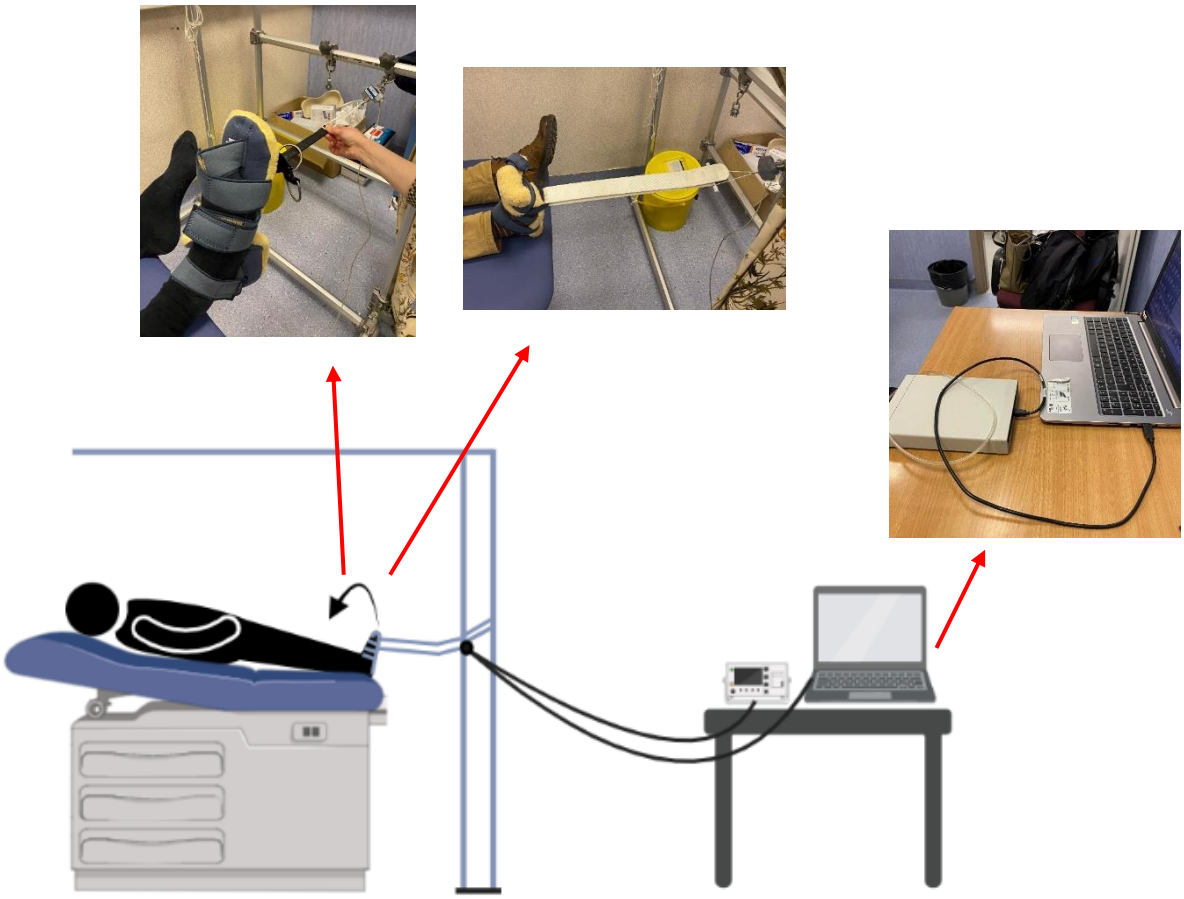


Figure 26. Quantitative Muscle Strength Assessment (QMA) and pulley system for measuring maximal voluntary isometric contraction (MVIC). *Figure created by author of this thesis using Biorender (BioRender).*

Participants were asked to dorsi-flex their ankle using their maximal strength against resistance for 5-seconds. This was repeated three times and the best of the three was taken as the participant's MVIC (kg). This was then repeated for the opposite leg. Muscle force measurements were conducted in a room at the Royal Hallamshire hospital separate to the MRI scanner due to the magnetic nature of the equipment used. The QMA system produces a force-time curve (Figure 27) which clearly shows a ''ramp time'' of approximately one second. This is the time it took for the muscle to initiate contraction from zero to maximal force. Therefore, the participant's MVIC was calculated from the average of the last four seconds of all three trials for consistency.

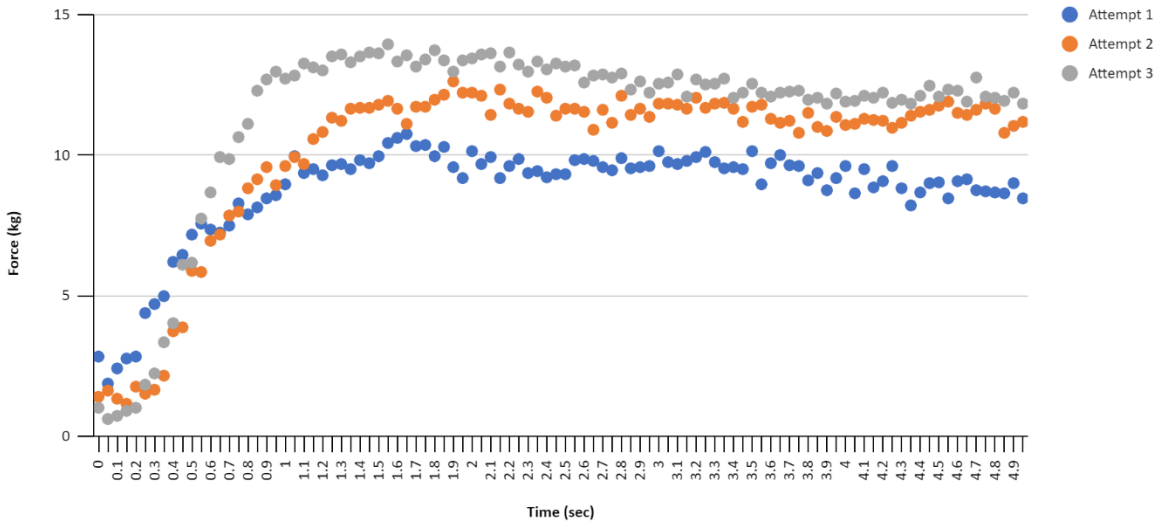


Figure 27. Example of a force-time curve for maximal isometric voluntary contraction of tibialis anterior

5.3.5.2 Magnetic resonance imaging and spectroscopy

5.3.5.2.1 Hardware

For muscle acquisitions, scans were conducted at 3 Telsa (3T) (Philips Ingenia, Philips Healthcare) using a transmit-receive ^{31}P surface coil (Philips Ingenia, Philips Healthcare).

5.3.5.2.2 Sequencing

Spectra were acquired from the proximal portion of the left and right ankle dorsiflexors encompassing tibialis anterior. The tibialis anterior was chosen because analysis of this muscle has been successful in prior studies of ^{31}P-MRS (Sassani et al., 2020) and is sensitive to T_2 weighted signal changes (Jenkins et al., 2018). The MR radiographer placed the top of the coil 2cm below the tibial tuberosity (Figure 28). Positioning of the surface coil was performed by the same radiographer for all subjects and was cross-checked to ensure consistency. This was important as oxidative capacity differs between proximal and distal portions of tibialis anterior (Boss et al., 2018). A pulse-acquire sequence was applied at rest (see figure 27 for a representative muscle spectrum acquired at rest). Muscle spectroscopy acquisition time for each leg was approximately 20 min (~40 min total).

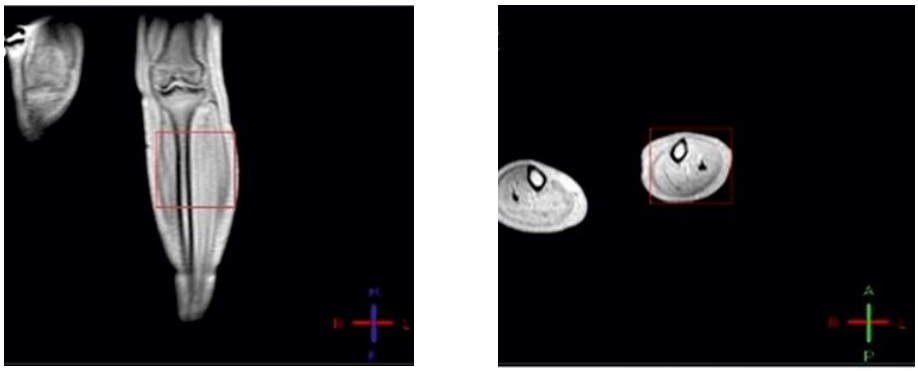


Figure 28. Anatomical localisation of tibialis anterior. *Copyright permissions obtained and available upon request.*

5.3.5.2.3 Spectroscopic data processing

Spectroscopic data processing and was conducted by Dr Matilde Sassani who was blinded to participant status, whilst muscle force measurements, statistical analysis and interpretation of the results was conducted by the author of this thesis. All participants were assigned a study ID number and all data was anonymised at acquisition. Spectroscopic analysis protocol has been published (Sassani et al., 2020), briefly, spectroscopic data processing involved manual phasing and frequency shifting. Signal was then fitted using the AMARES algorithm (available with jMRUI, http://www.jmrui.eu) (Vanhamme et al., 1997, Naressi et al., 2001, Stefan et al., 2009). Resonances were fitted for muscle as shown in Figure 29. Amplitudes were corrected for T_1 relaxation using published values (Meyerspeer et al., 2003, Bogner et al., 2009).

5.3.5.2.4 Reported parameters

For the purpose of this study, we chose to focus on ATP (quantified spectroscopically as the γ phosphate of ATP), phosphocreatine (PCr) and inorganic phosphate (Pi). Spectroscopic results were correlated with fatigue severity (FSS-7) and measures of physical function such as the 6MWT and MVIC.

ATP – ATP is the main energy currency of the cell and the sole fuel for muscle contraction. ATP is required for activity of key enzymes involved in myofilament cross-bridge cycling (myosin ATPase) (the process whereby skeletal muscle filaments actin and myosin which form

the contractile apparatus of skeletal muscle attach to one another), sarcoplasmic reticulum calcium handling (Ca^{2+} ATPase), and membrane excitability (Na^{+}/K^{+} ATPase). Continual supply of ATP is crucial for cellular processes and survival and to maintain normal contractile function.

Phosphocreatine – PCr is found predominantly in skeletal muscle and in brain. It acts as an energy buffer via its ability to regenerate ATP by transferring a high-energy phosphate to ADP. In skeletal muscle, PCr serves as an energy store and is depleted during exercise (Hargreaves and Spriet, 2020). PCr depletion is associated with reduced energy availability and PCr resynthesis relies on the continued production of ATP. Thus, reduced levels of PCr in skeletal muscle may be indicative of impaired mitochondrial metabolism and reduced rates of oxidative ATP synthesis (Kemp et al., 2007).

Inorganic Phosphate – Pi is a by-product of ATP hydrolysis (process by which ATP is broken down to release energy). Pi increases substantially during skeletal muscle fatigue and is associated with reduced force generation (Allen and Trajanovska, 2012). Pi interferes with cross-bridge performance and reduces calcium (Ca^{2+}) release during fatigue (vital for muscle contraction) (Allen and Trajanovska, 2012). Increased Pi in skeletal muscle may be indicative of impaired mitochondrial function.

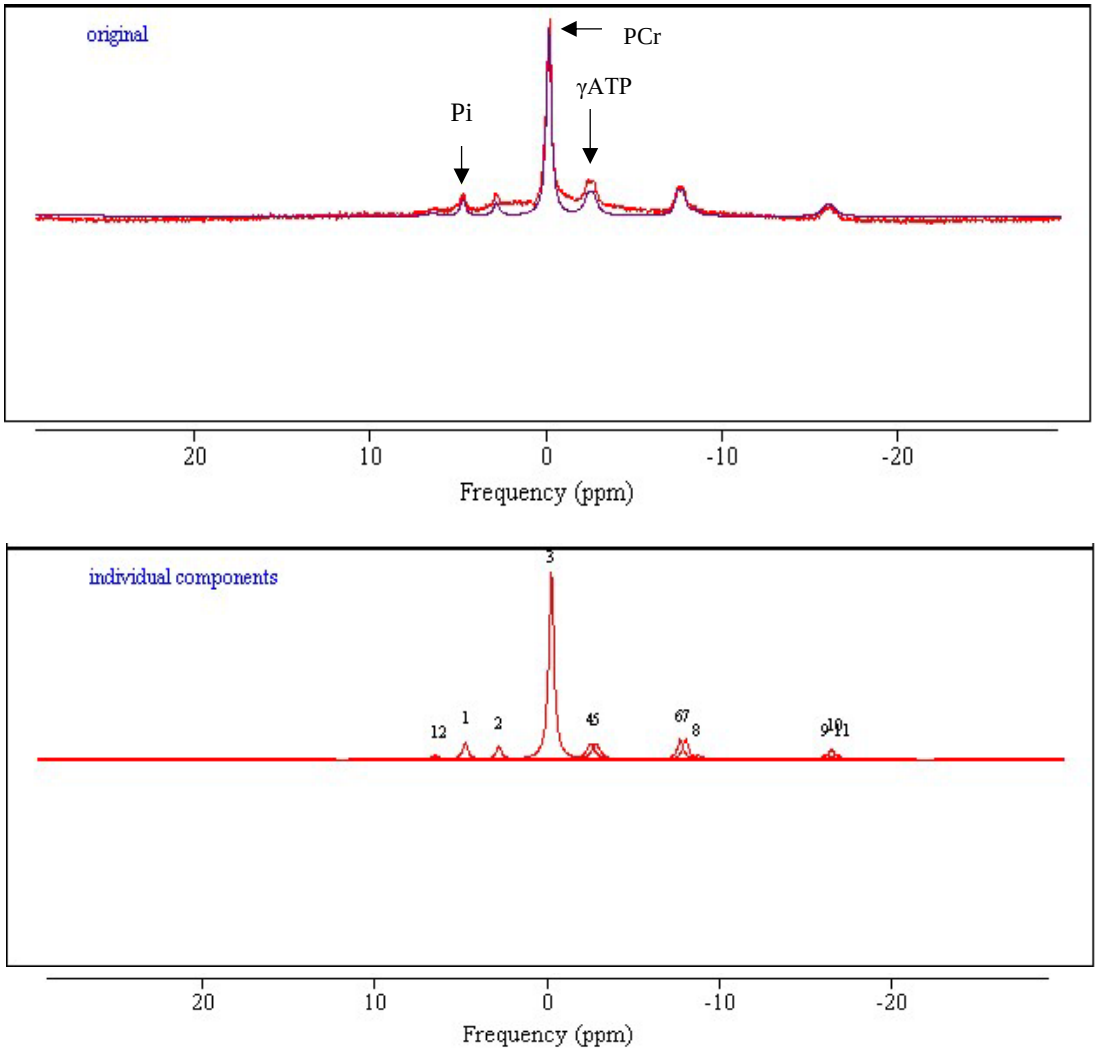


Figure 29. A representative muscle spectrum acquired from a stroke patient's tibialis anterior (stroke affected side) at rest. *(A) illustrates a spectral fitting: in red is the original spectrum and in purple is the estimated spectrum. Resolved peaks are (from left to right): inorganic phosphate (Pi), phosphocreatine (PCr), and gamma resonance of adenosine triphosphate (γATP). In (B) individual resonances are depicted: 1 =Pi, 3 = PCr, and 5 = γ phosphate of ATP.*

5.3.6 Statistical analysis

All data was analyzed using IBM SPSS Statistics (version 26) and GraphPad Prism (version 9). Sample size was small; hence analyses were exploratory. Analysis of the 3 spectroscopic parameters were mainly descriptive however, an exploration of any potential differences between the treatment and sham groups in relation to spectroscopic parameters was completed using a one-way ANCOVA. Like with other outcome measures in previous chapters (clinical, 6MWT, CPET) the participant's baseline scores and mRS were included as co-variates for all spectroscopic parameters. All assumptions for ANCOVA (see Chapter 3 section 3.3 *Statistical analysis)* were tested and satisfied before completion of the analysis. Spectroscopic parameters that did not meet these assumptions were also analysed and reported using a non-parametric Mann-Whitney U Test and were reported accordingly (median and interquartile range, IQR). Spectroscopic parameters were correlated with clinical and physical parameters including fatigue (FSS-7), MVIC and 6MWT. Correlations between variables were explored using a Pearson r correlational analysis and simple scatter plots.

5.4. Results

A total of 7 participants who completed the 6-week intervention (3 treatment, 4 sham) underwent ^{31}P-MRS at baseline and at 6-week follow-up. Demographic and clinical characteristics are summarized in *Table 32.* An additional eighth participant had a baseline MRI scan, however they withdrew from the study and did not have a follow-up scan. All data analyzed and included in the tables are from the 7 participants that had a baseline and follow-up MRI. Participants in the treatment and sham group were similar with respect to time since stroke. Participants in the treatment group were heavier with a mean difference of 21.73 kg, however this difference was not statistically significant (P=0.11). MVIC on the non-affected limb was similar between the two groups, however participants in the sham group were slightly stronger on their stroke affected side compared to the treatment group (MVIC 7.2kg vs 6.4kg, respectively) but this difference is not statistically significant (P=0.40).

Assumptions for a one-way ANCOVA on data for ATP, PCr and Pi on the stroke affected side were sufficiently met for parametric testing. For the non-affected limb, PCr and Pi sufficiently met the assumptions for parametric testing, however ATP was not normally distributed. Therefore, ATP in the non-affected limb was also analysed using a non-parametric Mann-

whitney U test. Due to the exploratory nature of the study, we reported the findings of both tests for completeness.

Table 32. Participant characteristics of participants who underwent [31]P-MRS.

Characteristic	All	Treatment	Sham
n	7	3	4
Age, years (mean; SD)	63.4 (5.9)	59.3 (2.9)	66.5 (5.9)
Sex	4 M: 3 F	1 M: 2 F	3 M: 1 F
Height, cm (mean; SD)	176.7 (6.1)	178.7 (6.0)	175.3 (6.6)
Weight, kg (mean; SD)	86.2 (16.3)	98.6 (11.4)	76.9 (13.2)
BMI (mean; SD)	27.5 (4.3)	30.9 (2.5)	24.9 (3.6)
Time since stroke (months) (mean; SD)	33.6 (19.6)	33.3 (19.7)	33.75 (22.7)
Stroke type (%)			
Ischaemic	85.7%	100.0%	75.0%
Haemorrhagic	14.3%	0.0%	25.0%
MVIC, kg (mean; SD)			
Stroke side	6.8 (3.2)	6.4 (1.8)[7]	7.2 (4.3)[7]
Non-affected side	10.7 (2.0)	10.5 (2.8)	10.8 (1.6)

[*31]P-MRS = [31]Phosphorus magnetic resonance spectroscopy; SD =standard deviation; BMI = Body mass index, MVC = Maximal voluntary isometric contraction. [7]Mann-whitney U test applied and difference in MVIC between the two groups on the stroke affected side is not statistically significant (*P*=0.40).

Resting spectroscopic parameters

ATP

ATP content of the tibialis anterior in all participants appeared greater in the non-affected limb compared to the stroke affected limb (Figure 30). Scatter plots suggested that ATP was correlated negatively with fatigue severity (FSS-7, Figure 31), and positively with walking distances (6MWT, Figure 32) and muscle strength (MVIC, Figure 33) with strengths of correlation stronger on the affected limb compared to the non-affected side.

Data from the affected stroke leg showed that all 4 participants in the sham group experienced reductions in ATP content of the anterior tibialis at 6 weeks follow-up while all 3 participants in the RIC group experienced increases in ATP content (Figure 34). The adjusted between group difference of 0.01 (95% CI 0.01 to 0.02) was statistically significant (*P*=0.004) *(Table 33).*

A similar effect was observed in the non-affected limb with an increase in ATP content at 6-weeks in the RIC group, but a reduction in the sham (Figure 35). However, this difference was not statistically significant (median difference 0.01, *P*= 0.63) (*Table 33).* Due to poor quality data acquisition in one participant's non-affected limb in the RIC group their data was discarded (Figure 35).

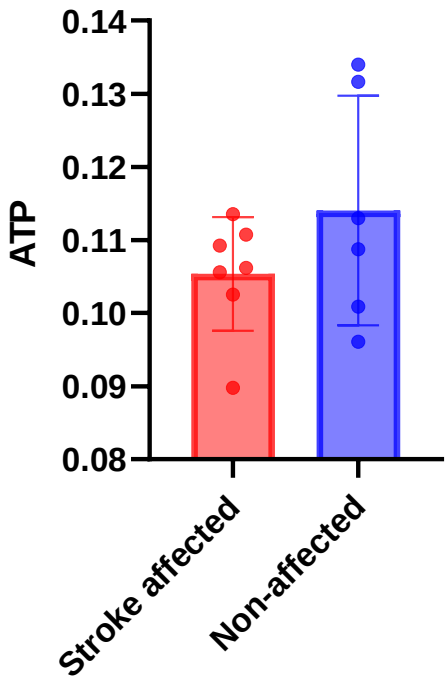
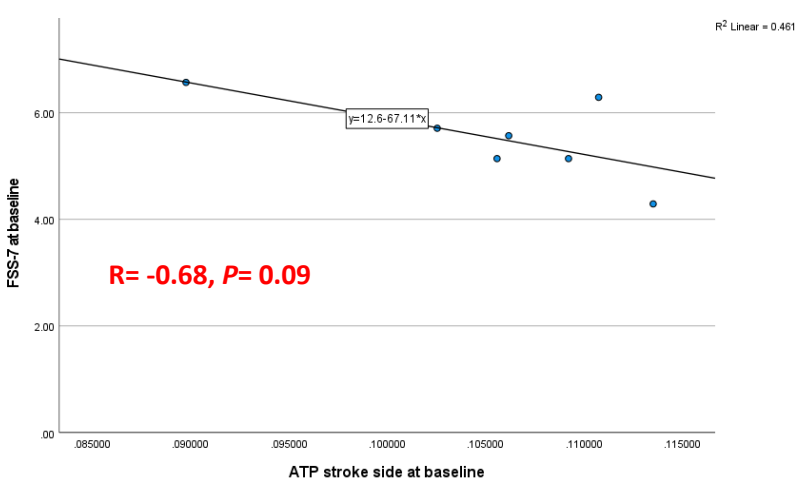
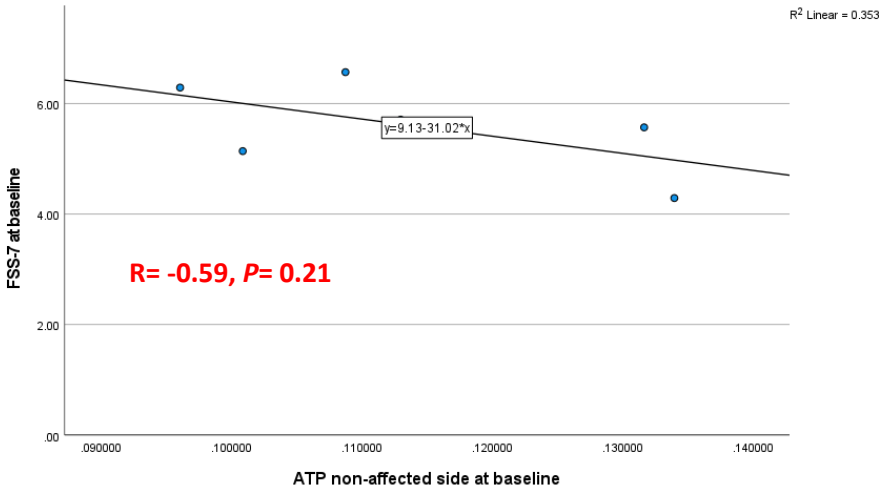


Figure 30. Baseline ATP in the tibialis anterior of the stroke affected (red) and non-affected (blue) leg at rest.

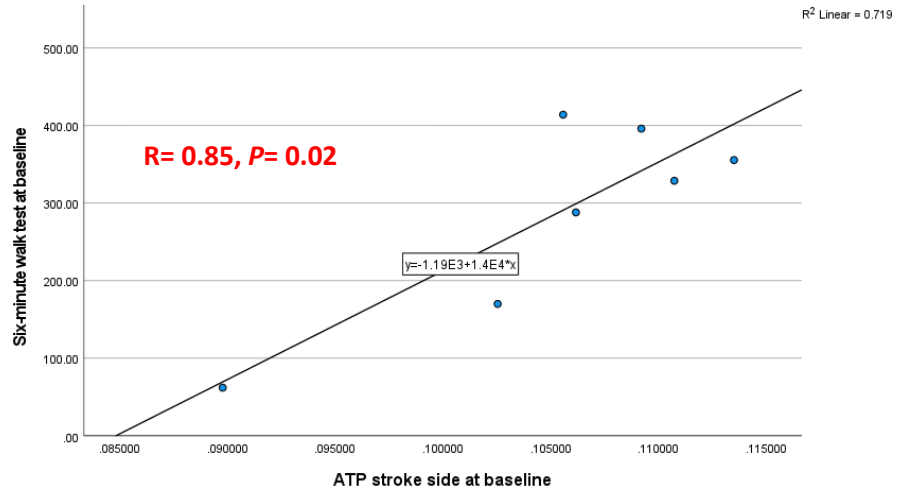


(A)

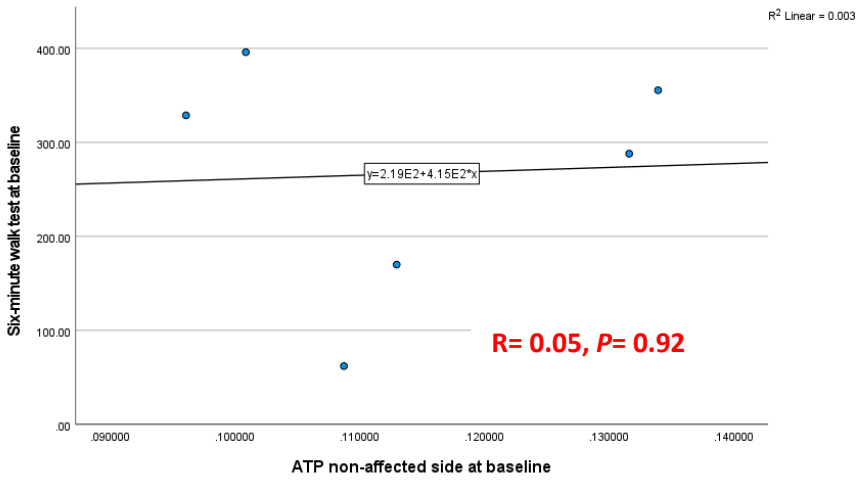


(B)

Figure 31. Relationship between fatigue severity (FSS-7) scores and ATP in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.



(A)



(B)

Figure 32. Relationship between six-minute walk test distance and ATP in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.

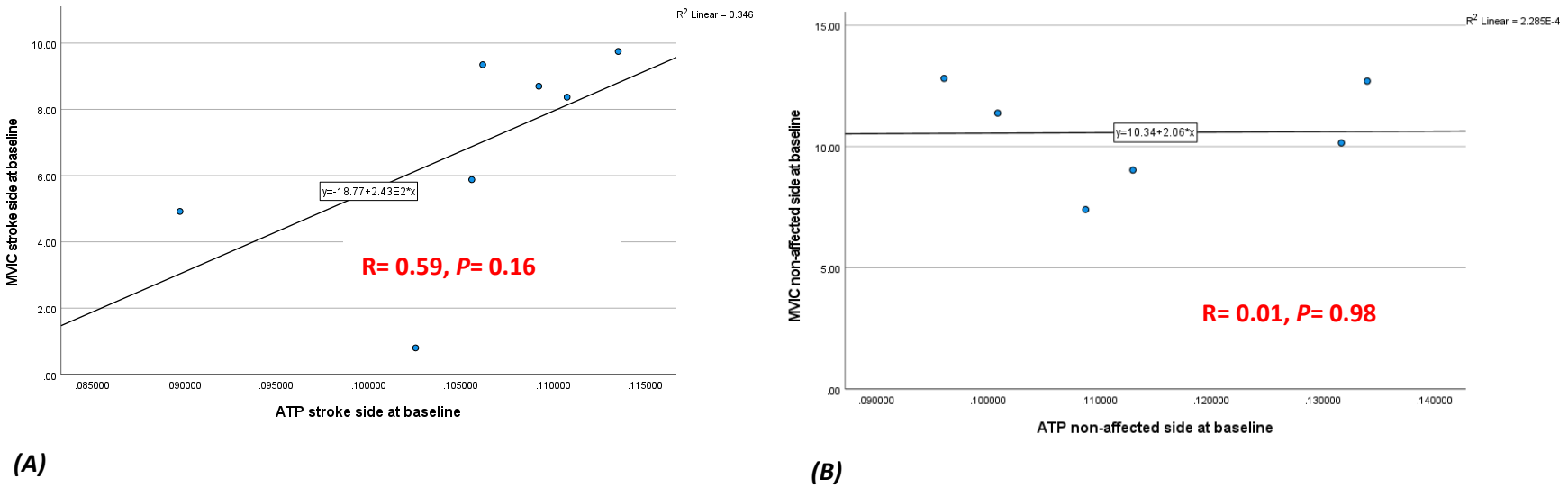


Figure 33. Relationship between MVIC and ATP in tibialis anterior on the participant's stroke side (a) and non-affected side (b) at baseline, at rest.

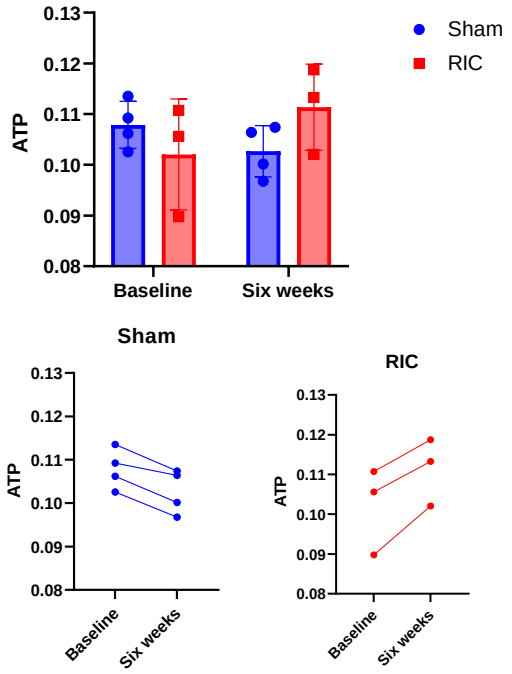


Figure 34. ATP in tibialis anterior in the stroke affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

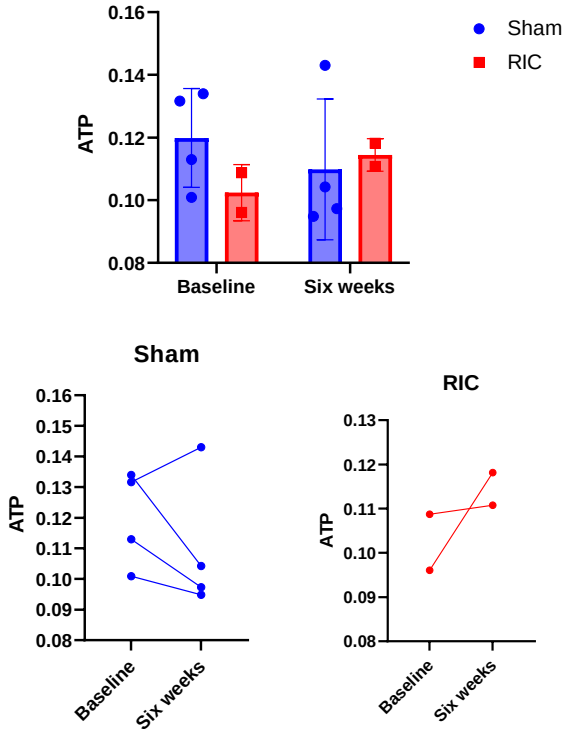


Figure 35. ATP in tibialis anterior in the non-affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

Table 33. Analysis of within group and between group differences in ATP measured in tibialis anterior at rest using a one-way ANCOVA at 6-weeks.

Outcome measure	Sham baseline (mean; SD)	Sham 6w (mean; SD)	RIC baseline (mean; SD)	RIC 6w (mean; SD)	Adjusted mean difference between the treatment and sham group (CI) (mean; SD)	P-value	Effect size
Stroke affected side							
ATP	0.11 (0.00)	0.10 (0.01)	0.10 (0.01)	0.11 (0.01)	0.01 (0.01 to 0.02)	**0.004**[#]	9.23
Non-affected side							
ATP	0.12 (0.02)	0.11 (0.02)	0.10 (0.01)	0.11 (0.01)	0.01 (-0.01 to 0.12)	0.72	0.17
ATP (median;IQR)*	0.12 (0.03)	0.10 (0.04)	0.12 (---)	0.11 (---)[8]	0.01[9]	0.63	0.56

*ATP= adenosine triphosphate; IQR = interquartile range. *Mann-Whitney U applied. [#]Significant at the 0.05 significance level.[8]IQR not available because sample size too small to calculate. [9]Unadjusted median difference provided.*

Phosphocreatine

Baseline PCr was slightly higher in the stroke affected limb compared to the non-affected leg (Figure 36). Phosphocreatine did not consistently correlate with either fatigue severity (FFS-7, Figure 37), walking distances / speed (6MWT, Figure 38) or anterior tibialis muscle strength (MVIC, Figure 39).

Similarly, there were inconsistent changes in PCr in response to RIC. In the stroke affected limb, there appeared to be a trend toward a reduction in PCr in the tibialis anterior in the stroke affected limb in both groups at 6-weeks (Figure 40). However, this difference between the two groups was not statistically significant (adjusted between group difference = 0.04 ,95% CI -0.06 to 0.14, *P*= 0.29) (*Table 34*). In contrast, PCr levels in the non-affected limb appeared to increase very marginally at 6 weeks albeit non-significantly (Figure 41).

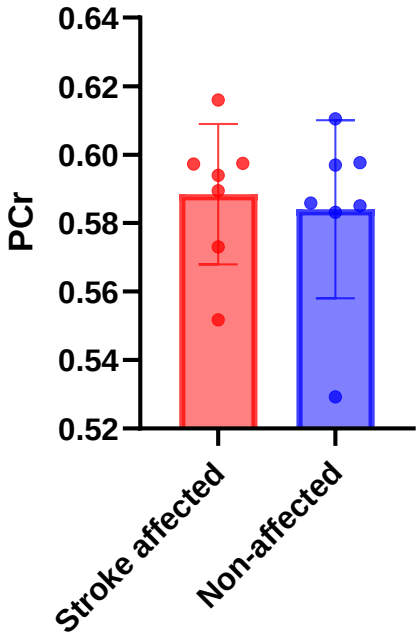


Figure 36. Baseline phosphocreatine (PCr) in the tibialis anterior of the stroke affected (red) and non-affected (blue) leg at rest.

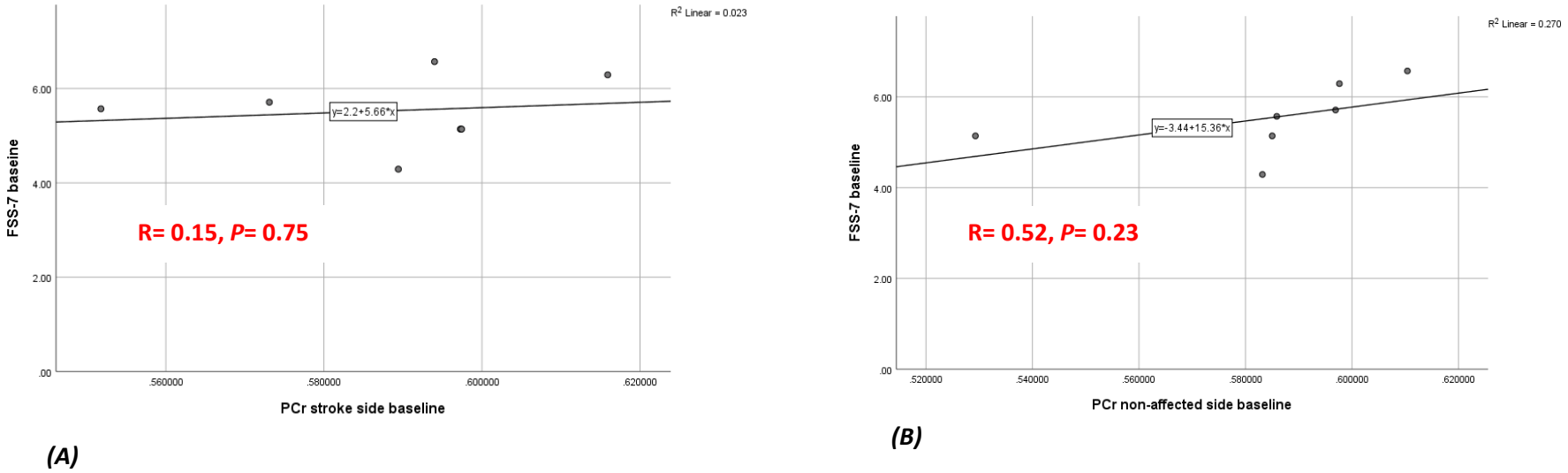
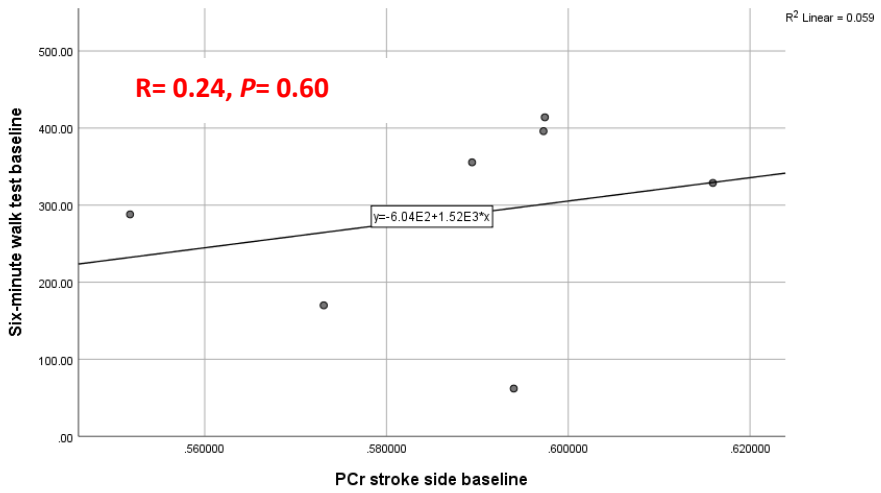
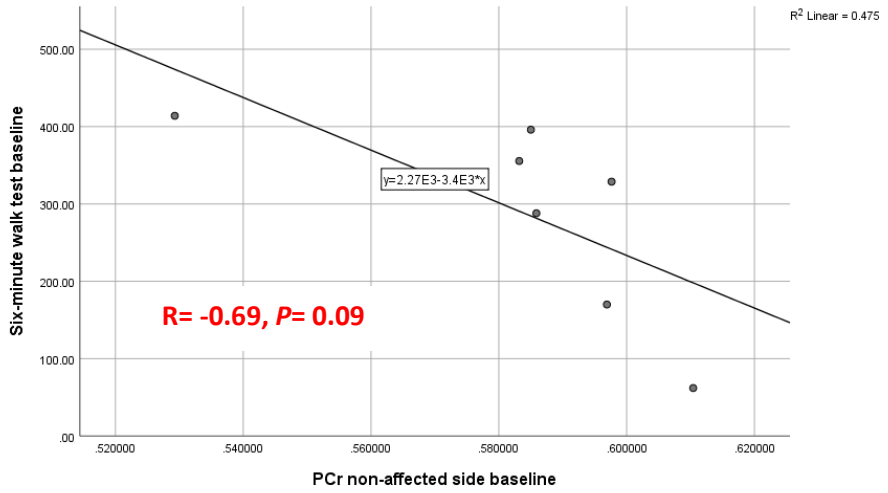


Figure 37. Relationship between fatigue severity (FSS-7) and PCr in tibialis anterior at baseline on the participant's stroke side (a) and non-affected side (b), at rest.



(A)



(B)

Figure 38. Relationship between six-minute walk test distance and PCr in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.

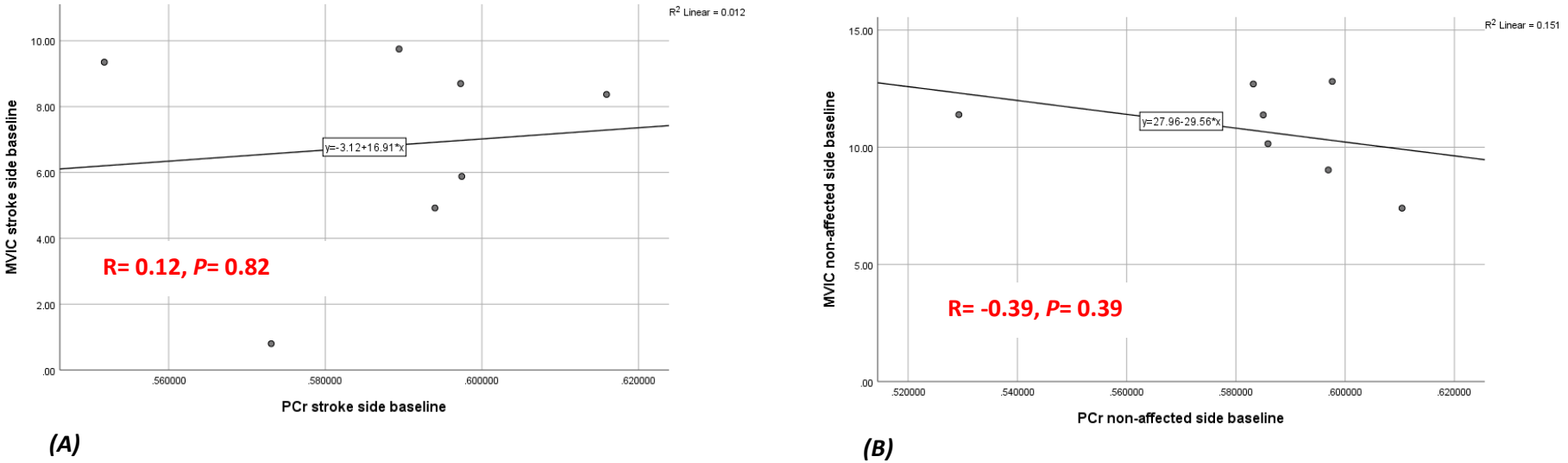


Figure 39. Relationship between MVIC and PCr in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.

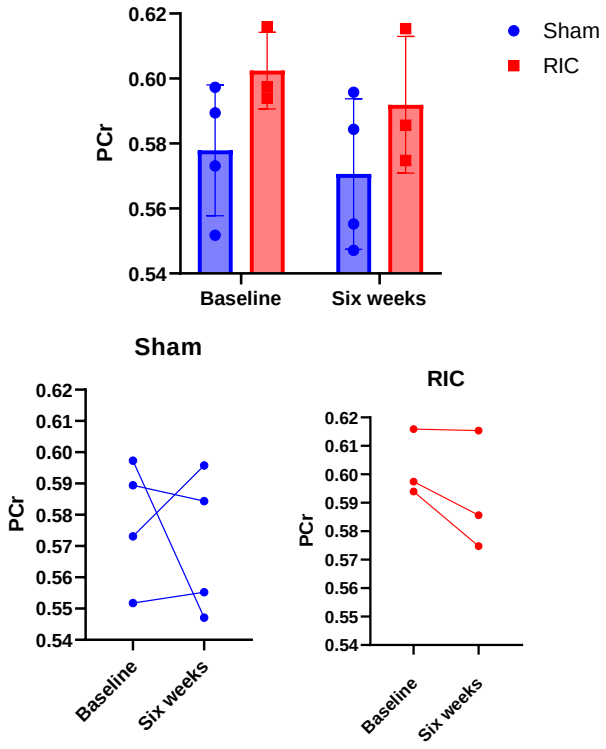


Figure 40. Phosphocreatine (PCr) in tibialis anterior in the stroke affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

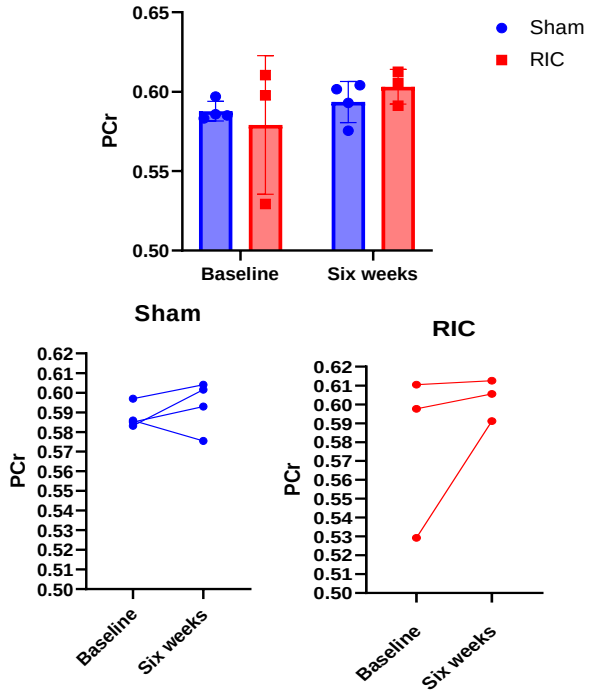


Figure 41. Phosphocreatine (PCr) in tibialis anterior in the non-affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

Table 34. Analysis of within group and between group differences in phosphocreatine measured in tibialis anterior at rest using a one-way ANCOVA at 6-weeks.

Outcome measure	Sham baseline (mean; SD)	Sham 6w (mean; SD)	RIC baseline (mean; SD)	RIC 6w (mean; SD)	Adjusted mean difference between the treatment and sham group (CI) (mean; SD)	P-value	Effect size
Stroke side							
Phosphocreatine	0.58 (0.02)	0.57 (0.02)	0.60 (0.01)	0.59 (0.02)	0.04 (-0.06 to 0.14)	0.29	0.88
Non-affected side							
Phosphocreatine	0.59 (0.01)	0.59 (0.01)	0.58 (0.04)	0.60 (0.01)	0.02 (-0.01 to 0.05)	0.15	1.69

Phosphocreatine is expressed as a proportion of total phosphorus.

Inorganic phosphate

Inorganic phosphate levels were higher in the stroke affected limbs compared to healthy limbs (Figure 42). At baseline there were positive correlations between FFS-7 and Pi, more strongly in the non-affected limb (Figure 43). Pi was inversely correlated with walking distances (6MWT, Figure 44) and muscle strength (MVIC, Figure 45).

There were no discernable differences in the effects of RIC on Pi compared to sham in either the stroke affected side (adjusted between group difference -0.00, 95% CI -0.02 to 0.01, P = 0.51, Figure 46), nor the unaffected limb (adjusted between group difference 0.01, 95% CI -0.02 to 0.03, P = 0.58, Figure 47) (*Table 35*).

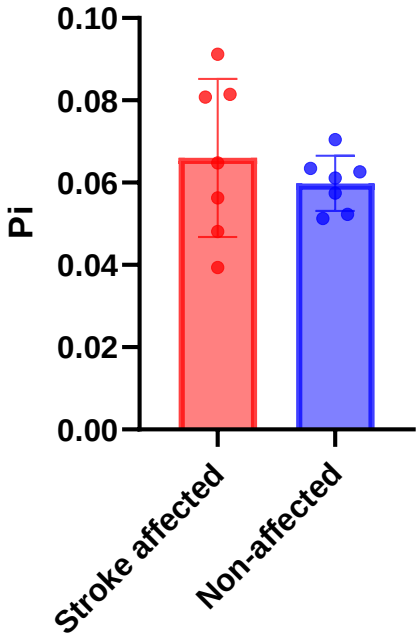
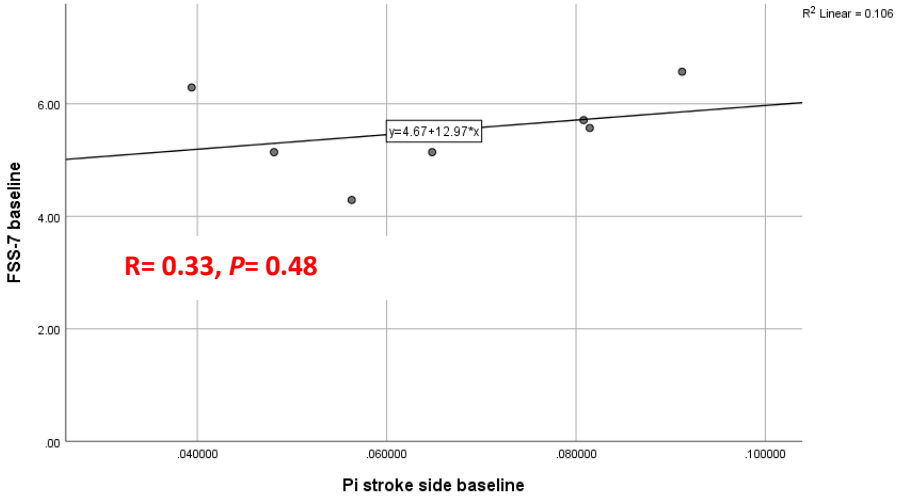
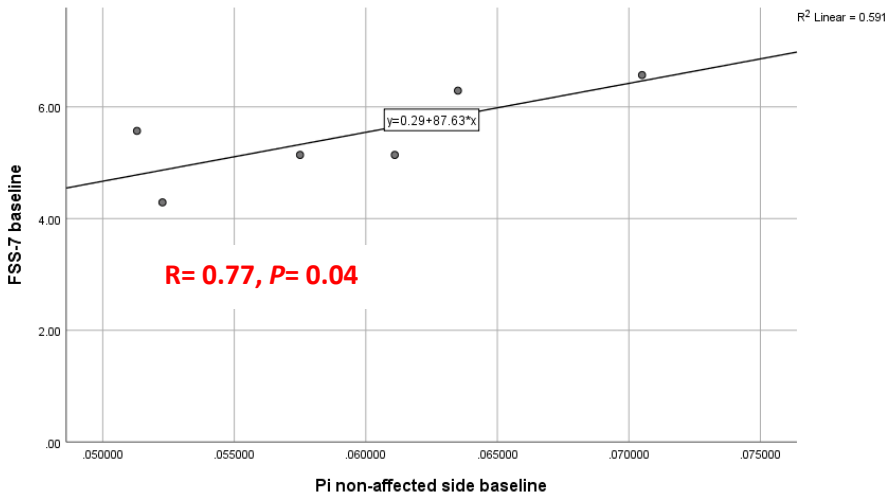


Figure 42. Baseline inorganic phosphate (Pi) in the tibialis anterior of the stroke affected (red) and non-affected (blue) leg at rest.



(A)



(B)

Figure 43. Relationship between fatigue severity (FSS-7) and Pi in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.

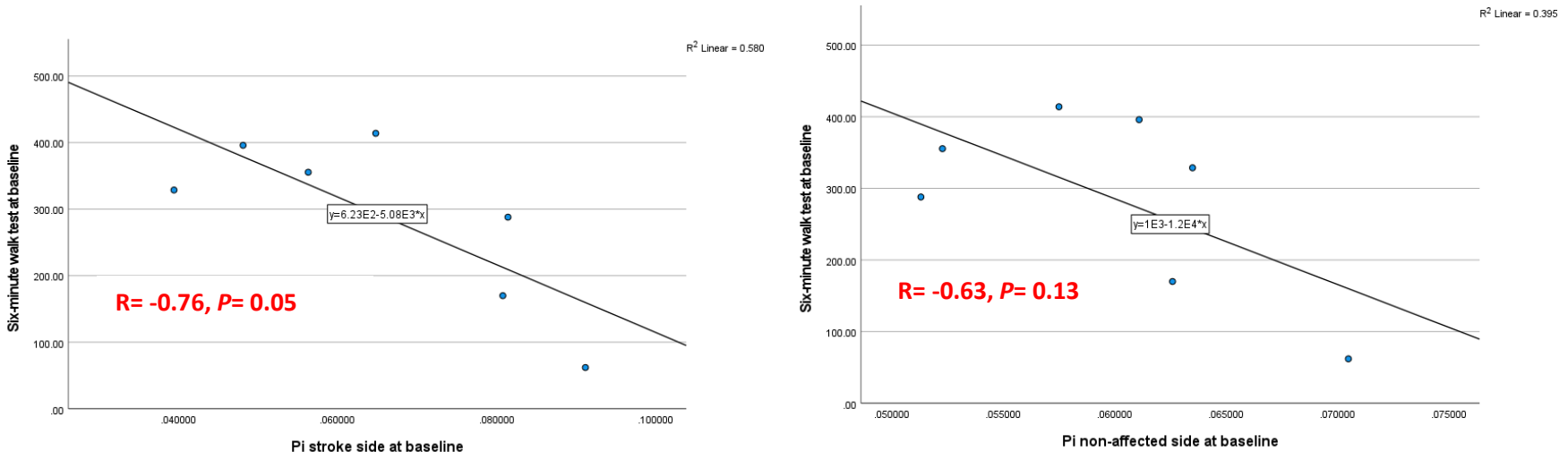


Figure 44. Relationship between six-minute walk test distance and Pi in tibialis anterior on the participant's stroke side (a) and (b) non-affected side at baseline, at rest.

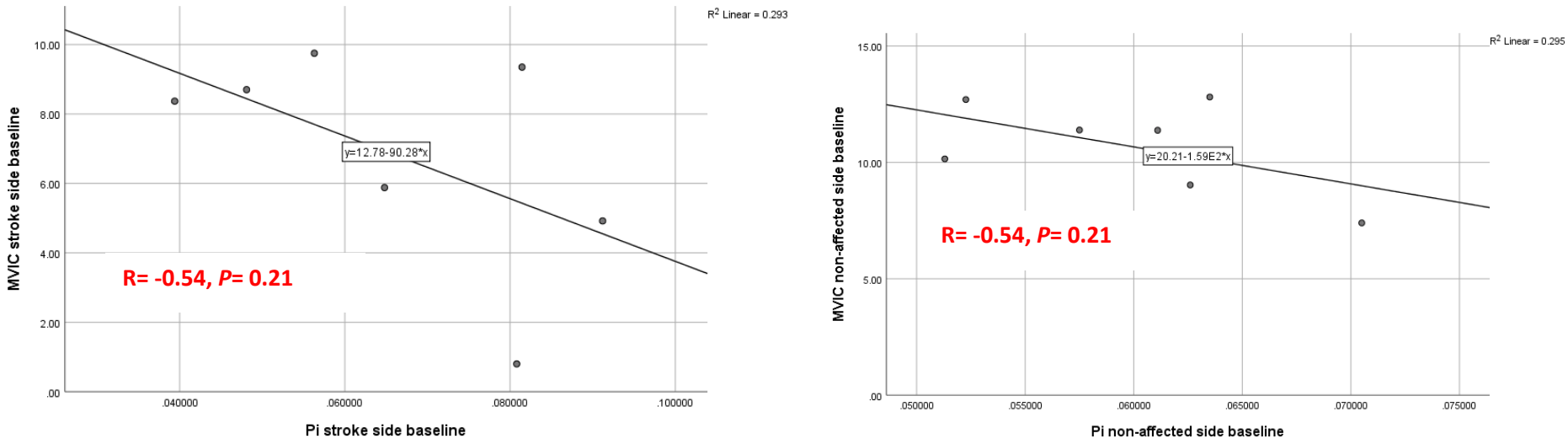


Figure 45. Relationship between MVIC and Pi in tibialis anterior at baseline on the participant's stroke side (a) and non-affected side (b), at rest.

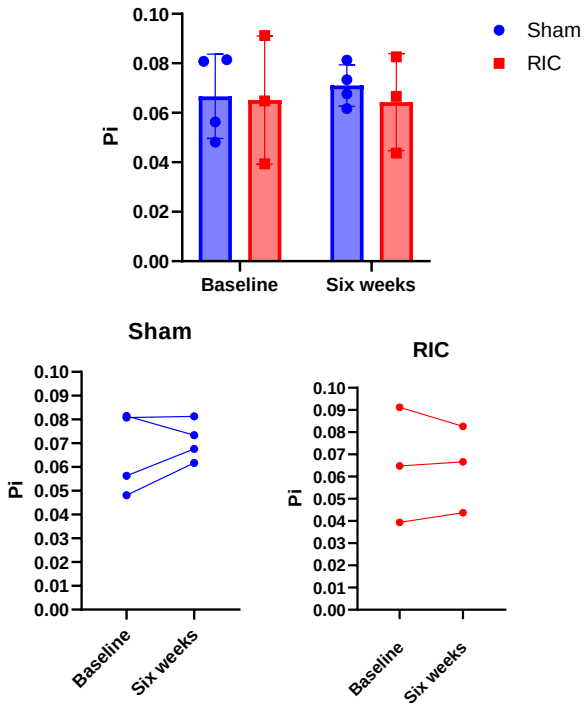


Figure 46. Inorganic phosphate (Pi) in tibialis anterior in the stroke affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

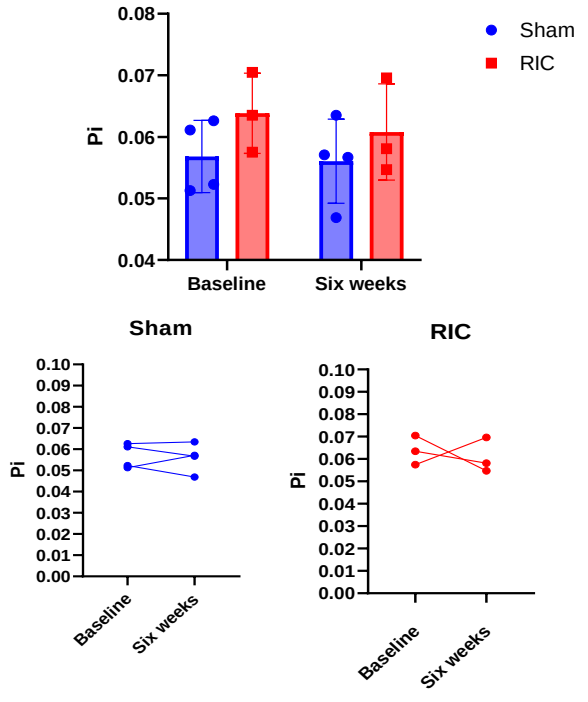


Figure 47. Inorganic phosphate (Pi) in tibialis anterior in the non-affected limb at baseline and at 6-weeks in the RIC and sham groups, at rest.

Table 35. Analysis of within group and between group differences in inorganic phosphate measured in tibialis anterior at rest using a one-way ANCOVA at 6-weeks.

Outcome measure	Sham baseline (mean; SD)	Sham 6w (mean; SD)	RIC baseline (mean; SD)	RIC 6w (mean; SD)	Adjusted mean difference between the treatment and sham group (CI) (mean; SD)	P-value	Effect size
Stroke side							
Inorganic phosphate	0.07 (0.02)	0.07 (0.01)	0.07 (0.03)	0.06 (0.02)	-0.00 (-0.02 to 0.01)	0.51	0.34
Non-affected side							
Inorganic phosphate	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.01 (-0.02 to 0.03)	0.58	0.24
Inorganic phosphate is expressed as a proportion of total phosphorus.							

5.5 Discussion

ATP

To the best of our knowledge this is the first study to investigate muscle cellular bioenergetics in patients with PSF using this advanced modality ^{31}P-MRS. Despite the small sample size, results were biologically plausible, providing initial evidence for altered bioenergetics in PSF and some amelioration by RIC. The results showed an association between the amount of ATP at rest in skeletal muscle of the affected lower limb in stroke and fatigue severity, walking distance/speed and MVIC strength. These findings suggest ATP is a potentially good biomarker for fatigue and physical function in stroke. Interestingly, although there appeared to be an association between ATP in the non-affected limb and fatigue severity, there was no association between ATP in the non-affected limb and walking distance/speed or MVIC. This may be related to the difference in mitochondrial function between healthy (i.e., non-affected limb) and diseased (stroke affected limb) tissues. This is consistent with anecdotal clinical observations as patients frequently report their abilities and endurance generally being limited by the weak leg rather than the function of the unaffected leg. Lower ATP levels have been implicated also in other pathologies. Muscle biopsy studies have found lower levels of skeletal muscle ATP concentrations in clinical populations affected by systemic diseases (e.g., sepsis, intensive care patients) compared to controls (Puthucheary et al., 2018). Another study employed this exact ^{31}P-MRS protocol to compare ATP in patients who had MND to healthy controls. Perhaps surprisingly, no between-group differences in ATP were found in MND. This may indicate that different pathophysiological mechanisms are taking place: in stroke there are upper motor neurone lesions which result in downstream disuse atrophy (and a possible consequent reduction in ATP), whereas denervation atrophy is the main mechanism taking place in MND, resulting in a different bioenergetic fingerprint (namely, elevated inorganic phosphate) (Sassani et al., 2020). If this is the case, then, in stroke, increased and improved levels of ATP might mean that mitochondrial function at the cellular level has improved by an intervention, e.g., by RIC.

Our results demonstrate how resting ATP in the tibialis anterior muscle of the stroke affected limb appeared to increase in every participant following RIC while levels fell in every participant in the sham group at 6-weeks. There also appeared to be an effect in the non-affected limb, however this was not statistically significant. Remote ischaemic conditioning might improve the function and efficiency of mitochondria via several pathways (*Table 31*)

(Hausenloy et al., 2005, Slagsvold et al., 2014, Thompson et al., 2014), and this may be why we are seeing the changes in our outcome measures. Skeletal muscle ATP concentration as well as ADP and Pi (which together form ATP), is essential for optimal functioning of the cell (Baker et al., 2010). Reductions in skeletal muscle ATP is associated with rapid fatigue development (i.e., reduction in the ability of muscle to produce power or force) (Bigland-Ritchie and Woods, 1984, Søgaard et al., 2006). Improved levels of ATP in the skeletal muscle in the treatment group at 6-weeks might be related to increased mitochondrial efficiency and metabolism and adaptations in mitochondrial function after RIC (Thaveau et al., 2007, Mansour et al., 2012). It may also reflect an improvement in the mitochondria's ability to regenerate ATP via mitochondrial respiration (Leung et al., 2014, Thaveau et al., 2007). Our findings of increased skeletal muscle ATP after conditioning are also consistent with studies that have found increases in skeletal muscle oxidative capacity after RIC (Andreas et al., 2011a, Jeffries et al., 2018a) (discussed in section 1.8.5.2).

The question then remains why we have seen significant changes in the stroke affected side as compared to the non-affected leg. One hypothesis that might explain this is that the effects of ischaemic conditioning may be greater in mitochondria that are abnormal as compared to mitochondria that are functioning at a more optimal level. Mitochondria from muscle tissue and cells in a leg that is functioning well (receiving nervous innervation, is active and receives adequate blood supply) such as a non-stroke affected leg, may already be working at a relatively normal and physiologic level. Mitochondria in tissues in a leg that is affected by stroke that is deconditioned, denervated and receives a reduced blood flow (Ivey et al., 2010) may be working at a suboptimal level and perhaps are much more susceptible to ischaemia or states of fatigue because of reduced resilience. This could contribute to why the patients who have stroke experience greater amounts of fatigue but if conditioning helps improve mitochondrial function, then it may do disproportionately in mitochondria that are abnormal or 'worse off' as opposed to mitochondria that function well.

Another fact that helps support this hypothesis comes from studies that examine the effects of conditioning on physical function in healthy populations or athletes (Incognito et al., 2017) compared to clinical populations (Hyngstrom et al., 2018). Systematic reviews and meta-analyses looking at the effects of RIC on performance in several healthy and athletic populations show mixed results (Marocolo et al., 2019, Caru et al., 2019), with many studies not demonstrating beneficial effects from RIC. Conditioning such populations who invariably

have ‘healthy’ mitochondria may have led to the self-selection of participants who may not respond to RIC. In contrast, studies that evaluate the effect of conditioning on muscle contraction and fatigability in stroke (Durand et al., 2019, Hyngstrom et al., 2018), MS (Chotiyarnwong et al., 2020) and cardiac patients (Hausenloy et al., 2015, Pryds et al., 2017, Chen et al., 2017) often demonstrate beneficial effects on outcomes such as muscle contraction, fatigue scores, and walking distances. These clinical populations are likely to have tissues with less ‘healthy’ mitochondria more susceptible to the effects of RIC.

Phosphocreatine

Prior studies that have evaluated ^{31}P-MRS in clinical populations (e.g., MND, diabetes mellitus) (Sassani et al., 2020, Ripley et al., 2018, Wu et al., 2012) have demonstrated lower levels of phosphocreatine in muscle or brain tissue compared to healthy controls. Studies have shown that lower levels of resting phosphocreatine correlate with lower rates of oxidative ATP synthesis (Kemp et al., 2007). Re-synthesis of phosphocreatine is dependent on mitochondrial oxidative phosphorylation and is a measure of oxidative capacity (Meyerspeer et al., 2020). In patients with stroke and fatigue one might hypothesise that tissue levels of phosphocreatine in the tibialis anterior would be lower than in healthy controls. Despite this we did not find this relationship between the stroke affected and non-affected legs, nor did we see a treatment response in PCr following RIC. This could just be because the magnitude of differences are too low to be detected in our small sample size, or it may be that this metabolite is not an outcome measure that reflects changes in muscle function or fatigue in patients with PSF. Indeed correlations between PCr and fatigue severity, 6MWT and MVIC were weak at best in our group of patients. Thus, the role of phosphocreatine as a biomarker in PSF and the effect of RIC is still yet to be elucidated.

Inorganic Phosphate

Some studies have shown that higher levels of inorganic phosphate in muscle tissue may be associated with reduced efficiency of cellular energetics or impaired mitochondrial function (Lodi et al., 1994, Martinelli et al., 2000, Sassani et al., 2020). Increased levels of Pi are associated with reduced muscle force and fatigue and suggest mitochondria are under stress (Westerblad et al., 2002, Pathare et al., 2005). We found that inorganic phosphate levels were slightly higher in the stroke affected side (i.e., weaker muscle) compared to the non-affected limb although our sample size was small (Figure 42). This is in line with prior studies that have found higher levels of skeletal muscle inorganic phosphate in clinical populations compared to

healthy controls inversely correlating with MVIC (Sassani et al., 2020, Zochodne et al., 1988). In our study, values of baseline inorganic phosphate in the tibialis anterior of the non-affected limb was similar to that found in healthy subjects (Sassani et al., 2020). Elevated inorganic phosphate in the stroke affected muscle suggests dysfunctional bioenergetic homeostasis and peripheral mitochondrial dysfunction in the diseased muscle. It may also indicate impaired mitochondrial responses to increased energy demand and inefficient ATP regenerative capacity of mitochondria (Nicholls and Ferguson, 2013). Inorganic phosphate is a product of ATP hydrolysis (Hargreaves and Spriet, 2020), therefore in times of increased energy demand inorganic phosphate levels increase.

Our data did not support a clear effect of RIC on Pi in either the affected nor unaffected limb, however increasing Pi was associated, albeit weakly, with increasing fatigue severity and more strongly with 6MWT and MVIC, suggesting that inorganic phosphate is associated with muscle function. This finding is in line with the study in MND which found that higher inorganic phosphate in the tibialis anterior was associated with reduced walking speed ($R = 0.77$, CI = 0.46 to 0.91, $P < 0.001$) and greater weakness on MVIC ($R = -0.57$, CI = −0.81 to −0.15, $P = 0.012$) (Sassani et al., 2020). Thus, inorganic phosphate may be an important biomarker for physical function in stroke and warrants further investigation.

5.6 Limitations

The main limitation of this study is the very small sample size. Replication of our preliminary findings in a larger powered trial is necessary to determine whether the findings are truly reflective of biological processes. In addition, ^{31}P-MRS in muscle can provide dynamic data, helpful to characterise further the bioenergetics of the tissue. In the future, analysis of such dynamic data might help shedding light on ATP, PCr and Pi response to muscle contraction in PSF. In this study, there are technical limitations related to muscle ^{31}P-MRS being a non-invasive technique that necessitates a surface coil with inherently limited depth sensitivity (Menon et al., 2021). Also, there may have been variability related to coil placement which may have increased the variance and perhaps reduced accuracy of data acquisition; nonetheless, all care was taken for the acquisition to take place in a standardised manner, including placement of coil being cross-checked by both the radiographer and Author of this thesis. Another drawback of ^{31}P-MRS limiting its use in clinical practice is the high cost, requirement

for a specialised coil that is not routinely available on most clinical MRI scanners and lack of fully automated and standardised analysis pathways.

5.7 Conclusion and Future directions

To our knowledge, this is the first study that has utilized ^{31}P-MRS to measure skeletal muscle metabolism in stroke patients with fatigue. It is also the first study we are aware of to correlate skeletal muscle spectroscopic parameters with measures of fatigue and physical function such as walking distances and MVIC in stroke. This is important as it might help encourage the development of this technique as a biomarker for physical function or fatigue in stroke. This study provides a first demonstration of the potential of skeletal muscle ATP as a biomarker for fatigue and physical function and sheds light on how the effects of RIC may occur. The disproportionate differences in effect of RIC on affected and non-affected stroke muscle groups may also help explain why studies in clinical populations have shown more promise for RIC compared to studies in healthy individuals or elite athletes. Our findings are preliminary and warrant further investigation. To test the validity of our findings this study needs to be repeated on a larger scale. We also plan to investigate the dynamic recovery of ATP following a protocol of isometric muscle contraction while acquiring ^{31}P-MRS data.

CHAPTER 6. QUALITATIVE EXPERIENCES OF REMOTE ISCHAEMIC CONDITIONING FOR FATIGUE AFTER STROKE

6.1 Introduction

The aim of this chapter was to explore individual expectations and experiences of the RIC intervention by conducting semi-structured qualitative interviews. Although studies evaluating chronic use of RIC have been conducted (Meng et al., 2015a, Meng et al., 2012, Shahvazian et al., 2017, Hansen et al., 2019, Durand et al., 2019, Vanezis et al., 2018, Kono et al., 2014, Chen et al., 2017, Kate et al., 2019) and have reported good adherence rates generally (Hansen et al., 2019, Meng et al., 2012, Chen et al., 2017) such adherence rates can vary considerably and may depend on the duration and frequency of the intervention, and the pressure protocols used. Interestingly there is a significant lack of data exploring how people feel about using what is sometimes viewed as a 'counter-intuitive' treatment. If a treatment like RIC is found to be effective for conditions like PSF, then it is imperative to identify common issues people face using it in order to optimise treatment utility moving forward. Further, prior studies investigating RIC on vascular outcome measures (Manchurov et al., 2020, Jones et al., 2015, Loukogeorgakis et al., 2005, Meng et al., 2012, Jeffries et al., 2018a, Kimura et al., 2007) or measures of muscle function (Hyngstrom et al., 2018, Durand et al., 2019) lack a qualitative component that details how any effects of RIC translate into everyday life for individuals. As such, we wanted to investigate what participants felt about the treatment (e.g., whether they thought it was uncomfortable, the duration and frequency of delivery) as well as whether participants felt as though they responded to the treatment (e.g., did they feel less tired) and if they would be prepared to have the treatment in the future. We also investigated the facilitators and barriers to adherence with the intervention and explored what it was like for participants who self-administered the intervention at home.

6.2 Aim and objectives

Aim: To investigate individual participant experiences with repeated RIC to treat fatigue after stroke.

Objective: To undertake and analyse semi-structured interviews with stroke patients with debilitating fatigue who had received RIC three times weekly for 6 weeks.

6.3 Methods

6.3.1 Sample

Eight participants in the active treatment group were sequentially offered the opportunity to participate in a semi-structured interview with the researcher.

6.3.2 *Procedure*

Informed consent was obtained at the beginning of the study (Appendix 6). Semi-structured qualitative interviews were conducted face-to-face or over the telephone (depending on covid-19 restrictions) using a topic guide (Appendix 21). The interview took place either during the last scheduled study visit or at another mutually exclusive time between the participant and researcher. Interviews were audio recorded using an Olympus Digital Voice Recorder VN-731PC and later transcribed verbatim. Participants were unaware of treatment allocation at the time of the interview to maintain treatment blinding at 3- and 6-month follow-up.

6.3.3 Confidentiality

Interview recordings and files containing the transcriptions of the interviews were stored on a password protected and encrypted laptop only accessible to the research team. Interview transcripts were anonymised and labelled with the participant's study ID. A printed copy of the interview transcripts was stored in the study site file, which is stored securely at the Royal Hallamshire hospital, Sheffield. Face-to-face interviews were conducted in a private room with only the participant and the interviewing researcher present and participants consented to the interview being audio recorded. Telephone interviews were also recorded (hands-free) using an Olympus Digital Voice Recorder VN-731PC and were transcribed and safely stored the same way as described above.

6.3.4 *Analysis*

Interview transcripts were analysed using thematic analysis following the six-phase process outlined in Braun and Clarke. (2006). These six phases are: (1) data familiarisation (includes

transcription of verbal data, repeated reading and note taking), (2) generating initial codes (identifying data that is interesting and meaningful), (3) search for themes (codes you have generated are grouped into overarching themes and subthemes), (4) review themes (refinement of themes), (5) defining and naming themes, (6) produce the report. We took a theoretical approach to thematic analysis (i.e., the analysis was driven by our interest in the research topic) (Braun and Clarke, 2006) and asked questions about the specific known issues we had information about relating to the treatment. The result of this process is a thematic map (see Figure 48). A second researcher reviewed the coded data and agreed the plausibility of the emerging themes. Recruitment of participants was intended to continue until it was felt that content saturation was reached.

6.4 Results

A total of eight participants in the active treatment group (4 male, 4 female) participated in semi-structured qualitative interview. Four of the participants had received in-hospital RIC and 4 participants had received home-RIC. The five main themes that emerged from thematic analysis were (1) participant expectations of the treatment, (2) positive treatment responses, (3) treatment side effects (4) facilitators and barriers to compliance with the intervention, and (5) willingness to have the treatment in the future. These themes and subthemes are depicted in Figure 48.

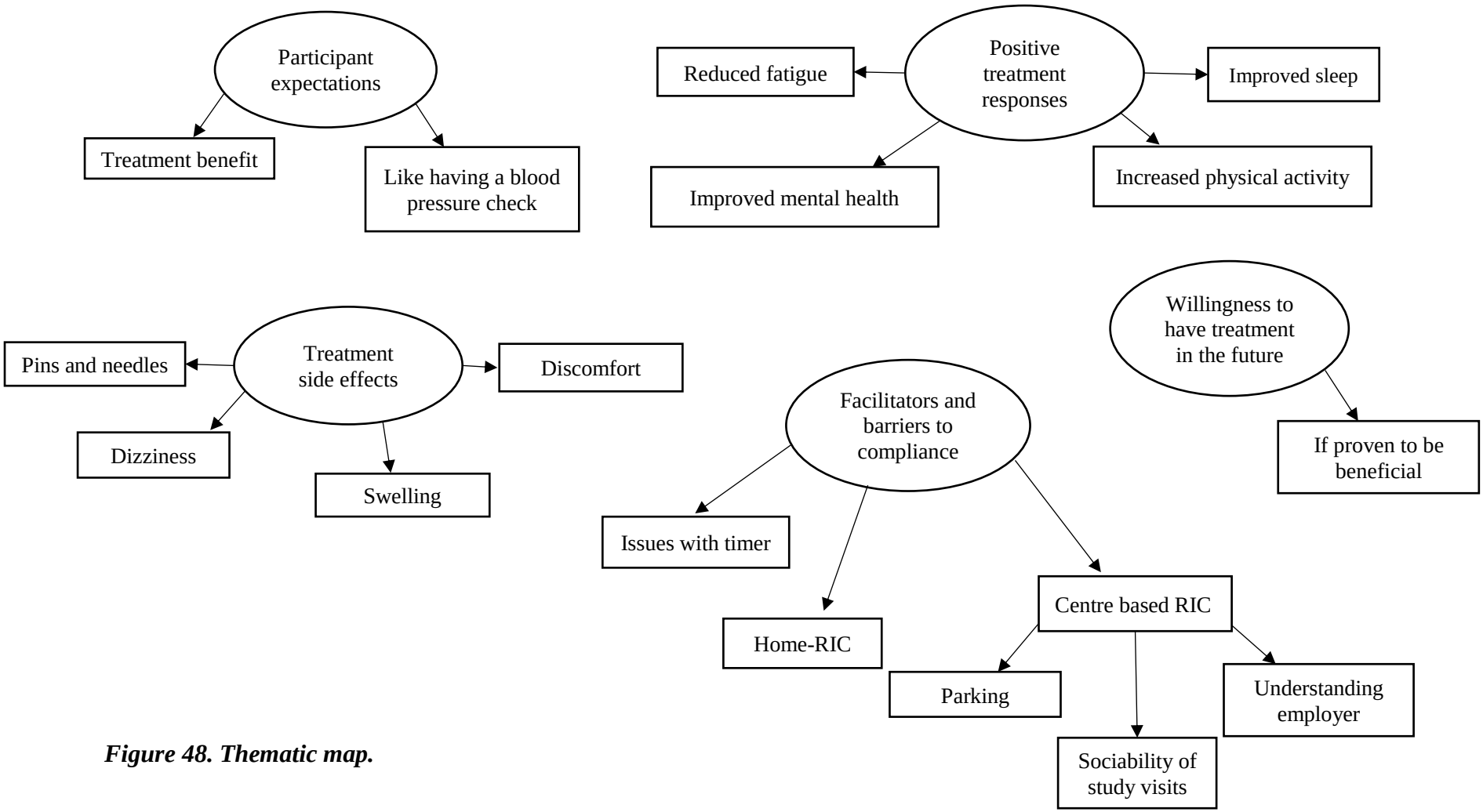


Figure 48. Thematic map.

Participant expectations

The first theme that emerged concerned participant expectations of the treatment. Subthemes included: (1) expected treatment benefits and (2) anticipation of discomfort. When asked what they expected the treatment to be like three participants said they thought it might help them to feel better and to improve their fatigue. This is likely the result of the information provided in the patient information sheet (Appendix 4). To our knowledge, only one participant who was in the home-sham group had read about the treatment before the study as they expressed disappointment when they were told what pressure they had to inflate the blood pressure cuff to.

> **RIC01:** *'I went hoping it would make me better and help. I was better for a while and think it did help.*' (Female age 62).

> **RIC05:** *'I just thought you were going to try and help improve my fatigue'* (Male age 47).

> **RIC23:** *'I thought it might help me out so that's why I went for the study.'* (Male age 56).

Participants expected it to be uncomfortable as they were familiar with the feeling of getting a blood pressure check. However, they had no other concerns and were intrigued to know if the treatment would work (although participants were aware when they consented to the study there was a chance they would be randomised to a 'dummy' treatment). They trusted the researchers would not ask them to do something that would cause them harm. The simple nature of the intervention and the fact it did not involve taking a drug was reassuring for participants.

> **RIC01:** '*I knew what it was like to get your blood pressure checked but I didn't find it too uncomfortable.'* (Female age 62).

> **RIC25:** '*Although I thought the level of it (referring to the pressure), I knew it would be uncomfortable, but I wasn't worried about it because you wouldn't be asking me to do something that is dangerous.*' (Female age 61).

RIC18: '*I wasn't worried about it because it didn't involve taking anything if you see what I mean. If it involved taking a drug, I would have been a bit more worried about it. The fact it was fairly straight forward it didn't strike me as being a difficult thing to ask of me really.*' (Female age 69).

Positive treatment responses

Overall, most participants felt as though they responded positively to the treatment. Most participants felt as though symptoms related to their fatigue improved, both from a physical perspective and from a cognitive one.

RIC01: *'I felt less tired. I think I did benefit from it. I felt less of a need to sleep in the day.'* (Female age 62).

RIC02: *'I'm not as tired and I feel better within myself. I'm in a good place... I feel like I can do more such as playing golf and going to the gym. I am concentrating better too.'* (Male age 44).

RIC18: *'Yes I did very much so. When I came to do the re-test (6-week follow-up), the cycling and things, I felt a lot better. I felt more energetic.'* (Female age 69).

RIC25: *'I'm able to do more. I'm less tired, less breathless and I'm not sleeping in the day as often as I was at the start.'* (Female age 61).

Interestingly, one participant said after they finished the treatment, they thought their fatigue went back to what it was like before the study.

RIC01: '*Since discontinuing slowly over time my fatigue has got worse but my arthritis is getting worse so it could be that… after the treatment my fatigue went back to what it was like before the study started.'*

Participants also reported increased level of physical activity, improved sleep and better mental health.

RIC02: *'I'm not sure if it was a placebo though. I have started to sleep better since.'* (Male age 44).

RIC22: *'The past few days I've walked a lot further than I have done in ages.'* (Female age 61).

RIC23: *'It made me feel a lot better in the mind because I thought it was doing me good. It made me feel better within myself.'* (Male age 56).

However, one participant did not feel as though the treatment helped them in any way.

RIC16: *'I don't think it helped at all. Especially the last few days, well you can probably tell with my voice how weak I am. That is the first thing that starts going when I am fatigued.'* (Male age 73).

Treatment side effects

Overall, participants tolerated the treatment very well. Participants expressed experiencing mild symptoms including slight swelling of the arm where the cuff had been inflated and pins and needles. One participant who received in-hospital RIC experienced dizziness one day after receiving the treatment, however they explained how they did not have any breakfast that morning which may have been a contributing factor.

RIC01*: 'A little bit of swelling but it wasn't problematic.'* (Female age 62)

RIC02: *'That one day where I experienced some dizziness, but I had no other side effects… there was an element of discomfort, but it wasn't that bad'* (Male age 44).

RIC16: *'Just a slight tingling in the arm at times but when the pressure pad (cuff) was taken off it was not a problem,'* (Male age 73).

One participant in the hospital-RIC group thought the treatment was a shock at first (referring to the pressure) but thought it got easier over time (based on feedback and experience with the participant over the 6-weeks).

> **RIC02:** 'It was a shock at first, I didn't expect it.' (Male age 44).

Facilitators to and barriers of compliance with the intervention

Overall participants felt it was acceptable to receive the treatment three times per week. One participant said the study was more time consuming than they anticipated but this was not a problem for them.

> **RIC02**: '*Three times a week was manageable.*' (Male age 44).

> **RIC16:** '*It was okay. It fit into my lifestyle alright no problem.*' (Male age 73).

> **RIC18:** *'It was probably more time consuming than I thought it would be, but it wasn't a problem.'* (Female age 69).

However, one participant highlighted how it was manageable because they were able to self-administer the intervention at home. They thought some people would have struggled if they had to travel to the hospital three times a week to receive the treatment.

> **RIC25:** '*It was manageable. I just think it was easier to be able to do it at home and I think a lot of people would have found it difficult to be travelling if they had needed to travel in each time.'* (Female age 61).

Although this may have been the case for some people, this was not a complaint expressed by participants in our study. Most of the participants who received in-hospital RIC were either out of work or retired, and free transport was available for all participants who required it. One participant in the treatment condition worked full-time but had an understanding employer who allowed them the time off work to participate in the study.

RIC02: *'Yes because work was very good and lenient.'* (Male age 44).

Parking was difficult at the Royal Hallamshire hospital due to limited car parking space and participants were also required to pay for parking (although this was reimbursed) unless they had a disability badge. However, at Sheffield Hallam University and at the Assessment and Rehabilitation centre (ARC) there was free parking.

RIC01: *'It was a lot easier coming to ARC than the Royal Hallamshire hospital. It was easier to park, and I found it easier getting in and out of the car.'* (Female age 62).

A couple of participants in the home-RIC group reported having issues with the timer. One participant said they sometimes forgot to turn the time on, the other participant said their wife operated the timer.

RIC18: *'The only thing I did do a couple of times is forget to switch the timer on then think oh bother now what do I do?'* (Female age 69).

RIC23: *'Not really. I would have struggled doing the timing and that, but my wife did it.'* (Male age 56)

One participant started the intervention in hospital but had to stop when covid-19 restrictions were introduced. When restrictions were lifted, and recruitment recommenced this participant was happy to re-start the intervention, but this time was trained on how to self-administer the intervention at home. Therefore, they had experience of in-hospital and home-RIC. They thought having it in hospital first made it easier for them to do it themselves at home.

RIC18: *'I think doing it at home (the treatment) on top of never having done it would have been quite difficult but actually having had the ARC experience it was very straight forward really. I could do it whilst watching the television. It wasn't a big deal really.* (Female age 69).

A key facilitator expressed by one participant in the hospital-RIC group was the social aspect of the study.

RIC01: *'It was sociable which is good if you are isolated. It was nice to see and chat to people.'* (Female age 62)

Willingness to have the treatment in the future

All eight participants said they would be prepared to have the treatment again in the future if it was shown to be effective.

RIC02*: 'Yes, the only issue was the time away from work.'* (Male age 44).

RIC16: *'Coming to ARC was no concern for me anyway but if there was a home treatment, I would certainly do that no problem'.* (Male age 73).

RIC22: '*Yes. If it is proved that it does help, then yeah.'* (Female age 61).

RIC23: '*Yes, I would yeah. If I'm thinking it's going to help me I would yeah.'* (Male age 56)

6.5 Discussion

To our knowledge, this is the first study to explore participant experiences of independently using RIC. Studies have used qualitive feedback to explore reasons for discontinuation of self- or care delivered RIC (Kate et al., 2019), however we are unaware of any studies that have performed a more in-depth exploration of participant experiences of RIC using semi-structured qualitive interviews.

Overall, the interview responses demonstrate that participants thought the RIC treatment was acceptable. Participants only reported experiencing mild symptoms/discomfort using the treatment that did not deter them from completing the study or considering ongoing future use if found to be effective for PSF. One way to mitigate the level of discomfort would be to utilise a different pressure protocol for RIC e.g., 30 mmHg above systolic pressure. This might be more tolerable but risks uncertainty about delivering true RIC as the participant's blood

pressure might increase during the procedure. Given that the side effects were mild it might be okay to continue with the current pressure protocol.

Participants had positive expectations of the intervention before starting the study. This is not uncommon and may have been due to the delivery of information about the study prior to or following recruitment and may have contributed to a placebo effect for the intervention and the sham group together. Improvement in fatigue were seen in both groups (RIC and sham) that may have mimicked the natural time course of PSF, however, while we believe the disproportionate treatment effect in the RIC group to be due to physiological effects of RIC, we cannot discount the effect that this positive expectation had on the outcome measures seen in the study.

It is evident that most participants interviewed felt as though the treatment benefitted them in some way. For example, participants reported feeling less tired with less daytime somnolence, improved night-time sleep and improved mood and physical activity. Importantly participants noted improvements in concentration demonstrating it effects more 'central' symptoms of fatigue rather than solely benefitting more 'peripheral' symptoms. These reports help link improvements in clinical outcome measures (FSS, 6MWT etc) to meaningful changes in the everyday lives of people with PSF. Of course, we cannot exclude the potential beneficial effect of participating in the study itself on these positive changes. The QoL measures (e.g., EQ5D-5L) did not change much over the course of the six-week intervention. This may be because the participants were already fairly highly functioning at the beginning of the study. However, even clinically modest improvements in mood or functioning can have substantial meaning to patients. From the interview responses it seems participants did feel as though they felt better after the treatment and that they were finding day to day activities easier (e.g., walking, going to the gym) which is reassuring.

Key facilitators to adherence expressed by participants in the hospital-RIC group was free parking at the research sites and having an understanding employer. They also enjoyed the sociability aspect of the study. Free transport arranged by the researcher was another key facilitator to participation in the study. At the beginning of the study participants were expected to pay for their transport and claim the money back. However, due to the frequency of study visits (three times weekly for 6-weeks) understandably people could not afford it. We were able to get a contract with a local taxi company which allowed the researcher to pre-book

transport. The introduction of home-RIC during the pandemic also facilitated recruitment and adherence. Although participants in the home-RIC expressed having trouble with the timer and required help from family members. These findings suggest that an ideal intervention would be one that is self-delivered at home. Home-delivered RIC is convenient, people can do it while watching the TV and it negates the barriers associated with centre delivered treatment. The timers were an issue thus development of an automated RIC machine with pre-set protocols will enhance ease of treatment delivery. It is really reassuring that all participants interviewed said they would be willing to have the treatment in the future if it was shown to be beneficial.

Studies looking at the qualitative aspect of conditioning is lacking. The study by Kate et al. (2019) discussed previously found that reasons for discontinuation of RIC therapy (33% of patients discontinued the study) included the length of the treatment (repeated two twice daily for 12-weeks), development of petechial rash, pain during the intervention, illness of caregiver and treatment associated anxiety. Two participants withdrew because either they found an alternative therapy or they felt better (Kate et al., 2019). A few qualitative studies have been conducted of stroke survivors' experience with PSF (Flinn and Stube, 2010, Barbour and Mead, 2012, Kirkevold et al., 2012, White et al., 2012b, Worthington et al., 2017). However, data is lacking on what it actually feels like when fatigue improves from a patient perspective. We cannot say whether people realistically knew whether they were in the treatment or control arm. However, we tried in the trial design to help avoid lack of blinding. For example, although participants were aware they would be randomised to ''treatment'' or ''dummy treatment'', they were just told there would be a difference in the pressure the cuff is inflated to. Also, study visits were arranged so that participants could not meet each other or discuss the study. In the hospital-RIC group, the researcher delivering the treatment avoided showing the participant the pressure to which they were inflating the cuff. However, participants in the home-RIC group had to perform the intervention themselves at home and were taught to inflate the cuff until they reached the coloured sticker on the Sphygmomanometer dial that was clearly over the 200-mmHg mark. Therefore, there is a greater chance of them being able to look up details of the intervention and expected pressures online. Prior studies that have used RIC in patients with stroke have shown that the sham is feasible and that most participants are unaware of treatment allocation. This was demonstrated in a study by England et al. (2019) of 60 stroke patients (31 RIC, 29 sham) who found that when asked which intervention participants thought they received, 93% did not know, 4% answered incorrectly and 4% were correct. However, the RIC protocol used in their study was slightly different (cuff inflated to 20 mmHg above SBP

rather than 200 mmHg for everyone) and was delivered using an automated RIC machine. It might be that going forward we can look at using a slightly different approach. This might make the treatment more tolerable for patients long-term, for example, an elderly patient with fatigue who has low blood pressure (e.g., 120 mmHg SBP) would not need to inflate the cuff to 200 mmHg. It would just make the intervention more intolerable for no added reason. A protocol of 20-30 mmHg above SBP might be more appropriate for those patients.

Results from our qualitative interviews will help optimise RIC treatment delivery in a larger definitive trial and will help in the planning of a full-scale RCT to test the efficacy of RIC for fatigue after stroke. For example, in a larger trial we can offer additional training on how to use the stopwatches and ensure they are as easy to operate. Using an automated device that automatically inflates/deflates the cuff rather than a manual device would prevent issues with timers and would be less effort for participants. In future trials we can try and use research sites with free accessible parking and ensure measures are in place before the study starts to be able to pre-book transport.

6.6 Strengths and limitations

A limitation of this study is that we only obtained responses from participants in the active treatment condition and did not interview participants in the sham group. It would have been good to explore whether there was a placebo effect in the sham group. This is because we wanted to explore the safety and acceptability of the intervention. The topic guides also focused a lot on acceptability (e.g., did you find the treatment uncomfortable? Did you have any problems?) and whether participants felt as though they 'responded to the treatment'. It is likely that we did not reach 'data saturation' (point during data collection where no new information is being obtained) (Saunders et al., 2017, Guest et al., 2020) due to the effects of the pandemic on trial recruitment, and future trials should also focus on obtaining participant views to help supplement the existing qualitative data on RIC experience. Despite this we were able to obtain interviews from an even split of participants who received hospital or home RIC and were therefore able to gain an insight into the experience of both types of treatment delivery. There was also an even split between the number of males and females interviewed. Participants were not aware of treatment allocation when they were interviewed to avoid this

impacting their responses to the interview questions and to maintain blinding at 3- and 6-month telephone follow-ups.

Thematic analysis is flexible and provides a rich and detailed account of research data (Nowell et al., 2017). It is quick to learn, does not require detailed prior knowledge of qualitative methods, and is useful for summarizing key features of large data sets (Nowell et al., 2017). However, this flexibility can lead to lack of coherence and inconsistencies when developing themes. Furthermore, thematic analysis relies heavily on the interpretation of the researcher conducting the analysis (Nowell et al., 2017, Kiger and Varpio, 2020). In our study a second researcher reviewed the data and agreed the plausibility of the identified themes. Both researchers also had experience of conducting thematic analysis previously.

6.7 Conclusion

In conclusion, the interview responses highlighted that most participants felt like RIC improved their life in some way, whether that was improved energy levels, mood, sleep, concentration or level of activity. The responses from these interviews show that improvements in fatigue can have a big impact on someone's day to day life. Centre based delivery of RIC was acceptable but home-based delivery may offer a way to circumnavigate a number of identified barriers to treatment utility going forward. This study helps fill a gap in our knowledge about participant experiences of RIC including home-delivered RIC.

CHAPTER 7. REMOTE ISCHAEMIC CONDITIONING FOR FATIGUE AFTER STROKE: CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Conclusion

RIC is a relatively novel treatment that may be helpful for PSF because of the mechanisms of action (improved mitochondrial function, improved tissue perfusion, reduced oxidative stress) and how they might counteract the mechanisms of development of PSF (impaired mitochondrial energetics, inflammation, physical deconditioning).

In chapter two we undertook the first known pilot, randomised, placebo-controlled trial evaluating whether 6-weeks of researcher-or-home delivered RIC three times weekly is safe and tolerable to undertake in stroke patients with debilitating fatigue. The results of our study showed 6-weeks of RIC was safe and acceptable in these patients and patients were able to be recruited into a RCT like this.

A pilot RCT aims to help identify if there are any treatment signals that come from the intervention. In Chapter three we analysed some secondary outcome measures that were primarily aimed at identifying this. We used one of the most commonly used fatigue scales in neurological disease and stroke and found that RIC delivered three times per week delivered at home or at the center seemed to result in a trend towards a benefit in fatigue scores that were mirrored by an improvement in their walking distances and walking speeds. This appeared to be independent of any changes in cognition or mood that might confound these findings. While it is important to know this is a very small study and that the limitations of such studies might make us question the validity of these results.

On a physiological basis one of the things we wanted to understand is whether there were physiological outcome measures that may help us understand why such an effect on fatigue severity occurred amongst these individuals in the treatment arm. In chapter four, we describe one of the mechanistic evaluations that utilized cardiopulmonary exercise testing to try and help understand any potential treatment effect. We found that out of the cardiopulmonary outcome measures VO_{2peak} is the most strongly correlated with fatigue and 6MWT and the correlation between VO_{2peak} and 6MWT was statistically significant. While no obvious effect

of RIC was observed on $VO_{2peak,}$ there were mild trends towards increased VAT and reduced VE/VCO_2 slope which may support a move towards improved CRF and efficiency of energy production in the treatment group.

In chapter five, we describe the results of an exploratory study using a technique that looks to evaluate cellular energetics at tissue level. The results suggest that ATP seems to be well correlated with fatigue and measures of physical function (6MWT, MVIC) and represents a promising biomarker for PSF that requires further investigation. Also, when we look at the intervention effects RIC seems to result in improved levels of ATP in the tissue, potentially reflecting improved capacity for energy generation. As a consequence of this it potentially sheds light on the mechanism through which RIC has an effect on peripheral energy utilization and development of fatigue. RIC might improve the way the mitochondria create energy within the muscle. If energy production mechanisms are more efficient and there is less tissue lactic acid production, then we might hypothesize there is less of a need to ventilate more CO_2 which is why the VE/VCO_2 slope reduces. It might also help explain why the VAT increases i.e., aerobic ATP generating potential of tissues and muscles increases, more prominently in the stroke affected side, before it has to switch into a less efficient anaerobic method. The overall result might be that people are able to walk further and do more before experiencing fatigue.

The qualitative discussion in Chapter 6 demonstrates how these changes people experience lead to meaningful changes in their day-to-day life. In this chapter we acquired a greater understanding of what facilitated treatment completion (e.g., home-RIC, parking assistance) and what might make compliance difficult for people (e.g., issues with timer in the home-RIC group). Thus, we have a clearer understanding of how to optimize the treatment for future delivery in a larger trial and potentially in clinical practice.

7.2 Future directions

On the basis of the results of this study we conclude there is sufficient evidence to warrant a larger definitive research study looking at the efficacy of RIC for PSF. We have a better understanding of how to try and optimize the treatment for successful delivery and we understand which clinical outcome measures would be useful and practical to measure in a full-scale study. Further, we glean information as to which biomarkers may be helpful in understanding more about the mechanism through which any effects of RIC occur.

Appendices

Appendix 1

Section/Topic	Item No	Checklist item
	1a	Identification as a pilot or feasibility randomised trial in the title
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial
	2b	Specific objectives or research questions for pilot trial
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons
Participants	4a	Eligibility criteria for participants
	4b	Settings and locations where the data were collected
	4c	How participants were identified and consented
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial
Sample size	7a	Rationale for numbers in the pilot trial
	7b	When applicable, explanation of any interim analyses and stopping guidelines
Randomisation:		

Sequence generation	8a	Method used to generate the random allocation sequence
	8b	Type of randomisation(s); details of any restriction (such as blocking and block size)
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how
	11b	If relevant, description of the similarity of interventions
Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective
	13b	For each group, losses and exclusions after randomisation, together with reasons
Recruitment	14a	Dates defining the periods of recruitment and follow-up
	14b	Why the pilot trial ended or was stopped
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group
Numbers analysed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)
	19a	If relevant, other important unintended consequences
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility
Generalisability	21	Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence

	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments
Other information		
Registration	23	Registration number for pilot trial and name of trial registry
Protocol	24	Where the pilot trial protocol can be accessed, if available
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders
	26	Ethical approval or approval by research review committee, confirmed with reference number

Appendix 2

FATIGUE SEVERITY SCALE (FSS)

Date _____ Name _____

Please circle the number between 1 and 7 which you feel best fits the following statements. This refers to your usual way of life within the last week. 1 indicates "strongly disagree" and 7 indicates "strongly agree."

Read and circle a number.	Strongly Disagree →						Strongly Agree
1. My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
2. Exercise brings on my fatigue.	1	2	3	4	5	6	7
3. I am easily fatigued.	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
8. Fatigue is among my most disabling symptoms.	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7

Are you happy to be contacted in the future about future research studies? Yes/No

Appendix 3

Department of Geriatrics and Stroke Medicine

Faculty of Medicine, Dentistry & Health

Dr Ali Ali
Consultant Physician
Department of Geriatrics and Stroke Medicine
Sheffield Teaching Hospitals NHS Foundation Trust
Glossop Rd
Sheffield S10 2JF
United Kingdom

Telephone: +44 (0) 1142713789
Fax: +44 (0) 114
Email: ali.ali@sth.nhs.uk

Dear

I am writing to you to invite you on behalf of the Stroke Research team at the Sheffield Teaching Hospitals to consider taking part in a study that will be investigating whether applying a blood pressure cuff around your arm for short periods of time **(Remote Ischemic Conditioning)** is acceptable to patients after stroke, helps reduce feelings of fatigue, and improves physical performance. This study is funded and organised by the Ryder Briggs Neuroscience Research Fund, Sheffield Biomedical Research Centre and the Department of Neuroscience, Sheffield Teaching Hospitals.

Fatigue can affect up to 75% of individuals after stroke and can have severely negative impacts on rehabilitation and quality of life. Effective treatments are scarce and thus research looking at ways to treat fatigue are urgently needed. This study will look to see if Remote Ischemic Conditioning (RIC) can be undertaken **3 times a week for a period of 6 weeks** and whether it can help improve fatigue and physical performances in people who suffer stroke. To do this we aim to recruit patients who have had a stroke and suffer with fatigue.

What will be involved?

Patients that wish to be involved in the study will be asked to attend the Royal Hallamshire Hospital or the Assessment and Rehabilitation Centre (ARC) at Nether Edge Hospital to complete a consent form to participate in the study. During this visit you will also have a fitness test (we will see how far you can walk in 6-minutes) and will be asked to complete a series of questionnaires regarding your fatigue, mood, memory and quality of life. The researcher (PhD researcher from the University of Sheffield) will also take a blood sample and a heart tracing (ECG) for research purposes. You may be selected to have a specialised MRI scan of the legs, however this will be optional and you can opt out of this if you wish.

At a second visit, you will be asked to complete another fitness test but this time on a seated cycling machine. Following this you will either receive the **RIC treatment** or a **'dummy' treatment** which will involve inflating a blood pressure cuff around the upper arm for 5 minutes and then deflating it for 5 minutes. This cycle will be repeated 4 times in total **(40 minutes)**. The treatment will be delivered **three times a week for 6-weeks** at the Assessment and Rehabilitation Centre (ARC) at Nether Edge Hospital. In exceptional circumstances (e.g. you work full-time, COVID-19 restrictions), you may be given the option to self-administer the intervention independently at home. If you opt for home delivery then you will be trained on how to attach and inflate the remote ischaemic conditioning cuff. Alternatively, the researcher can arrange weekly home visits to administer the intervention to you at home.

At the end of the 6-week study period the same tests and questionnaires completed at the beginning will be repeated. You may also be asked to take part in a short interview, however this is optional. The researcher will then complete telephone follow up assessments at 3, 6 and 12 months. You are free to withdraw from the study at any time without giving a reason and all information gathered will be stored securely and treated as strictly confidential.

We have enclosed a copy of the patient information sheet and would be grateful if you would read it and consider whether or not you would wish to take part.

Will I get paid for the time I spend as part of the team?

Unfortunately we do not have enough resources to pay individuals directly for their time, but we will reimburse travel expenses or car parking charges that you incur through taking part in this study up to a maximum of £20 per study visit. Refreshments will also be provided at each study visit.
If you are interested in taking part, please ring the telephone number above or send an email with your contact details to bjemoyle1@sheffield.ac.uk, and member of the research team will get back to you with further information.
Yours sincerely,

Dr Ali Ali MBChB, MRCP, MSc
Chief Investigator
Sheffield Teaching Hospitals

Bethany Moyle
PhD researcher
University of Sheffield

Appendix 4

PARTICIPANT INFORMATION SHEET

Study title: Remote Ischemic Conditioning to improve Fatigue after Stroke (RICFAST) – a pilot randomised, single-blind, placebo controlled trial

Principal Investigator: *Dr Ali Ali* **STH 19508**

You are invited to participate in a research project to find out whether applying a blood pressure cuff around your arm for short periods of time (remote ischemic conditioning), is acceptable to patients after a stroke, helps reduce feelings of fatigue, and improves physical performance.

Before you decide whether to take part it is important that you understand why the research is being done and what it will involve. Please take time to read the following information sheet carefully and discuss it with friends, or relatives, if you wish.

Please ask us if there is anything that is not clear to you or if you would like more information.

1. What is the purpose of the study?

Fatigue can affect up to 75% of individuals after stroke and can have severely negative impacts on rehabilitation and quality of life. Effective treatments are scarce and thus research looking at ways to treat fatigue are urgently needed.

Remote ischemic conditioning (RIC) is a technique where a blood pressure cuff is inflated around a limb (e.g. an arm) for short periods of time. This may stimulate improvements in the blood flow to organs such as the brain and muscles, but also may improve the way in which cells use oxygen. The treatment has been safely used in patients with heart disease and stroke previously, mainly for its potential effects in reducing the amount of tissue damage after a blood vessel becomes blocked. It has also been used in athletes to help improve their physical performance.

This study will look to see if RIC can be feasibly undertaken 3 times a week for a period of 6 weeks and whether it can help improve fatigue and physical performances in patients who have suffered stroke.

2. Why have I been invited to take part?

You have been invited because you had a stroke and suffer with fatigue.

3. Do I have to take part?

Your participation in the study is entirely voluntary. Once enrolled in the study you can still withdraw at any time without giving a reason. If you decide not to take part, the medical care you receive will not be affected.

4. What will happen to me if I take part?

You will be asked to attend the Assessment and Rehabilitation Centre (ARC) at Nether Edge Hospital or the Royal Hallamshire Hospital to complete a consent form to participate in the study. You will then be asked to complete a questionnaire regarding the fatigue you suffer. If you score high enough and all the other eligibility criteria are met then you will be included in the study. The researchers will collect some personal and clinical details from you such as your diagnosis, your medications, age as well as some details about your stroke (type, size, location, test results). They will then assess how far you can walk in 6 minutes and will help you complete some questionnaires assessing your mood, memory, and quality of life. The researcher will also take a few blood samples and a heart tracing (ECG) for safety and research purposes. One blood sample will be stored for analysis of blood markers including immune and genetic tests at a later date. The first visit will take approximately 1.5 hours to complete.

In a second visit, you will be asked to complete a fitness test on a seated cycling machine. This will take around 20 minutes and will involve cycling at gradually more difficult level throughout the test until you cannot go any further. For part of the test you will be asked to breathe through a facemask. Once you have recovered completely from the cycle test (and your pulse rate has become normal again) the researcher will deliver the **RIC** or **'dummy'** treatment **(Figure 1)**. This will involve inflating a blood pressure cuff around the upper arm for 5 minutes and then deflating it for 5 minutes. This cycle will be repeated 4 times in total (40 minutes). The *'dummy'* treatment will involve inflating a blood pressure cuff around the arm, but to a different pressure. You will not know which treatment you are allocated until the results are analysed at the end. This procedure will be performed **three times a week for 6 weeks** at the Assessment and Rehabilitation Centre (ARC) at Nether Edge Hospital. At the end of the second visit you will be given a symptom diary to keep track of any side effects or problems that you encounter. As well you will be given a wrist monitor that measures your level of physical activity. This should be worn as much as possible during the study period.

In exceptional circumstances (e.g. you work full-time, COVID-19 restrictions) you will be given the option to self-administer the treatment independently at home. Alternatively, the researcher can arrange three times weekly home visits to administer the intervention to you at home. If you choose to administer the intervention yourself then during the first visit you will be trained on how to correctly attach the remote ischaemic conditioning cuff and inflate it to the correct pressure. The researcher will then provide you with a cuff and a digital timer for a trial period of at least 48 hours so you can practise using the device. During visit two the researcher will assess whether you can use the device properly. If you are able and willing to perform the intervention with competency you will be asked to use the device on your arm three times weekly for 6 weeks. You can perform the treatment at any time of the day. The researcher will call you twice weekly at your own convenience to check for any problems, troubleshoot issues with the device, and ensure you are happy to continue.

You may also be selected to have an MRI scan of the legs, however this is optional and you can opt out if you wish. If you are selected for an MRI and would like to take part you will first complete a questionnaire to make sure you have no metal fragments in your body. Next, you will be asked to perform small flexion/extension exercises of the knee, after which you will be asked to lie down on a table in the scanner. The scanner itself can be noisy but is usually well tolerated. The scan will take about 40 minutes.

Figure 1. Chronic remote ischemic conditioning being undertaken on a patient.
Ref: https://www.healthline.com/

Each week the researchers will ask how the treatment is going and if you have had any untoward symptoms or problems with the activity monitors. They will be able to help with any problems or questions you have. They will also ask if you are happy to continue in the study.

At the end of the 6 week study period you will not need to continue the RIC treatment. The researchers will repeat all the tests and questionnaires that you completed at the start of the study. We will aim to repeat the MRI scan, which may mean a short trip from the Sheffield Hallam University to the Royal Hallamshire Hospital. You will be asked if you are happy to have a short interview that will be recorded as well as audio recorded, about your experiences of the RIC treatment, however this will be optional.

The researchers will follow you up at 3, 6 and 12 months via telephone to see how you are getting on. After this time your involvement in the study will be complete.

A table summarising the study schedule:

Visit Number	Week	Assessment	Therapy	Duration (hrs)
1	1	• Screen eligibility. • Consent. • Walking test (6MWT). • Blood tests. • ECG. • Demographic and clinical details. • Healthcare utilisation. • Outcome measure assessments. • Training on self-administration of RIC (home delivery participants only)	• Nil	1.5 hours
2	1	• Cycle test (VO2 max) • Outcome measure assessments. • Delivery and explanation of symptom diary. • Explanation of activity monitor. • MRI of the legs – 8 participants. • Assessment of competency in self-administration of treatment (home delivery participants only)	• Treatment (RIC/dummy treatment).	1.5 hours
(3-19)	1-6	• Acceptability of treatment (RIC/dummy treatment). • Symptom diary review. • Activity monitor review. • Twice weekly telephone follow-up (home delivery participants only)	• Treatment (RIC/dummy treatment)	1 hour
20	6	• Cycle test (VO2 max). • Walking test (6MWT). • Blood tests. • ECG. • Demographic and clinical details. • Outcome measure assessments. • Activity monitor review. • Healthcare utilisation. • MRI of the legs – 8 participants.	• End of study intervention.	2.5 hours
21	*7*	• *Qualitative interview.*	• *Nil*	*0.75 hours*
(11) Telephone call	12	• Questionnaires. • Healthcare utilisation.	• Nil	1 hour
(12) Telephone call	26	• Questionnaires. • Healthcare utilisation.	• Nil	1 hour
(13) Telephone call	52	• Questionnaires. • Healthcare utilisation.	• Nil	1 hour

5. What are the possible disadvantages and risks of taking part?

Previous studies have shown that RIC is safe and well tolerated. The main side effects include discomfort and a 'pins and needles' feeling during cuff inflation in about 1 in 10 participants and minimal bruising associated with this (less than 1 in 30 participants).

Attending the study visits, having the tests and undertaking weekly RIC can be inconvenient and burdensome, but we will try to be as flexible as we can with these arrangements.

Some people may find it difficult to have an MRI scan (claustrophobia, panic), however, participants can opt out of having this test.

You are free to withdraw from this study if you find it too demanding on top of your other activities.

6. Will the treatment affect other symptoms of my stroke?

The researchers in this study will be undertaking memory tests to further evaluate any effects seen in this study. While there is no reason to think that RIC will affect any other symptoms caused by stroke, you will be asked about any changes in your health state at each study visit. If you have any concerns about the treatment, you are free to withdraw from the study at any time.

7. Will my participation affect my medical care in any way?

No, your medical care, including any physiotherapy you are undergoing on the NHS or privately can continue as normal, even if you are taking part in this study.

8. What if something goes wrong?

If you have a concern about any aspect of the study, you should ask to speak to the researchers who will do their best to answer your questions.

If you wish to complain formally, you can do this by writing to the study principal investigator (Dr Ali Ali, Sheffield Teaching Hospitals NHS Foundation Trust, Glossop Road, Sheffield S10 2JF) or telephone the NHS Complaints Procedure on 020 8672 1255.

Alternatively if you wish to speak to someone not directly involved in the study, you can contact the Patient Services at Royal Hallamshire Hospital (tel. 0114 2712400) or email PST@sth.nhs.uk.

9. Will I be reimbursed for expenses for taking part in the study?

Yes, we will reimburse travel expenses or car parking charges that you incur through taking part in this study up to a maximum of £20 per visit. Refreshments will also be provided at each study visit.

10. Will my taking part in this study be kept confidential?

Information relevant to your medical condition and results of your assessments will be treated as strictly confidential and securely stored on a password protected laptop, in a locked room at the Royal Hallamshire Hospital.

If you take part in the feedback interviews with the researchers, these will also be recorded. The recordings will only be listened to by a member of the research team and the words on the recording will be typed up and stored on a computer so that they can be analysed. Once this has been done, the recording will be erased. The written record of the interview will not contain your name or other personal details. Both the recording and the written record will be

kept secure at all times. We may use direct quotes from your recording when we explain the study results to others; however, no-one will be able to identify these as your comments.

Your GP will be informed by letter that you are taking part in the study. In addition, if any of your questionnaire responses raise concern about your medical condition, your GP will be informed.

If any other information is released outside the study office this will be done in a coded form with your name removed from the records so that confidentiality is strictly maintained. Data derived from the study will be stored in a secure computer database here at the Royal Hallamshire Hospital for a maximum of 15 years.

11. Will students be involved in this study?

Yes. A PhD student at the University of Sheffield will undertake the majority of the study treatments. Also, students doing their neurology MSc at the University of Sheffield will be involved in undertaking activities such as assisting with questionnaire completion and data analysis. They will also be involved in undertaking the qualitative interviews with participants. They will have received all the appropriate training to undertake all these activities before the study began.

12. Who is organising and funding the study?

This study is funded and organized by the Ryder Briggs Neuroscience Research Fund (Charity number 245122) and the department of Neurosciences Sheffield Teaching Hospitals.

13. How can I receive results of the study?

If you would like to receive the results of the study when they are available, please let one of the research team know and we will arrange for a summary to be sent to you by post.

Thank you for taking the time to consider participating in the study. Further information can be obtained from Dr Dr Ali Ali, Sheffield Teaching Hospitals NHS Foundation Trust, Glossop Road, Sheffield S10 2JF. Telephone 0114 271 1768. Alternatively, you can sent an email with your contact details to bjemoyle1@sheffield.ac.uk and a member of the research team will get back to you with further information.

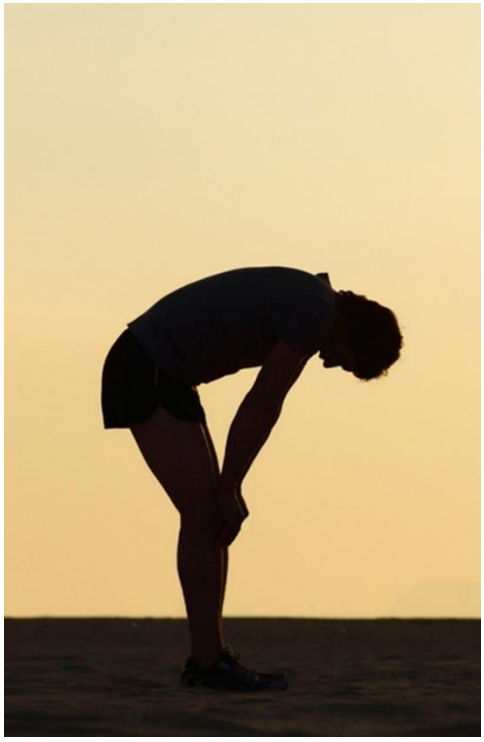
Appendix 5

Remote Ischaemic Conditioning for Fatigue After STroke (RICFAST). A randomised, single-blind, placebo controlled trial.

Patient Information Leaflet

Fatigue

Fatigue can affect up to 75% of patients who suffer a stroke. It can have negative impacts on rehabilitation, recovery and quality of life. Effective treatments for fatigue are lacking and research in this area has been highlighted as a priority.



Remote Ischaemic Conditioning

Remote Ischaemic Conditioning (RIC) is a technique where a blood pressure cuff is inflated around a limb (e.g. upper arm) for short periods that do not cause harm but stimulate changes that may improve blood flow to tissues and how efficiently cells use oxygen.

It has been used safely in patients with heart disease and stroke.

Study Questions?

- Is it feasible to use RIC daily after stroke?

- Can RIC help improve fatigue after stroke and help improve physical performance?

Why have you been chosen?

- You have been chosen because you have had a stroke.

Do I have to take part?

- No – your participation is entirely voluntary.

What will happen if I take part?

- researcher will contact you to arrange a time to meet.

- They will record some details such as your diagnosis, your medications, age as well as some details about your stroke (type, size, location, test results).

- You will have some blood tests, a heart tracing (ECG), and be asked to complete some questionnaires about what you can do, your mood and memory and levels of fatigue.

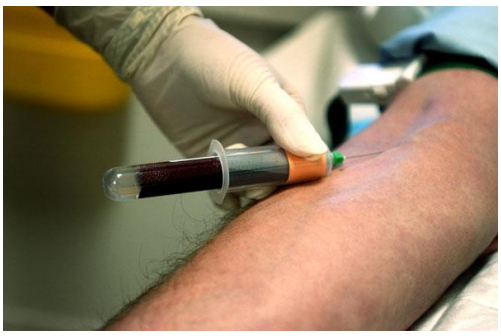
- The researchers will see how far you can walk in 6 minutes and test you on a seated bicycle machine. We will arrange for you to attend the Assessment and Rehabilitation Centre or Sheffield Hallam University to do this.

What will happen next?

- Some of the blood samples will be stored for analysis later on.

- You may be selected to have a special MRI scan of the legs.

    - You can however ***opt out*** of having this test if you like.



What will happen next?

- The researchers will give you a wrist monitor that measures your level of physical activity.

- They will demonstrate the RIC treatment with you

- This will be placed around the upper arm or leg.

- It will inflate for 5 minutes and then relax for 5 minute.

- This will be repeated 4 times in total.

What will happen next?

- We will arrange for you to attend the Assessment and Rehabilitation Centre or the Royal Hallamshire Hospitals 3 times a week to have this performed.

- In exceptional circumstances (e.g. you work full-time, COVID-19 restrictions) the treatment may be self-administered at home. You will be trained on how to do this.

- Alternatively, the researcher can arrange weekly home visits and deliver the intervention in your home if more convenient.

What will happen next?

- Each week the researchers monitor you and ask how the treatment is going.

- They will ask you to keep a diary of problems.

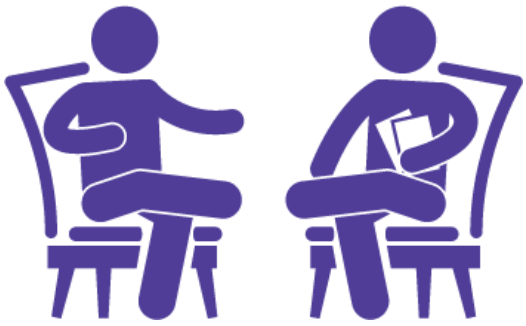
- They will ask if you are happy to continue.

- At the end of the 6 week study period the researchers will repeat all the tests and

questionnaires you completed at the start of the study.

What happens after that?

- The researchers will ask if you would be happy to have a short interview about the treatment (RIC).
- This will be recorded using a digital recorder.
- We will aim to find out what difficulties people found.



Follow up?

- The researchers will call you at 3, 6 and 12 months after the study to find out how you are getting on.

What are the risks of taking part?

- The RIC treatment may be uncomfortable and burdensome.

- Some people may find it difficult to have an MRI scan.

- Attending the study visit and interview can be inconvenient and burdensome.

- However, our researchers will be as flexible with you as they can.

What are the possible benefits of taking part?

- If RIC is effective at reducing fatigue then you may have benefited from the treatment.

- You will be contributing positively to our knowledge of stroke and this treatment.

- This may help future patients who suffer stroke.

What if something goes wrong?

- If you have any concerns about the study, then the researcher will be there to help.

- If you become distressed or unwell during the study, or you would like to stop for any reason that is fine.

- Your legal and medical rights will not be affected.

Will my taking part be kept confidential?

- Yes – all information collected about you during the study and the recorded interviews will be anonymised and kept on an encrypted laptop, secured in a locked office.

Who is organising the study?

- The department of Neurosciences, Geriatrics, and Stroke Medicine at Sheffield Teaching Hospitals.

- The Principle Investigator for this study is Dr Ali Ali, a stroke consultant at the stroke unit, Royal Hallamshire Hospital, Glossop Rd, Sheffield S10 2JF.

- Telephone: 01142711768.

Thank you for taking the time to consider this study.

Appendix 6

Study ID Number

PARTICIPANT CONSENT FORM – Version 3 13.04.20

Study title: Remote Ischaemic Conditioning to improve Fatigue after Stroke (RICFAST) – a pilot randomised, single-blind, placebo controlled trial

Principal Investigators: *Dr Ali Ali*

Please initial box

1) I confirm that I have read and understood the information sheet (version X dated XX/XX/XX) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2) I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected. ☐

3) I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4) I understand that some personally identifiable information (my name, address, date of birth and telephone number) will be collected but these data will be stored in a locked office in the hospital and be kept separate from other data collected during the study. ☐

5) I agree to have a blood sample taken which I understand will be stored and used for analysis of a number of blood markers and genetic tests related to future medical research projects. I understand that this is given as a gift. ☐

6) I agree to have an MRI scan at the start and at the end of the study treatment which will look at metabolic functions of the leg muscles (optional). ☐

7) I understand that my General Practitioner will be informed about my participation in the study and of any concerns about my medical condition which arise as a result of my participation ☐

8) I agree to take part in an audio recorded interview with one of the research team after the treatment is complete for feedback about how I found the treatment (optional). ☐

9) I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of witness (For patients who cannot write)	Date	Signature
Name of Person taking consent	Date	Signature

Appendix 7

Sheffield Teaching Hospitals
Royal Hallamshire Hospital
Glossop Road
Sheffield S10 2JF,
United Kingdom

Telephone: +44 (0) 114 2711768
Fax: +44 (0) 114
Email: ali.ali@sth.nhs.uk
ali.ali@sheffield.ac.uk

(Date)

Dear Dr

Re: (Name) (Date of birth)

The above patient from your practice kindly agreed to take part in a research study entitled:

"Remote Ischaemic Conditioning for Fatigue after Stroke (RICFAST) – a pilot randomised, single-blind, placebo controlled trial"

Your patient has agreed to participate in the above study. They will attend the Assessment and Rehabilitation Centre at Nether Edge Hospital for baseline assessments and to receive a physical activity monitor and will have RIC or sham performed 3 times weekly. This will involve inflating a blood pressure cuff around the upper arm for 4 cycles of 5 minutes and will be used for a total of 6 weeks. Follow up assessments will occur at 3, 6 and 12 months after the start of the study.

Remote ischaemic conditioning has been used safely in patients with cardiac disease and has been tolerated well. All patients will undergo an ECG tracing and routine blood tests (full blood count, electrolytes, liver function tests, inflammatory markers, coagulation profile). Any significant abnormalities highlighted will be communicated with you and a member of our research team will contact you to discuss management.

We ask that patients keep a diary of any additional physiotherapy they receive outside the study during the 6 week intervention period, but please feel free to refer your patient for any therapy that you consider as part of their clinical care.

Please feel free to ask us any questions you have about the study or raise any concerns you may have about your patient taking part. Our team can be contacted at the address above.

Yours sincerely,

Dr Ali Ali
MBChB, FRCP, MSc Chief Investigator

Appendix 8

Assessment of Competency in Delivering RIC/Sham Intervention

Step	Competent	Not competent
Applies blood pressure cuff securely around the bare upper arm		
Inflates device to designated pressure setting		
Sets digital timer for 5 minute interval and presses start		
Deflates blood pressure cuff at the end of the 5 minute interval		
Describes the intervals of further cycles (5 minutes on, 5 minutes off for 4 cycles)		

Appendix 9

Symptom diary for Remote Ischaemic Conditioning

Please score on a scale of 1-5 where **1= none and 5= extremely severe**, how would you rate your experience of the following symptoms/side effects during the therapy sessions **you have just had**?

Symptom	**Score**				
Overall discomfort	1	2	3	4	5
Skin irritation / redness	1	2	3	4	5
Pain	1	2	3	4	5
Weakness after	1	2	3	4	5
Pins and needles	1	2	3	4	5
How easy was it to use the Auto-RIC?					
Ease of use	Very easy	Easy	Fairly manageable	Difficult	Very difficult

Did you experience **any other** unwanted effects? Please give details and score 1-5 as detailed above.

Symptom / Problem	**Score**

Appendix 10

Remote Ischaemic Conditioning for Fatigue after Stroke (RICFAST)
Compliance monitoring diary

How many days did you complete a full RIC treatment cycle (40 mins) in the last week?

……..

How many days did you do an incomplete session (< 40 minutes) in the last week?

…….

Did you miss any days altogether? Yes / No *(circle)* How many? …….

Appendix 11

PHQ-9 Depression

Over the last 2 weeks, how often have you been bothered by any of the following problems?

(Use "✔" to indicate your answer"

	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things.....	0	1	2	3
2. Feeling down, depressed, or hopeless.....	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much.....	0	1	2	3
4. Feeling tired or having little energy.....	0	1	2	3
5. Poor appetite or overeating.....	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down.....	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television.....	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving .around a lot more than usual.....	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way.....	0	1	2	3

Column totals **___ + ___ + ____ + ___**

= Total Score _____

Appendix 12

GAD-7 Anxiety

Column totals: ___ + ___ + ___ + ___

Over the last 2 weeks, how often have you been bothered by the following problems? *(Use "✔" to indicate your answer"*	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3

= Total Score _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

Appendix 13

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about □

I have slight problems in walking about □

I have moderate problems in walking about □

I have severe problems in walking about □

I am unable to walk about □

SELF-CARE

I have no problems washing or dressing myself □

I have slight problems washing or dressing myself □

I have moderate problems washing or dressing myself □

I have severe problems washing or dressing myself □

I am unable to wash or dress myself □

USUAL ACTIVITIES (e.g. work, study, housework, family or leisure activities)

I have no problems doing my usual activities □

I have slight problems doing my usual activities □

I have moderate problems doing my usual activities □

I have severe problems doing my usual activities □

I am unable to do my usual activities □

PAIN / DISCOMFORT

I have no pain or discomfort □

I have slight pain or discomfort □

I have moderate pain or discomfort □

I have severe pain or discomfort □

I have extreme pain or discomfort □

ANXIETY / DEPRESSION

I am not anxious or depressed □

I am slightly anxious or depressed □

I am moderately anxious or depressed □

I am severely anxious or depressed □

I am extremely anxious or depressed

Appendix 14

MODIFIED RANKIN SCALE (MRS)

Rater Name: _____

Date: _____

Score Description

0 No symptoms at all

1 No significant disability despite symptoms; able to carry out all usual duties and activities

2 Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance

3 Moderate disability; requiring some help, but able to walk without assistance

4 Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance

5 Severe disability; bedridden, incontinent and requiring constant nursing care and attention

6 Dead

TOTAL (0–6): _____

Appendix 15

FEEDING
0 = unable
5 = needs help cutting, spreading butter, etc., or requires modified diet
10 = independent _____

BATHING
0 = dependent
5 = independent (or in shower) _____

GROOMING
0 = needs to help with personal care
5 = independent face/hair/teeth/shaving (implements provided) _____

DRESSING
0 = dependent
5 = needs help but can do about half unaided
10 = independent (including buttons, zips, laces, etc.) _____

BOWELS
0 = incontinent (or needs to be given enemas)
5 = occasional accident
10 = continent _____

BLADDER
0 = incontinent, or catheterized and unable to manage alone
5 = occasional accident
10 = continent _____

TOILET USE
0 = dependent
5 = needs some help, but can do something alone
10 = independent (on and off, dressing, wiping) _____

TRANSFERS (BED TO CHAIR AND BACK)

0 = unable, no sitting balance
5 = major help (one or two people, physical), can sit
10 = minor help (verbal or physical)
15 = independent _____

MOBILITY (ON LEVEL SURFACES)

0 = immobile or < 50 yards
5 = wheelchair independent, including corners, > 50 yards
10 = walks with help of one person (verbal or physical) > 50 yards
15 = independent (but may use any aid; for example, stick) > 50 yards _____

STAIRS
0 = unable
5 = needs help (verbal, physical, carrying aid)
10 = independent _____

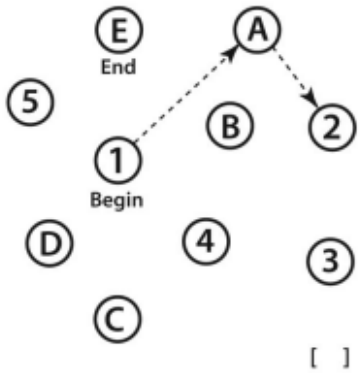
TOTAL (0–100): _____

Appendix 16

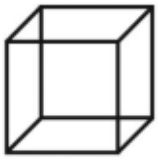
MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education :
Sex :
Date of birth :
DATE :

VISUOSPATIAL / EXECUTIVE



Copy cube



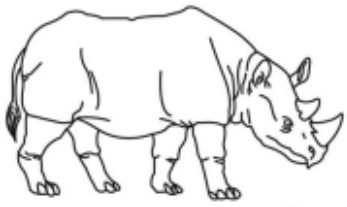
[ ] [ ]

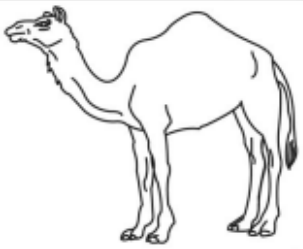
Draw CLOCK (Ten past eleven) (3 points)

[ ] Contour [ ] Numbers [ ] Hands

POINTS ___/5

NAMING





[ ] [ ] [ ]

___/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4

Subject has to repeat them in the backward order [ ] 7 4 2

___/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors

[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

___/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

___/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

___/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

___/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

___/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
		[ ]	[ ]	[ ]	[ ]	[ ]	
Optional	Category cue						
	Multiple choice cue						

___/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

___/6

© Z.Nasreddine MD **www.mocatest.org** Normal ≥ 26 / 30

TOTAL ___/30

Add 1 point if ≤ 12 yr edu

Administered by: _____

Appendix 17

Description of activity or Title of research project:	**Location(s): Collegiate Crescent Campus,**
Returning to research activity for 'the 'Remote Ischaemic Conditioning for Fatigue After Stroke (RICFAST) study' **(Funded by the University of Sheffield, and sponsored by Sheffield Teaching Hospitals NHS Foundation Trust).** **Participants/visits** -Participants – n=24 with stroke >6 weeks ago - No more than one participant per day -Each participant will attend twice; a baseline visit and follow-up visit six weeks later (2 hours per session). -Each maximal cardiopulmonary exercise test, including setup/equipment fitting will last up to 30 minutes. The CPET itself will last up to 12 minutes. Other procedures to last up to 90 minutes. -Quantitative research only **Environment** There is natural ventilation through windows. An antiviral fogger will be used after each testing session. **Equipment involved in maximal cardiopulmonary exercise testing** -Static cycle ergometer -Sterile silicone face mask -Washable facemask strap	**RA ref:** This risk assessment is to be used in conjunction with the risk management principles outlined in the BRATS02 bridging risk assessment. Staff working on this project will be required to familiarise themselves with both risk assessments before returning to research activity.

- Computer **Equipment involved in venipuncture** **-**Venipuncture needles -Vacutainers -Centrifuge -Freezer **Equipment involved in a walk test** - Pen/paper - Stopwatch	

Activity	Who could be harmed?	Risk	Initial risk level	Control measures to be implemented to minimise risk	Revised risk level
Put in this box the activity which may cause harm.	*e.g. participant / investigator / bystanders*	**Risk** of [*place in here the harm that may be caused*] **caused by** [*put the hazard (source of danger) in here*]	*Risk level **before** control measures are considered: Low, medium or high*	*What control measures will be put in place to minimise this risk?*	*What is the level of risk **following** the implementation of control measures*

Maximal cardiopulmonary Exercise Testing	Participants, investigators, other lab users	COVID-19 transmission Transmission may through Contaminated objects, such as CPET face masks, or contaminated surfaces. Person to person transmission hazard via respiratory droplets e.g. through breathing heavily during CPET	Likelihood of occurrence - 4 Hazard Severity – 5 Risk level = 20 (High)	Participants will be recruited and undergo the majority of baseline tests at Sheffield Teaching Hospitals, by a study doctor, prior to visiting Sheffield Hallam University. During baseline testing, participants will be asked whether they have had any symptoms of COVID-19 including: • A fever • New onset cough • Muscle aches • Loss of taste They will also be asked whether they have had contact with anyone that has COVID-19, or is awaiting the results of a COVID-19 test. After answering these questions, they will have their temperature taken and documented. If they answer yes to any of these questions or have a tympanic temperature >37.8˚C, recruitment will be delayed and local COVID-19 control protocols will be followed. Individual vulnerabilities will then be assed using the following resource, as a guide https://www.nhs.uk/conditions/coronavirus-	Likelihood of occurrence - 2 Hazard Severity – 5 Risk level = 10 (medium)

				covid-19/people-at-higher-risk/whos-at-higher-risk-from-coronavirus/ After screening, participants will be referred for their cardiopulmonary exercise test at Sheffield Hallam University, which will be completed within approximately 7 days. We will attempt to minimise this duration between testing, to lower the likelihood of participants contracting COVID-19 before they come to Sheffield Hallam University. Only one participant will be tested, per day. They will be advised to come ready for all testing, and will not use changing facilities. Participants will be asked to minimise contact with people outside of their immediate family until they have attending testing at Sheffield Hallam University. The minimum number of staff required to undertake research activity, safely, will be present on the day of testing. On the day of testing, two staff members (one where possible), will be wearing face masks (Type IIR surgical face mask; EN 14683 or equivalent), to greet the participant. Where more than one person is required to meet a participant, a managed process, including social distancing and other control measures outlined in BRATS02, will be put in place.	

				Entirely avoiding busy periods is impractical. However, participants will be asked to arrive for testing at 20 past the hour, to avoid busy transition periods which typically occur at the end of an hour, or at half past the hour. Participants attending for a cardiopulmonary exercise test will be met at the main lobby of Collegiate Hall, and will be advised to stay 2m away from other people, in the unlikely event that they arrive before the researchers. Patients will not be allowed to move beyond the entrance lobby until the following measures have been confirmed. 1) They are wearing a face mask, unless contraindicated. 2) They have immediately washed, or sanitise their hands. 3) Their tympanic temperature is <37.8˚c (investigator to measure), measured using a disposable ear inserts. 4) They have not had any COVID-19 symptoms in the last 14 days, including a fever, new onset cough, muscle aches, and loss of taste 5) They have not had contact with anyone that has COVID-19, or is awaiting the results of a COVID-19 test, within the last 14 days.	

				If these factors are not confirmed, participants will be asked to go home immediately. A stock of face covers, will be kept in the lab in case patients forget their own. Participants will be asked to self-isolate and book a COVID-19 test if they present with COVID-19 symptoms. If participants are sent home, the investigator will wash their own hands and sanitise the room that the patient was in, and inform their line manager, local PI, and study CI. Minimising the time between conducting a cardiopulmonary exercise test and leaving the lab is a practical and proportional risk to reduce the risk of COVID-19 transmission. Cardiopulmonary exercise testing must therefore, wherever possible, be the last investigation conducted on the day of testing, because it is the research activity that poses the greatest environmental risk. Investigators will wear a FFPIII face masks, whilst setting up and conducting the cardiopulmonary exercise test. Investigators will have received instructions on how to fit and remove masks safely, their limitations and how to dispose them safely. Disposable nitrile gloves and surgical gowns will also be provided by Sheffield Teaching Hospitals. Only when this PPE has been fitted will	

				patients be asked to remove their face mask, so that the cardiopulmonary exercise testing equipment, including a sterile, silicone face mask, can be fitted. During a cardiopulmonary exercise test, air is expelled from the front the face mask. Thus, the investigator will stand at least one meter away from the participant. They will also stand to the side, or behind the participant. This will minimise the risk of airborne virus transmission. It is not possible to stand further away than 1m because the investigator needs to operate the equipment attached to the patient, and monitor patients' health during the test. When the maximal cardiopulmonary exercise test is complete, the participant will be asked to remain on the exercise bike. The investigator will remove the silicone mask, whilst standing behind the participant. The patient will be ask to refit their own face mask. The silicone mask and other cardiopulmonary exercise test equipment will be disconnected. Equipment will be cleaned as outlined in the BRATS02 assessment. The silicone face mask will be immediately placed in bowl of pre-prepared sterilisation fluid. All other surfaces, including the end of the	

Maximal Cardiopulmonary Exercise Testing	Participants	Risk of musculoskeletal injury caused by extra demand placed on the musculoskeletal system.	Likelihood of occurrence: 3 Hazard severity: 3 Risk rating = 9 (Medium)	sample line, will be cleaned after 20 minutes, 'using a suitable virucidal spray or wipe, as described in BRATS02. The investigator will support the patient whilst dismounting the bike, and ask them to sit down at least two meters away from the investigator. Whilst the patient is recovering from the exercise test, the investigator will dispose of the nitrile gloves used during the maximal cardiopulmonary exercise test, and replace them with clean gloves. They will then open the windows, immediately, and sterilise each surface in the room, including the exercise equipment, computer keyboards, mouse, waste bin lid, and door handles/plates. After this, the investigator will remove and dispose of the surgical gown and nitrile gloves in a designated clinical waste bin. The patient will then be escorted off the premises, following the one-way system. Lab technicians will then use an 'antiviral fogger' in the room. The time when the laboratory next becomes available will be indicated on the door.	Likelihood of occurrence: 2 Hazard severity: 3 Risk rating = 6 (Low)
Maximal Cardiopulmonary exercise testing	Participants	Risk of cardiovascular complications caused by extra strain placed on the cardiovascular system due to exercise.	Likelihood of occurrence: 3 Hazard severity: 5 Risk rating: 15 = (High)		Likelihood of occurrence: 2 Hazard severity: 5 Risk rating = 10 (Medium)

				A pre-test medical questionnaire is used to screen out anyone who, for health reasons should not undertake the test. Participants are instructed in the correct technique before the test, including the correct adjustment of saddle height. Participants perform an appropriate warm up and cool down. Patients undertaking maximal cardiopulmonary exercise testing will be referred by a consultant physician. A pre-test medical questionnaire is used to identify anyone who, for health reasons should not undertake the test. The following are contraindications which preclude an individual from exercising (with the exception of allowances as determined in consultation with an appropriate doctor/GP or cardiologist) (ACPICR, 2015): - Unstable angina - Uncontrolled hypertension, that is, resting systolic blood pressure (SBP)	

Maximal Cardiopulmonary exercise testing	Participants	Risk of injury due to participant falling from cycle ergometer	Likelihood of occurrence: 3 Hazard severity: 4	>180mmHg, or resting diastolic blood pressure (BP) (DBP) >110mmHg - Orthostatic blood pressure drop of >20 mmHg with symptoms - Significant aortic stenosis (aortic valve area <1.0 cm^2) - Acute systemic illness or fever - Uncontrolled atrial or ventricular arrhythmias - Uncontrolled sinus tachycardia (HR>120 bpm) - Acute pericarditis or myocarditis - Uncompensated HF - Third degree (complete) atrioventricular (AV) block without pacemaker - Recent embolism - Acute thrombophlebitis - Resting ST segment displacement (>2 mm) - Uncontrolled diabetes mellitus - Severe orthopaedic conditions that would prohibit exercise - Other metabolic conditions, such as acute thyroiditis, hypokalaemia, hyperkalaemia or hypovolaemia (until adequately treated)	Likelihood of occurrence: 1 Hazard severity: 4 Risk rating = 4 (Low)

			Risk rating = 12 (Medium)	- Severe grade 3 rejection (cardiac transplantation recipients Appendix N). If individuals present with any of the above contraindications, the study doctor will be consulted, and the participant will be removed from the study as appropriate. Reference: ACPICR. (2015). Standards for Physical Activity and Exercise in the Cardiovascular Population, 2015. Retrieved from: https://www.acpicr.com/data/Page_Downloads/ACPICRStandards.pdf Participants perform an appropriate warm up and cool down exercise. Participants will be monitored visually, and using verbal communication, throughout the test, and during the test recovery period. Heart rate will be continuously monitored to ensure that the participant does not exercise beyond the intensity specified in the protocol.	Likelihood of occurrence: 1

Maximal Cardiopulmonary exercise testing	Participants, investigators, other laboratory users.	Risk of injury due to tripping on trailing cables.	Likelihood of occurrence: 3 Hazard severity: 4 Risk rating = 12 (Medium)	A first aid trained member of staff must always be present or in close proximity. A defibrillator is located in close proximity to the exercise test. Ensure the participant has a period of familiarisation before the test if they are unaccustomed to cycling. Check that the saddle and handlebars are properly secured. Participants are monitored throughout the test for signs of syncope. Help the participant to get down from the cycle immediately if they start to feel faint, and immediate lay them down on an examination bed, with feet elevated. Secure participants shoelaces and any loose clothing out of the way in order to avoid getting anything caught in moving parts of the cycle. Staff member to stand in close proximity to participant to provide assistance if participant falls from cycle ergometer, whilst remaining socially distanced.	Hazard severity: 4 Risk rating = 4 (Low) Likelihood of occurrence: 1 Hazard severity: 3 Risk rating = (Low) Likelihood of occurrence - 1
Maximal Cardiopulmonary exercise testing	Investigators	Risk of injury due to moving cycle ergometers.	Likelihood of occurrence: 4 Hazard severity: 3 Risk rating = 12 (Medium)		
	Investigators /Participants	COVID-19 Transmission	Likelihood of occurrence - 3		

Blood Sample/venepuncture			Hazard Severity – 5 Risk level = 15 (High)	All trailing cables in areas where people are expected to pass are to be taped to the floor or blocked using obstacles. In the event that this is not possible participants are informed of the trailing cable and the area is not to be entered, unless this is unavoidable.	Hazard Severity – 5 Risk level = 5 (Low)
Blood Sample/venepuncture	Participant	Bruising/embolism/nerve damage.	Likelihood of occurrence - 2 Hazard Severity – 4 Risk level = 8 (Medium)	Cycle ergometers are tilted on to their transport wheels when they are being moved. Persons should seek assistance rather than attempting to move cycle ergometers that are too heavy by themselves. Staff that regularly move ergometers (technical staff) to attend manual handling training.	Likelihood of occurrence - 1 Hazard Severity – 3 Risk level = 3 (Low) Likelihood of occurrence - 1
Blood Sample/venepuncture	Investigator	Risk of (non-COVID-19) infection from coming into contact with someone else's	Likelihood of occurrence - 2		

Blood Sample/venepuncture	Participant	contaminated blood. Puncture site becoming infected by general contamination from surroundings	Hazard Severity – 5 Risk level = 10 (Medium) Likelihood of occurrence - 2 Hazard Severity – 4 Risk level = 8	Participants will be wearing a face mask. Investigators will be wearing a FFPIII face, gloves, and a surgical gown. Once blood samples have been taken and stored, the investigator will safely remove their gloves, dispose of them in a clinical waste bin, and put a new pair on before resuming other testing activity. Participant Only those who have trained and hold up to date certification for venepuncture are allowed to collect venous blood.	Hazard Severity – 5 Risk level = 5 (Low) Likelihood of occurrence - 1 Hazard Severity – 4 Risk level = 4(Low)

Blood Sample/venepuncture	Participants/Investigators	Risk of injury from sharps (potentially contaminated with blood) used during the collection	Likelihood of occurrence - 3 Hazard Severity – 5 Risk level = 15 (High)	A medical questionnaire is used to screen out anyone who is not allowed to have blood taken. In addition, investigators with blood borne infectious diseases are excluded from collecting venous blood. Investigators check that their Hepatitis B immunisation is up to date. Appropriate PPP, including nitrile/latex gloves will be worn.	Likelihood of occurrence - 1 Hazard Severity – 5 Risk level = 5 (Low)
Blood Sample/venepuncture .	Participants	Risk of participant fainting/feeling nauseous due to phobia of needles or blood.	Likelihood of occurrence - 2 Hazard Severity – 3 Risk level = 6 (Medium)	All equipment used during the collection of blood is kept within the designated biohazard area. Investigator washes their hands and covers any wounds with plasters before the collection. Investigator wears disposable gloves throughout all procedures. A fresh pair is used for each participant and gloves are also changed if they become contaminated with blood. The investigator should wear either a disposable apron or lab coat. The venepuncture site is wiped with an alcohol	Likelihood of occurrence - 1 Hazard Severity – 3 Risk level = 3 (Low)

Six minute walk test	Participants/investigators	COVID-19 Transmission	Likelihood of occurrence - 3 Hazard Severity – 5 Risk level = 15 (High)	swab before the needles is inserted. Any blood spillages are immediately wiped up and all contaminated surfaces (including surfaces of equipment) are disinfected. The puncture site is covered with a plaster when the sample has been collected. Sharps and other consumable items are disposed of in the appropriate clinical waste container immediately after use.	Likelihood of occurrence - 2 Hazard Severity – 5 Risk level = 10 (Medium)
Six minute walk test	Participants	Risk of injury due to tripping on trailing cables.	Likelihood of occurrence: 3 Hazard severity: 4 Risk rating = 12 (Medium)	Blood is collected with the participant either seated or lying down. Where possible, this will be done with researchers stood to the side of participants. Anyone who feels faint is immediately helped to lay with legs raised.	Likelihood of occurrence: 1 Hazard severity: 4 Risk rating = 4 (Low)

				The investigation will be conducted within the same laboratory as all other investigations. Participants will walk between two cones, spaced 10m apart, for six minutes. Participants will wear a face mask whilst walking. The investigator will also wear a face mask and clean nitrile gloves. Investigators will be sit or stand more than 2m away from participants during the test. After the test, participants will be asked to sit back down on their chair. Participants to conduct test along a path without trailing cables	

All activity				**Contact tracing** If participant-PI interactions within the 1-metre envelop are kept to less than 1 minute, then, should of a participant returns a positive test for COVID19, the PI would not be classed as 'contact', thus allowing him/her to carry on working as s/he would not have to self-isolate. The same applies to interpersonal interactions lasting more than 15 minutes within the 2-metre envelope.	

Emergency Procedures
• Alert and inform appropriate medical/emergency services if adverse reactions manifest in participant due to any aspect of experimental procedures. • Stop immediately all testing activity if participant endures an accident/injury or intolerable discomfort arises from trial activities. • Testing investigators are hospital, immediate life support trained, and will act if/when necessary. • A spillage kit is always present to swab up sweat, saliva, blood or vomit

Monitoring Procedures
Continual monitoring of patient throughout testing and a 30-minute, post-testing period for signs of discomfort or adverse reactions to physical exertion. Ongoing monitoring of project to highlight any problems or areas of concern. Verbal and visual monitoring of participants by principle investigator and one other laboratory technician/physiologist to be performed continuously throughout the testing periods and with frequent monitoring visits to participants. Outside of trial contact time participants will be advised to seek medical attention if they experience any unwanted symptoms they may feel have resulted from any aspect of the data collection protocols used.

Communication of significant findings		
Method of communication (describe): *How will the findings of this assessment and its recommended control measurements be communicated to those who may be affected by the risks?* Risks and associated control measures will be recorded within the participant information sheet. A verbal reinforcement will be conducted prior to each laboratory trial to participants and testers.	**Person/people to communicate findings**: *Who will be responsible for communicating these findings?* . Dr Simon Nichols - Local PI. Dr Ali Ali – Study CI	Target date(s): 09/09/2020 to 30/04/2021

Approval - must be approved by Director of Studies / Line Manager / other appropriate person			
Carried out by: Dr Simon Nichols	Post: Senior Research Fellow	Signature: S Nichols	Date: 29/10/2020
Approved by:	Post:	Signature:	Date:

Review of risk assessment (not applicable to time-framed activities such as research projects of less than 12 months in duration)
The frequency of the review is (refer to guidance): every 12 months, or when any adverse event occurs.

Review date :	Carried out by:	Signature:

Review date :	Carried out by:	Signature:
Review date :	Carried out by:	Signature:

Appendix 18

Rating of Perceived Exertion (RPE) Category Scale

6

7 Very, very light

8

9 Very light

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard

20

Borg G. Borg's Perceived Exertion and Pan Scales. *Champaign, IL: Human Kinetics, 1998.*

Appendix 19

THE UNIVERSITY OF SHEFFIELD ACADEMIC UNIT OF RADIOLOGY

Magnetic Resonance Imaging Unit at the Royal Hallamshire Hospital

PATIENT & VOLUNTEER SCREENING FORM

Please complete this form prior to having your scan. Please circle the appropriate answer.

Have you ever had any surgery to your heart or chest e.g., cardiac pacemaker, replacement valves, stents or filters inserted? Yes No

Have you ever had any operation to your brain, e.g., aneurysm clips or shunts inserted?

Yes No

Have you EVER had any metal fragments in your eyes? Yes No

Are you or could you be pregnant? Yes No

Do you have an electronic or breast implant in your body? Yes No

Have you had any surgery of any type in the last 2 months? Yes No

YOU MUST RING THE UNIT IF YOU HAVE ANSWERED 'YES' TO ANY OF THE ABOVE QUESTIONS.

FAILURE TO DO SO MAY MEAN THAT YOU CANNOT BE SCANNED. TELEPHONE NO. 0114 2159595

Do you suffer from any heart disease or rhythm disorder? Yes No

Do you have any hearing problems, e.g. tinnitus? Yes No

Do you have any kidney problems? Yes No

Do you wear any removable metal dental work? Yes No

Do you suffer from epilepsy or diabetes? Yes No

Do you have any allergies? Yes No

Do you have any other metallic object in your body, e.g. metal fragments or surgical clips? Yes No

If so, please specify

Please remove all credit cards and loose metallic objects, e.g. watches, wallets, keys, money, glasses, jewellery (including body piercing), hearing aids, hair clips and skin patches. Lockers for your valuables are provided in the waiting area.

How much do you weigh? How tall are you?

If you have read and understood the above restrictions please sign below.

Print Name Signature Date

Appendix 20

PLEASE NOTE – INCORRECTLY COMPLETED REQUEST FORMS WILL BE RETURNED

THE UNIVERSITY OF SHEFFIELD

REQUEST FOR MAGNETIC RESONANCE IMAGING

MRI Number

Completed requests should be sent to
University MRI Unit, Academic Radiology, Floor C, Royal Hallamshire Hospital, Sheffield S10 2JF
Telephone: 0114 215 9595 Fax: 0114 271 1714

Appointment: Tuesday 22nd June 3pm

Surnam[redacted]
First Name[redacted]
Address:[redacted]
Date of Birth[redacted]
Telephone Number: (Home)
(Work)
(Mob[redacted]

Hospital: RHH
Ward/Dept: OPD IP/OP
Hospital No[redacted]
Referring Consultant: DR ALI ALI
Speciality: STROKE GERIATRICS CONSULTANT
NHS / PRIVATE / M/L / (Research)

Previous MRI Scans
Hospital Year

RELEVANT CLINICAL HISTORY/DIAGNOSIS

Stroke.
RICFAST STUDY

Continue on back of card if necessary

AREA TO BE SCANNED

- ☐ Brain
- ☐ Cervical Spine
- ☐ Thoracic Spine
- ☐ Lumbar Spine
- ☐ Pulmonary [functional]
- ☐ Pulmonary/MRA
- ☐ Cardiac
- ☐ Obstetric

JOINT / LIMB (please state) Legs

OTHERS (please state)

URGENCY – Urgent Requests not discussed with a CONSULTANT RADIOLOGIST will be treated as "soon"

URGENT	SOON	(ROUTINE)

IT IS **ESSENTIAL** THAT THE FOLLOWING INFORMATION IS GIVEN

Does the patient have any of the following? YES/NO If yes please specify

- ☐ Cardiac pacemaker/ history of cardiac surgery
- ☐ Intracranial aneurysm clip/ history of cranial surgery/ programmable shunt
- ☐ Metallic foreign body
- ☐ Metallic/electronic implant following surgery

FOR MRI USE ONLY
Sequences:

Is the patient pregnant? YES / (NO)

NB: THE SCANNER WEIGHT LIMIT IS 150kg

Referrer's Signature: Name (Block Letters)
Referrer's Status: Date:
Bleep No: Sec. Tel. No:

Appendix 21

Topic Guide for semi-structured interviews

Thank you for agreeing to take part in this study and thank you for agreeing to discuss your experience. Can I just check before we start that you are still happy to do this interview and that you are happy to have the interview recorded.

1) When you volunteered for this study, what were you expecting the treatment to be like?

- (Were you at all worried about the thought of using ischaemic conditioning?)

- (Did you expect it to be uncomfortable?)

- (What sort of therapy were you expecting?)

2) Tell me about the treatment you received in this study.

- (Were you able to do the conditioning every day?)

- (Did you experience any problems with the treatment?)

- (How did you feel about having ischaemic conditioning at home?)

3) Do you feel as though you responded to the treatment?

- (Do you think it made you any better or any worse? In what way?)

- (Did you feel like you could do more activity?)

- (Do you feel it helped with levels of fatigue?)

4) Would you be prepared to have this treatment again?

5) How do you feel about how frequently and for how long you had the treatment?

6) Is there anything else you would like to mention or discuss about your experience with the remote ischaemic conditioning?

Thank you for sharing your experience

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