# Exploring time-efficient strategies to improve fitness in older adults

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# Statement of Authenticity

I declare that I have completed this thesis myself, under the supervision of Mr Jonathan Lund and Dr Bethan Phillips at the Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, University of Nottingham.

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# Outputs from this thesis

## Peer reviewed publications

**Herrod PJJ,** Blackwell JEM, Moss BF, Gates A, Atherton PJ, Lund JN, Williams JP, Phillips BE. The efficacy of 'static' training interventions for improving indices of cardiorespiratory fitness in premenopausal females. *Eur J Appl Physiol* 2019*:***119**:645-52

**Herrod PJJ**, Doleman B, Blackwell JEM, O'Boyle F, Williams JP, Lund JN, Phillips BE. Exercise and other nonpharmacological strategies to reduce blood pressure in older adults: a systematic review and meta-analysis. *J Am Soc Hypertens.* 2018;**12**:248-67

## Published abstracts

**Herrod PJJ**, Lund JN, Phillips BE. Exploring time efficient strategies to improve fitness for surgery in older adults: A randomised trial. *BJS* 2018; **105**(S1):21

**Herrod PJJ**, Doleman B, Blackwell J, O'Boyle F, Lund JN, Phillips BE. Non-pharmacological strategies to reduce blood pressure in older adults: a systematic review and meta-analysis. *Lancet* 2017; 390:S43

## International and national oral presentations

**Herrod PJJ,** Blackwell JEM, Boereboom CL, Williams JP, Lund JN, Phillips BE. The time course of physiological adaptations to high intensity interval training (HIIT) in healthy older adults. European College of Sports Science, Prague (July 2019)

**Herrod PJJ,** Torkington AL, Blackwell JE, Battacharjee D, Sian TS, Williams JP, Lund JN, Phillips BE. Two weeks of time-efficient high-intensity interval training (HIIT) does not appear sufficient to elicit cardiorespiratory fitness gains in older adults. Europhysiology, London (September 2018)

**Herrod PJJ**, Lund JN, Phillips BE. "Exploring time efficient strategies to improve fitness for surgery in older adults: A randomised trial." Society of Academic and Research Surgery, Nottingham (January 2018)

## National Poster presentations

**Herrod PJJ**, Doleman B, Blackwell J, O'Boyle F, Lund JN, Phillips BE. Non-pharmacological strategies to reduce blood pressure in older adults: a systematic review and meta-analysis. UK Public Health Science Meeting. London (November 2017)

# Abstract


**Background**

Exercise interventions have the potential to reverse some of the health deficits associated with aging and improve the fitness of older adults, however rates of exercise uptake and adherence are strikingly low with "lack of time" a commonly cited reason.

**Methods**

This thesis carried out a systematic review of all non-pharmacological interventions to address one of these deficits (blood pressure), before describing a randomised controlled trial of three, time-efficient physical activity interventions: high-intensity interval training (HIIT), isometric handgrip (IHG) training and remote ischaemic preconditioning (RIPC) with the primary outcome of reducing resting systolic blood pressure.

**Results**

Meta-analysis demonstrated that physical activity interventions such as aerobic or resistance exercise can lead to significant reductions in resting blood pressure, however the majority of interventions require a minimum of 3 months. The

randomised controlled trial described in this thesis showed that 6 weeks of either HIIT or IHG can reduce resting systolic blood pressure by 9 mmHg, with HIIT also increasing anaerobic threshold (a marker of cardiorespiratory fitness) by 2ml/kg/min.

**Conclusions**

Six weeks of either the HIIT or IHG protocols described in this thesis can lead to both statistically significant and clinically relevant reductions in resting systolic blood pressure in healthy older adults in 6 weeks, with HIIT having additional benefits on markers of cardiorespiratory fitness. These protocols have the potential for use as physical activity interventions in older adults to improve various markers of health.

# Abbreviations

| | |
|---|---|
| ABP | Ambulatory blood pressure |
| AET | Aerobic exercise training |
| ANOVA | Analysis of variance |
| AT | Anaerobic threshold |
| BMI | Body mass index |
| BP | Blood pressure |
| CEUS | Contrast enhanced ultrasound |
| CI | Confidence interval |
| COM training | Combined aerobic and dynamic resistance exercise |
| CPET | Cardiopulmonary exercise test |
| CRF | Cardiorespiratory fitness |
| CVD | Cardiovascular disease |
| DBP | Diastolic blood Pressure |
| DEXA | Dual-energy X-ray absorptiometry |
| DICOM | Digital imaging and communications in medicine |
| ECG | Electrocardiogram |
| ERAS | Enhanced recovery after surgery |
| FMD | Flow mediated dilatation |
| FM% | Body fat percentage |
| HIIT | High intensity interval training |

| | |
|---|---|
| HOMA-IR | Homeostatic model assessment of insulin resistance |
| HR | Hazard Ratio |
| IET | Isometric exercise training |
| IHG | Isometric handgrip training |
| LBF | Leg blood flow |
| LM | Whole-body lean mass |
| LLM | Leg lean mass |
| MAP | Mean arterial pressure |
| MBF | Microvascular blood flow |
| MBV | Microvascular blood volume |
| MFV | Microvascular flow velocity |
| MVC | Maximum voluntary contraction |
| NICE | National Institute for Clinical Excellence |
| NHS | UK National Health Service |
| OGTT | Oral glucose tolerance test |
| RCT | Randomised controlled trial |
| RET | Dynamic resistance exercise training |
| RIPC | Remote ischaemic preconditioning |
| RPE | Rating of perceived exertion |
| SBP | Systolic blood pressure |
| SD | Standard deviation |
| VE | Minute ventilation |
| $VCO_2$ | Volume of carbon dioxide production |

| | |
|---|---|
| $VO_2$ | Volume of oxygen uptake |
| $VO_{2Peak}$ | Peak volume of oxygen uptake |
| $W_{Peak}$ | Peak workload |
| WHO | World Health Organisation |

# Statement of contributorship

I declare that I have completed this thesis myself, under the supervision of Mr Jonathan Lund and Dr Bethan Phillips.

For the systematic review that comprises chapter 2 of this thesis I sought assistance from Dr Brett Doleman (BD) and Dr James Blackwell (JB) who assisted with independent, blinded review of full texts for inclusion in the review and independent, blinded data extraction respectively.

I personally recruited and tested all participants in the studies listed in chapters 3-7 and conducted the majority of exercise training intervention sessions, however I was grateful for the assistance of some lab colleagues who covered a minority of the training sessions when I was unavailable. The same is true for the majority of the participants listed in chapter 8, with the exception of the "4-week" cohort, who were a cohort who had been previously recruited and trained by Miss Catherine Boereboom.

# 1 Introduction

## 1.1 Ageing population

The steady and continued rise in life expectancy that we are experiencing has been labelled a major health issue for the 21st century. The percentage of people aged >65 y in the UK increased from 15% in 1985 to 17% in 2010, an increase of 1.7 million people(Office for National Statistics 2012). By 2035 it is projected those >65 y will account for 23% of the UK population, while those >85 y will account for >5% (House of Lords Science and Technology Committee 2005). This shift towards an aged population presents numerous socio-economic and clinical issues for society and policy makers.

### 1.1.1 Socio-economic consequences of population ageing

An increase in the age of the population presents two significant socio-economic problems. Firstly, as the ratio of those of working age and in employment to those who are retired falls, the tax burden falls on a reduced number of working age adults, who must pay for older, retired citizens who may in turn make greater demands on social and health care (Spijker and MacInnes 2013). The UK government has steadily been increasing the retirement age and currently plans to further increase the state pension age to 67 between 2026

and 2028 (Age UK 2019), which may help to offset this imbalance. However the slowing in the economic growth of developed countries from the reduction in the working age populations of these countries is predicted to continue (Lee and Mason 2017).

Secondly, ageing is also associated with reductions in independence and an increased reliance on carers and potentially institutional care (Kingston et al. 2018). Complex modelling of the UK's projected care needs of older adults has predicted that the numbers of older adults requiring higher dependency care (requiring at least daily help to perform activities of daily living) will almost double between 2015 and 2035 (Kingston et al. 2018), whilst the UK Competition and Markets Authority, who conducted a report into the care home sector in 2017, estimated that the rise in population age may lead to an increase in demand for institutional care of between 14%-34% between 2015 and 2025 (UK Competition and Markets Authority 2017). The financial implications of this are vast, with the countries of the European Union projecting an approximate increase in spending of 1% of GDP on long term institutional care needs and a 1.5% of GDP increase in

healthcare spending over the next 30 years (Rechel et al. 2013).

In addition to the financial cost to the government from the increased need for publicly funded social care, there is an increasing amount of informal, unpaid care provided by friends and family, currently estimated to be worth between £57 billion to £100 billion per year (Kelly and Kelly 2018). Of note, only half of adults who identify themselves as carers are also in employment (Office for National Statistics 2019), and this may disadvantage them both financially and socially (Kelly and Kelly 2018).

### 1.1.2 Clinical consequences of population ageing

An increased age of a population brings increased prevalence of a wide range of diseases (Jaul and Barron 2017), which may have an impact on both the quality of life of an individual and their life expectancy, and also leads to primary care physicians managing increasing numbers of patients with multiple chronic conditions (Baird et al. 2016).

Diseases associated with ageing include hypertension (Knott and Mindell 2012), type 2 diabetes mellitus (Bullard et al. 2018) and sarcopenia (Shafiee et al. 2017), all of which also have a lifestyle component to their pathogenesis which may include sedentary behaviours (Leahy 2005; Beevers et al. 2001; Rolland et al. 2008). Sedentary behaviour has previously been demonstrated to increase with advancing age in population based studies (Diaz et al. 2016) and this could potentially be one mechanism by which all these disease processes become more prevalent with advancing age.

Low levels of physical activity in older adults have been hypothesised to be key cause of a large proportion of chronic disease and disability in later life (Booth et al. 2012) and conversely, a systematic review of 23 longitudinal cohort studies concluded that increasing levels of physical activity were associated with "healthy ageing" (albeit in this review healthy ageing encompassed a vastly heterogeneous number of outcomes including physical performance, survival to a certain age or the presence/absence of certain diseases) (Daskalopoulou et al. 2017).

## 1.2 Non-communicable impairments and disease in older adults and their impact

### 1.2.1 Blood pressure control and hypertension

There is a well-described trend towards increasing blood pressure of a population with age (Knott and Mindell 2012). Mechanisms are thought to mainly involve an age-related stiffening of large arteries (Pinto 2007) which often leads to isolated systolic hypertension, the most prevalent form of hypertension in older adults (Bavishi et al. 2016). Raised blood pressure is strongly linked to both the development of and death from cardiovascular disease, with meta-analysis of over a million patients robustly demonstrating this (Lewington et al. 2002).

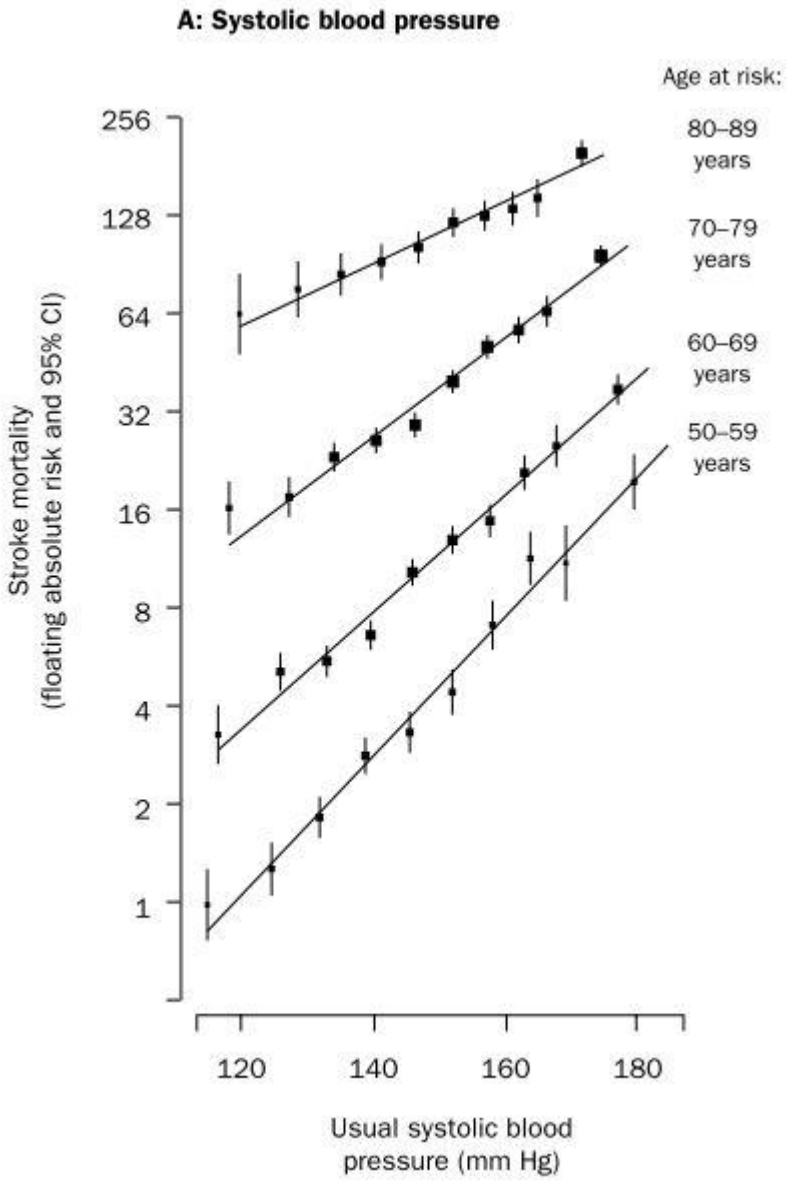


*Figure 1 Stroke mortality in each age decade versus systolic blood pressure (Figure taken from Lewington* et al. 2002)

Based on this well-established link between hypertension and cardiovascular disease, the control of hypertension remains the cornerstone of the management of cardiovascular risk throughout national guidelines (NICE 2011; Eckel et al. 2014). In support of this premise, the largest meta-analysis to date

of antihypertensive therapy, comprising 464,000 patients from 147 randomised controlled trials showed that a 10 mmHg reduction in systolic blood pressure or a 5 mmHg reduction in diastolic blood pressure was associated with a 22% reduction in coronary heart disease events and a 41% reduction in stroke events. This reduction was independent of the patient's initial blood pressure measurement, down to a threshold of 110 mmHg systolic and 70 mmHg diastolic, below which reduction had no further benefit (Law et al. 2009).

Lifestyle modification is recommended as the first line intervention for the treatment of hypertension in both the UK NICE guidelines (NICE 2011) and the guidelines from the American Heart Association (Eckel et al. 2014). Lifestyle modification strategies broadly include: i) increasing physical activity, ii) maintaining a healthy body weight, and iii) reducing salt intake (Appel 2003), although it must be noted that the majority of evidence for these recommendations come from studies in much younger adults and as with many other clinical guidelines, are vague in their advice in older adults (Jansen et al. 2015). The most recent UK National Health Service (NHS) recommendations on exercise lists exactly the same advice for

adults aged 65 and over as it does for those aged 19-64 (NHS 2018).

Although this first line intervention is given, as is the case across the adult population *per se,* uptake and adherence to lifestyle guidelines is poor (Mellen et al. 2008) and as such hypertension is most often managed pharmacologically with a variety of classes of medication (NICE 2011; Mancia et al. 2013; Ross and Macleod 2005). Antihypertensive medications have proven efficacy in the lowering of blood pressure and prevention of cardiovascular disease (Baguet et al. 2007), however compliance remains an issue as many patients dislike taking medication for an asymptomatic disease or experience problematic side effects (Misselbrook and Armstrong 2001; Ross et al. 2001).

The possibility of adverse events from pharmacological management for hypertension also potentially limit their use. The traditionally accepted cut-off for starting drug treatment in most clinical guidelines is >140 mmHg systolic or >90 mmHg diastolic (NICE 2011; Williams et al. 2018), however the latest version of the joint guidelines (2018) of the American College of Cardiology and the American Heart

Association recommends a lower threshold of >130 mmHg systolic or >80 mmHg diastolic, in adults with an estimated 10-year atherosclerotic cardiovascular disease risk of 10% or higher (Whelton et al. 2018).Reasons for setting the threshold for treatment at the level of 140/90 mmHg, despite evidence from large meta-analyses that cardiovascular disease risk may be reduced further by lowering blood pressure to 110/70 mmHg (Law et al. 2009), are related to the potential for increased incidence of side effects at lower blood pressures (Tinetti et al. 2014) or even reversal in risk direction in a phenomenon called the "J-curve" due to poor organ perfusion (Kang and Wang 2016). Recognised side effects associated with some medication, whilst aiming for stricter blood pressure control include acute kidney injury, electrolyte disturbances and potential for orthostatic hypotension and falls (Weiss et al. 2017).

The potential for benefit from more stringent and lower blood pressure targets in older adults has been investigated by the both the SPRINT trial and ACCORD BP trials, which demonstrated conflicting results from pursuing a more stringent systolic BP target of 120 mmHg compared to the

standard target of 140 mmHg, (The SPRINT research Group 2015; The ACCORD Study Group 2010).

In the SPRINT trial, which recruited hypertensives aged over 50 (but with an increased cardiovascular disease risk, but excluding diabetics), the arm randomised to intensive treatment achieved a mean systolic BP of 121 mmHg at 1 year, compared to 136 mmHg in the standard treatment arm. This was associated with fewer primary outcome events (a composite of myocardial infarction, other acute coronary syndrome, stroke, acute heart failure or cardiovascular death) in the intensive treatment arm with a hazard ratio (HR) of 0.75(95%CI: 0.64-0.89), with a number needed to treat of 61. However, this intensive treatment was also associated with more serious adverse events classified as related to treatment (including acute kidney injuries, syncope, electrolyte abnormalities and falls leading to injury) with a HR of 1.88.

The beneficial effect of intensive systolic BP lowering was however not demonstrated in the ACCORD study, which enrolled only diabetic patients. In this study the intensive treatment arm achieved a systolic BP of 119 mmHg at one year compared to 134 mmHg in the standard treatment arm, but

this did not lead to a significant difference in the composite primary outcome (HR0.88, 95% CI: 0.73-1.06). As with the SPRINT trial though, intensive treatment was associated with significantly more serious adverse events.

Thus, it may be that reducing systolic BP aiming at a stringent target of 120 mmHg, particularly in non-diabetic patients may lead to a reduction in cardiovascular disease events. As the majority of the adverse events are known side effects of the antihypertensive agents used in both studies, rather than resulting from the lower blood pressures *per se* (Lackland et al. 2018), achieving this through non-pharmacological means remains an attractive option.

### 1.2.2 Cardiorespiratory fitness

As with blood pressure, cardiorespiratory fitness (CRF) is a known parameter that declines with advancing age and is negatively associated with morbidity and premature mortality (Mandsager et al. 2018; Jackson et al. 2009). CRF is the ability of the circulatory, respiratory and muscular systems to supply oxygen during sustained physical activity (Lee et al. 2010a). CRF displays large inter-individual variation throughout a population (Laukkanen et al. 2009), but gradually decreases

with advancing age, with age-related declines demonstrated through both large repeated measures cohort studies (Jackson et al. 2009; Rogers et al. 1990) and large cross sectional studies (Hakola et al. 2011; Baur et al. 2012). High levels of cardiorespiratory fitness have been demonstrated to be associated consistently with improved life expectancy (Mandsager et al. 2018; Clausen et al. 2018) and conversely low levels are associated with poor survival in a range of patient cohorts including diabetics (Wei et al. 2000) and patients with ischaemic heart disease (Nichols et al. 2018).

Reduced cardiorespiratory fitness is also associated with a risk of becoming physically dependent in older age. For example in a longitudinal cohort of 373 older adults, low cardiorespiratory fitness at baseline was found to be an independent predictor of dependency over 8 year follow up (Paterson et al. 2004). This may lead to increased need for institutional care (McNally et al. 2017), with the socio-economic burden of this already outlined in section 1.1.1.

Cardiorespiratory fitness is also a major factor in determining an individual's ability to cope with and recover from a

physiological insult such as trauma or surgery (Older et al. 1999), and is often used to stratify preoperative surgical risk.

There are no guidelines for the management of low CRF in older adults, save for the WHO physical activity guidelines for all adults (World Health Organisation 2015) which will be discussed further in section 1.3.

### 1.2.3 Vascular health

Impairment of the peripheral vasculature is another physiological decline which becomes more prevalent with advancing age (Shammas 2007). These impairments found at both the macrovascular and microvascular level (Scioli et al. 2014) and are linked with the pathogenesis of arterial hypertension. These impairments at their worst can also lead to symptomatic peripheral vascular disease and atherosclerosis, with patients presenting with various sequelae ranging from intermittent claudication to limb threatening critical limb ischaemia (Shammas 2007).

Even in the absence of these symptoms, older adults exhibit reductions in whole limb blood flow in response to exercise

compared to younger adults (Donato et al. 2006). This age-related deficit is also found in studies examining microvascular blood flow both at rest and after exercise (Durham et al. 2010). These reductions in limb and microvascular blood flow may contribute to the pathogenesis of insulin resistance (another age-associated condition; see section1.2.5) as they may lead to reductions in peripheral insulin-mediated glucose uptake by skeletal muscle (Julius et al. 1992). Conversely acute exercise has previously been shown to increase both leg muscle microvascular blood flow in response to insulin and glucose uptake within the leg, compared to the contralateral unexercised limb (Sjøberg et al. 2017). In addition, a 6- week whole-body resistance exercise training (RET) programme has been shown to improve both muscle microvascular blood flow and insulin sensitivity in middle aged diabetics, further highlighting the interplay between the microvasculature of the skeletal muscle and insulin resistance (Russell et al. 2017).

The function of the arterial endothelium is another important component of vascular health. Abnormalities in the function of large arteries have long been known to be present in asymptomatic, middle-aged individuals with no overt vascular disease, and are significantly correlated with known risk

factors for the future development of vascular disease (such as smoking) (Celermajer et al. 1994). The measurement of arterial endothelial function (discussed in depth in section 1.4.3.1), also has the ability to predict future cardiovascular disease events, with a large meta-analysis of 14 studies (comprising 5547 participants ) demonstrating that impaired function is significantly associated with these (Inaba et al. 2010). Endothelial function is also known to reduce with age, with this having been demonstrated in several comparative cohort studies which have compared older and younger subjects after controlling for other confounding variables (Rodríguez-Mañas et al. 2009; Skaug et al. 2013).

Although no specific guidelines for improving vascular health exist, indeed neither macrovascular or microvascular blood flow nor endothelial function are routinely measured in the clinical setting, many health promotion and public health measures have previously been demonstrated to have a beneficial effect on the peripheral vasculature. For example, regular physical exercise is known to be beneficial to all measures of vascular health described above. For example, in a systematic review and meta-analysis 485 participants in observational studies comparing endothelial function in older

adults who performed regular aerobic exercise with sedentary controls demonstrated significantly greater endothelial function (Campbell et al. 2019). However, the same review also performed a meta-analysis of 4 intervention studies in sedentary older adults, where participants performed regular aerobic exercise for with intervention durations ranging from 2-12 weeks with no significant benefit to endothelial function (Campbell et al. 2019).

As for endothelial function, macrovascular blood flow both at rest and in response to exercise may be changed by exercise. Unlike endothelial function, it appears that regular physical activity may not have an impact on resting limb blood flow; in a study which compared this between three cohorts of older adults of varying habitual exercise there was no difference in resting limb blood flow (Dinenno et al. 2001). However several short term intervention studies have demonstrated both RET (Anton et al. 2006) and aerobic exercise (Lim et al. 2017) can improve resting limb blood flow. Additionally, short term aerobic exercise interventions have been demonstrated to increase leg blood flow in response to exercise in older adults (Beere et al. 1999). Finally, intervention studies for both aerobic exercise (Hurley et al. 2019) RET (Phillips et al. 2015)

in older adults have been demonstrated to improve microvascular blood flow.

### 1.2.4 Body composition and sarcopenia

In addition to functional changes relating to the cardiovascular and respiratory systems, body composition is also known to change with advancing age (Guo et al. 1999; Goodpaster et al. 2001). One of the most notable changes in body composition with advancing age is termed sarcopenia (Cruz-Jentoft et al. 2010); the age-associated loss of muscle mass (and function). Sarcopenia was defined by the 2010 European consensus statement as the presence of both low muscle mass and either low muscle strength or low physical performance (Cruz-Jentoft et al. 2010). This definition was revised in 2018, with the addition of low muscle quality as a defining criterion, thus meaning that a diagnosis of sarcopenia is now defined by both low muscle strength and either low muscle quantity or quality (Cruz-Jentoft et al. 2019). Sarcopenia is an incipient condition which in most individuals begins in the 4$^{th}$ decade of life, with a marked acceleration after the age of 70 years  Sarcopenia has an estimated worldwide prevalence 10% in those over 60 years of age (Shafiee et al. 2017), rising to 21% in those over 85 years (Dodds et al. 2017).

Although the role of muscle mass is often underappreciated in whole-body health, with many still only considering skeletal muscle to be important for locomotion and skeletal support, skeletal muscle has a vast range of physiological roles crucial for the maintenance of whole-body health (McLeod et al. 2016). For example, skeletal muscle is the largest glucose disposal site in the body and skeletal muscle insulin resistance is hypothesised to be a key component in the development of type 2 diabetes mellitus (DeFronzo and Tripathy 2009). Skeletal muscle is also the body's largest store of amino acids, which may be required to be mobilised during recovery from critical illness (Bloch et al. 2012) whilst cohort studies of critically ill patients have demonstrated sarcopenia to be an independent predictor of mortality in critical care (Moisey et al. 2013). Finally, muscle mass in older adults may be correlated with overall mortality. This has been demonstrated by follow up from the ilSIRENTE study, showing that both the presence of sarcopenia (defined in this study as a combination of reduced handgrip strength and a reduced mid arm muscle circumference) or low muscle mass alone were associated with all-cause mortality (Landi et al. 2010, 2013).

Sarcopenia is also important in older adults as it may lead to or co-exist with physical frailty (Cesari et al. 2014), which then in turn may lead to physical disability (Makizako et al. 2015). Frailty is a syndrome, defined by Fried and colleagues in 2001 which requires 3 from the following 5 criteria : unintentional weight loss, self-reported exhaustion, weakness (as measured by grip strength), slow walking speed and low physical activity in order to make a diagnosis (Fried et al. 2001).

Sarcopenia may be also be associated with a reduced ability to cope with and recover from physiological insults such as surgery (Friedman et al. 2015; Jones et al. 2017; Herrod et al. 2019b). In a systematic review of 24 studies comprising 5267 patients having major abdominal surgery, radiologically defined sarcopenia was associated with an increased risk of major complications, 30-day mortality, and 1-year, 3-year and 5-year survival (Jones et al. 2017) . This association is not only limited to surgical patients, in a cohort of 378 patients admitted to both medical and surgical wards in Amsterdam, low muscle mass was an independent predictor of both post-discharge mortality and additionally post-discharge falls (Reijnierse et al. 2018) , In older fallers, it has been shown that low skeletal muscle mass may also lead to an increased

risk of further falls and a potential loss of independence, functional decline and hospital admissions (Beaudart et al. 2017). Finally sarcopenia is associated with increased risk of all-cause mortality in both community-dwelling older adults and those in nursing home care (Landi et al. 2013; Zhang et al. 2018).

Juxtaposed to the issue of sarcopenia and also related to body composition, the United Kingdom is also currently suffering from an "obesity crisis", with the prevalence of obesity of 27% in 2015, having increased from 15% in 1993 (NHS Digital 2017). In older adults, the prevalence of obesity is estimated to be as high as 30% of 65-84 year olds, whilst up to 69% of this age group are overweight (NHS Digital 2015). Factors behind this rise in obesity on a population level are multi-factorial but are likely associated with reductions in physical activity from sedentary lifestyles and an increase in the availability of cheap, high calorie density foodstuffs (NHS 2019).

Obesity in older adults raises similar health issues as that in younger adults, leading to an increased risk of developing cardiovascular disease, hypertension, type 2 diabetes mellitus

and premature mortality (Zamboni and Mazzali 2012). In addition, large cohort studies have shown that obesity pre-existing before the age of 65 years is associated with the development of disability later in old age (Backholer et al. 2012).

Specifically in relation to older adults, one term that has been used in the literature in recent years is that of "sarcopenic obesity" (Stenholm et al. 2008), whereby sarcopenia and obesity co-exist. This co-condition seemingly has a worse prognosis for older adults than either sarcopenia or obesity in isolation (Baumgartner 2000) with individuals studies showing this for in both the postsurgical setting (Kamo et al. 2018; Carneiro et al. 2016) and in a meta-analysis of over 50,000 participants in prospective cohort studies of both community dwelling and hospitalised older adults (Zhang et al. 2019).

### 1.2.5 Insulin resistance

With clear links to both low muscle mass (Srikanthan and Karlamangla 2011) and increased adiposity (Kahn and Flier 2000), insulin resistance is also an age-associated phenomenon (Fink et al. 1983). Insulin resistance is the resistance of cells to insulin stimulated glucose uptake and is

a key step in the pathogenesis of Type 2 diabetes (Reaven 1988). In the UK there is an ongoing progressive increase in the prevalence of Type 2 diabetes (Diabetes UK 2018) as well as other precursors of diabetes, such as impaired glucose tolerance and impaired fasting glucose, which are linked to the increasing prevalence of obesity (Mainous et al. 2014). Currently 9% of the adult population of the UK, around 3.8 million people are diagnosed with diabetes (Public Health England 2016). Insulin resistance is also linked to the development of other non-communicable diseases including hypertension, hyperlipidaemia and atherosclerosis (Reaven 1988). Insulin resistance, as measured by HOMA-IR (a simple model of insulin resistance based on paired fasting glucose and insulin concentrations) can independently predict the development of cardiovascular disease in diabetic patients (Bonora et al. 2002).

Current management strategies for insulin resistance start with lifestyle modification interventions, which if not strictly adhered to may be ineffective (McAuley et al. 2002) and then rely on drug treatment with either metformin (Rena et al. 2017) or a newer antidiabetic agent such as a thiazolidinedione (Lebovitz and Banerji 2001). However, as with anti-

hypertensive medication, adherence to medication for an often-asymptomatic condition is poor (Polonsky and Henry 2016).

## 1.3 Current lifestyle modification recommendations

Lifestyle modification involving diet and exercise is the first-line intervention recommended in the management of hypertension (NICE 2011) and type 2 diabetes (NICE 2017) by the UK National Institute for Health Care and Excellence (NICE). Both guidelines are fairly vague in the recommendations they make for this, however particular emphasis is given to a reduction in sodium intake in the guideline for hypertension (NICE 2011), whereas emphasis is given to weight loss in the guideline for diabetes (NICE 2017). Regular physical activity is also recommended by the World Health Organisation (WHO) for the prevention of non-communicable diseases (World Health Organisation 2015). Current WHO physical activity guidelines for adults over 65 recommend a minimum of 150 minutes of moderate aerobic exercise or 75 minutes of vigorous exercise per week, in combination with some resistance exercise to maintain muscle function (World Health Organisation 2015). As with the NHS

recommendation for physical activity mentioned in section 1.2.1, the WHO physical activity guideline contains few differences between its recommendations for adults aged 18-64 and for adults aged 65 and over. This guideline does however recommend that aerobic exercise should be performed in bouts of a minimum of 10 minutes duration, raising the possibility of time-efficient, interval training exercise modalities which will be further discussed in section1.6.1. Unfortunately however, the adherence to many physical activity interventions and the WHO guidelines in older adults is consistently described as poor, with rates of adherence reportedly as low as 10% (Nawrocka et al. 2017; Jefferis et al. 2014; Luzak et al. 2017).

Reasons why older adults may not engage in regular physical activity have been previously investigated and although the main reason may be already poor physical health (Newson and Kemps 2007; Moschny et al. 2011), another key reason may be a perceived lack of time (Booth et al. 1997; Macera et al. 2017).

## 1.4 Measurement of deficits in health-related physiology in older adults

### 1.4.1 Measurement of Blood pressure

The first recorded measurement of blood pressure was made in 1733 by Stephen Hales, a clergyman, when he made measurements of the blood pressure of a horse by cannulating a crural artery and connecting this to a glass tube, observing the rise and fall of the blood in the tube (Booth 1977).

Non-invasive blood pressure measurement was developed a century later by Samuel von Basch and later refined by Scipone Riva-Rocci into a sphygmomanometer similar to that in use today (Booth 1977).

The Riva-Rocci method, which involved manual palpation of the radial pulse to determine systolic blood pressure, was further developed by Nikolai Korotkov, who, in 1905 described auscultating the artery distal to the occlusion caused by a Riva-Rocci cuff to determine both systolic and diastolic blood pressure accurately (Konstantinov 1998).

This method, which is still in routine clinical practice today, initially utilised mercury sphygmomanometers, but these have now mostly been replaced by aneroid sphygmomanometers (due to safety and toxicity concerns from mercury exposure) (A'Court et al. 2011).

However, increasingly in the modern health service blood pressure is measured by oscillometry using digital devices (A'Court et al. 2011), with numerous brands of device available. Oscillometry works by measuring the vibrations caused by blood flow beginning to return through an artery as a cuff is gradually reduced in pressure below the systolic pressure (Ogedegbe and Pickering 2010; Berger 2001; Geddes et al. 1982). Most devices directly measure mean arterial pressure (MAP) and use an algorithm to determine systolic and diastolic pressure indirectly (Babbs 2012)

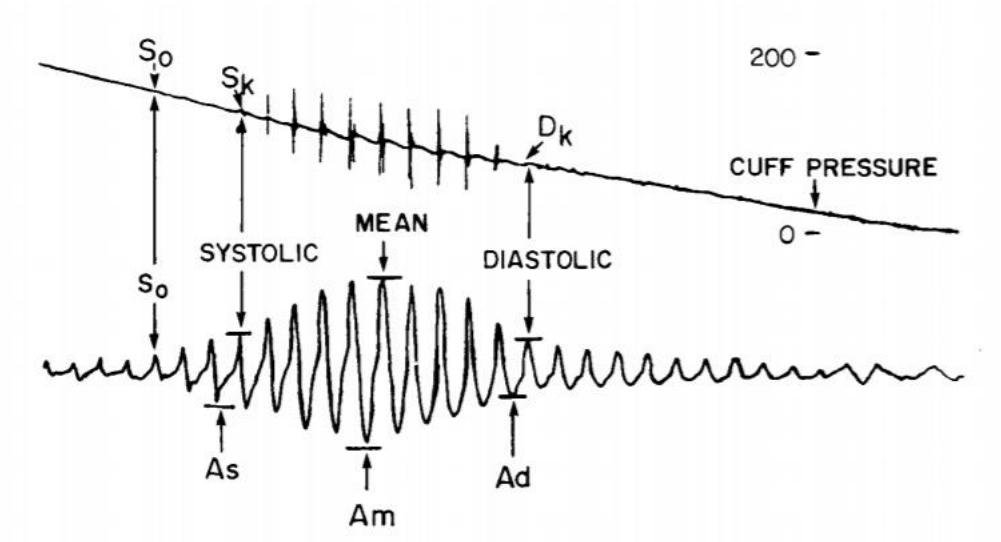


*Figure 2 Measurement of blood pressure using oscillometry, compared to the use of Korotkoff sounds superimposed above.*

*(Figure taken from Geddes et al. 1982).*

Digital devices have become the most commonly used method of measuring blood pressure in both primary (A'Court et al. 2011) and secondary care (Carney et al. 1999) in the UK with many devices validated and recommended for use by the British and Irish Hypertension Society (British and Irish Hypertension Society 2019).

Invasive measurements of blood pressure are still made in certain clinical situations by an arterial line, usually in a critical care environment or during anaesthesia where constant, real-time measurements of blood pressure is required to titrate vasoactive drugs (Lamia et al. 2005).

Blood pressure is by its nature a variable dynamic measurement that will be influenced by numerous factors including patient anxiety, posture, duration of preceding rest and which arm is used (O'Brien et al. 2003).

Therefore, national and international specialty associations have made recommendations to standardise blood pressure measurement in order to minimise measurement error (British and Irish Hypertension society 2017). The recommendations made by the British and Irish Hypertension Society are displayed in Figure 3.

# Blood Pressure Measurement

## Using Automated Blood Pressure Monitors

BIHS
British and Irish Hypertension Society
Committed to preventing cardiovascular disease

- The patient should be seated in a chair with a back rest and feet on the floor for at least 5 minutes and relaxed and not speaking
- Check the pulse. If any irregularity is found, use a manual device. A manual method of measurement should always be accessible
- The arm should be supported at the level of the heart, resting on a cushion, pillow or arm rest. Ensure no tight clothing constricts the arm
- Place the cuff on neatly 2cm above the brachial artery and aligning the 'artery mark'. The bladder should encircle at least 80% of the arm but not more than 100%




- Use the cuff size recommended by the manufacturer of the monitor
- Some monitors allow manual blood pressure setting selection where you choose the appropriate setting. Other monitors will automatically inflate and re-inflate to the next setting if required. Warn the patient that this might happen to avoid alarm
- Repeat 3 times and record measurement as displayed. Initially test blood pressure in both arms and use arm with highest reading for subsequent measurement

### Points to note:

It is good practice to occasionally check the monitor against other validated devices, using the same patient at the same time and not connecting the two machines together.

It is important to have the monitor serviced and calibrated according to the manufacturer's guidelines

*Figure 3: British and Irish Hypertension Society recommendations for blood pressure measurement*

*(From British and Irish Hypertension society, 2017)*

#### *1.4.1.1 Ambulatory blood pressure measurement*

The acute variations that may exist in blood pressure measurements made in clinic may also be affected by the phenomenon of white coat hypertension. This is defined as an increase in blood pressure measurements made by a clinician, compared to those made in the patients' own environment in the absence of a clinician (Cobos et al. 2015) and was first described in 1988 by Thomas Pickering (Pickering et al. 1988).

Subsequent to this, advances in technology led to device manufacturers developing monitors that were lightweight and small enough to be easily portable to facilitate ambulatory measurement of a patient's blood pressure (Head 2014). Because of this the UK NICE guidelines on hypertension recommended the use of ambulatory blood pressure monitoring when making a diagnosis of hypertension, in order to minimise over diagnosis owing to white coat syndrome (NICE 2011).

The most recent systematic review of studies comparing ambulatory blood pressure to clinic measured or home measured blood pressure showed that these one-off blood pressure readings may over diagnose hypertension in a

substantial number of patients and recommended they be confirmed by ambulatory readings before beginning treatment (Hodgkinson et al. 2011).

### 1.4.2 Measurement of cardiorespiratory fitness

A variety of methods exist for measuring cardiorespiratory fitness which range from very simple and easy to perform, walking measures for example, to advanced clinical measurements such as a cardiopulmonary exercise test (CPET). Simple measures, such as the validated 6-minute walk test (Moriello et al. 2008) have minimal equipment requirements (a stop watch and a corridor)(American Thoracic Society 2002) and can give a global measure of a subject's functional exercise capacity (Casanova et al. 2011), however these measures do not give much insight into underlying physiological processes.

Conversely, a CPET the gold standard measure of exercise capacity (Tran 2018) is fairly laborious for both patient and operator. The key feature of a CPET, which distinguishes it from other exercise testing methods (such as a stress ECG) is the measurement of respiratory gas volumes and concentrations either through a mask or mouthpiece which

allows a deeper insight into a subjects underlying physiological responses to exercise (Albouaini et al. 2007). Computer performed breath-by-breath analytical techniques for the measurement of inspired and expired gasses (first described by the laboratory of Wasserman and Whipp (Beaver et al. 1973)) allows real time measurement during exercise. Prior to this development, gas exchange had been measured by the Douglas bag technique, which limited the frequency of measurements and was prone to error (Shephard 1955).

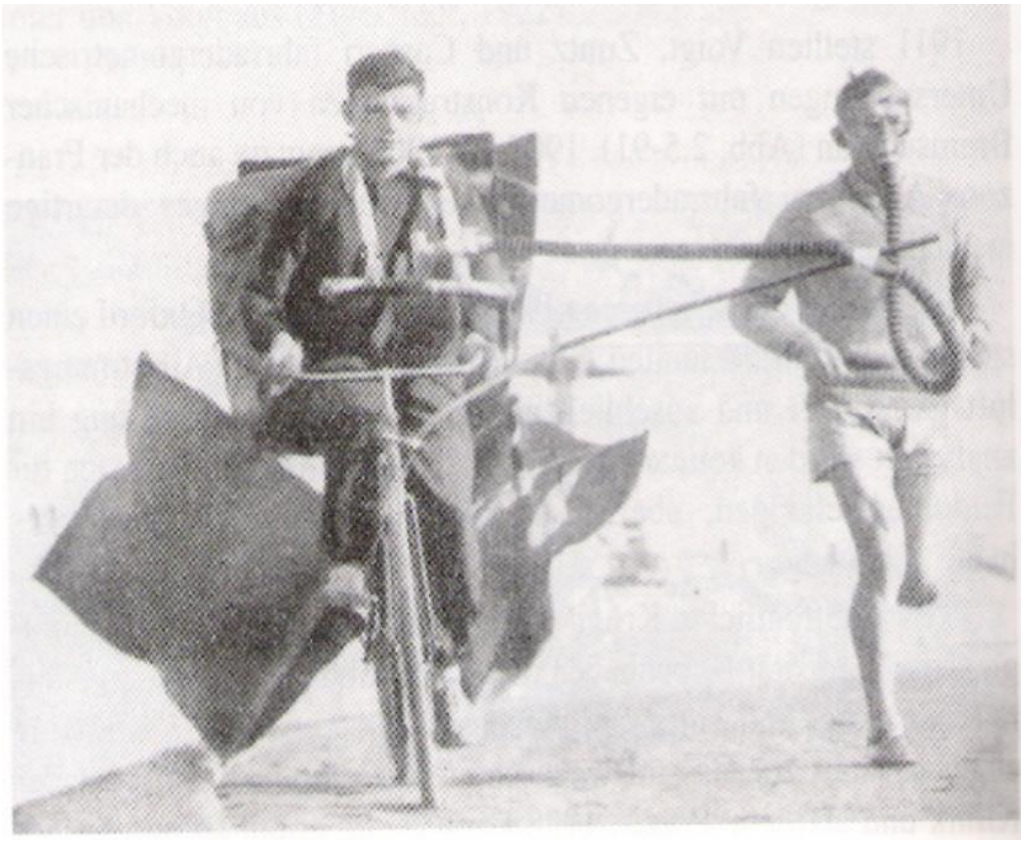

*Figure 4 Collection of expired respiratory gas using the Douglas bag technique.*

*Image taken from [http://www.pftforum.com/history/wp-content/uploads/2015/01/Douglas_Bag_1928_Exercise.jpg](http://www.pftforum.com/history/wp-content/uploads/2015/01/Douglas_Bag_1928_Exercise.jpg) [accessed 18/8/19)*

A variety of protocols exist for conducting a CPET, however in a clinical context they are usually conducted in accordance with the guidelines set by the American Thoracic Society and American College of Chest Physicians in 2003 (Weisman et al. 2003), with similar guidelines for the UK recently produced by the UK Perioperative Exercise Testing and Training Society (Levett et al. 2018).

Briefly, the fundamental procedure for performing a CPET involves continuous monitoring of a subject, through pulse oximetry, non-invasive blood pressure measurement, continuous ECG monitoring and breath-by-breath respiratory gas measurement before, during and after a period of continuous, supervised, incremental exercise (Albouaini et al. 2007).

Different exercise modalities for CPET have been described. The most commonly utilised modalities are treadmill running (Holm et al. 2014), upright cycle ergometry (Reeves et al. 2018) and recumbent cycle ergometry (Cornelis and Buys 2016), however some laboratories may use an arm-crank

ergometer for subjects with impaired mobility of their lower limbs (Mitropoulos et al. 2017).

Although treadmill running represents the modality utilised in a large number of clinical and healthy volunteer studies in the USA (Balady et al. 2010), the majority of UK clinical centres (and healthy volunteer research groups) use upright cycle ergometry to conduct their CPETs (Reeves et al. 2018), with treadmill tests most commonly performed by athletic populations (Losnegard and Hallén 2014; Kalapotharakos et al. 2011).

The exercise modality used for performing a CPET must be controlled when comparing results. Tests conducted on a treadmill may recruit more muscle mass and lead to higher values in the same individual when compared to tests carried out with cycle ergometry (Millet et al. 2009). This is likely due to differences in the muscle recruitment patterns between the exercise modalities, with cycling utilising predominantly the quadriceps and plantar flexors, whilst running recruits far more lower limb muscles (Bijker et al. 2002) and the upper body (Millet et al. 2009). However this difference can be eliminated and then reversed in trained cyclists, such that the VO2 max

they obtain measured during cycle ergometry may exceed that from a treadmill test (Ricci and Léger 1983). This makes comparison between values obtained from CPETs using different modalities difficult.

Most gas exchange values obtained from a CPET are reported as relative to unit bodyweight, with the most commonly reported unit in the literature being ml/kg/min (Levett et al. 2018). This standardisation is necessary as larger subjects have a larger muscle mass and will therefore utilise more oxygen and produce more carbon dioxide both at rest and at exercise (Weisman et al. 2003). The limitation of reporting values in this way is that it will underestimate the fitness of overweight and obese individuals, whose excess body fat whilst not participating in exercise through increasing respiration, will raise the denominator (Weisman et al. 2003). This can be addressed by standardising to fat free mass instead of total mass (Goran et al. 2000).

In addition to the choice of exercise modality, the choice of incremental exercise protocol used may also affect results generated by a CPET. Broadly, protocols can be divided into stepwise or ramp based protocols (Mezzani 2017) where the

workload steadily increases in incremental "steps" or gradually in a ramp-like fashion (Figure 5). Step protocols were initially vital when expired respiratory gases were being collected by the Douglas bag method in the early to mid-20th century, however the development of computer analysed breath by breath analysis of respiratory gases allowed the development of ramp protocols (Murias et al. 2018).

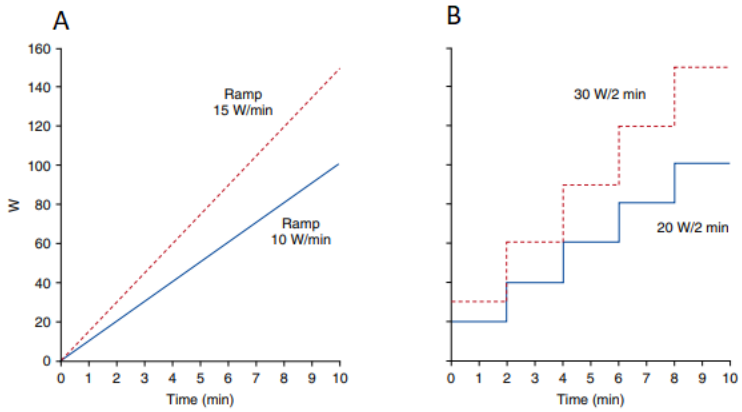


*Figure 5: (A) Ramp incremental and (B) incremental stepwise CPET protocols (Mezzani 2017)*

Although both ramp and step protocols are still in use in laboratories, ramp protocols are often preferred as they do not contain sudden increases in resistance which may make interpreting gas exchange data more challenging (Mezzani 2017). As such, ramp protocols are recommended by the

POETS guideline for use in clinical populations (Levett et al. 2018).

Regardless of the incremental pattern chosen, several key variables can be determined by a CPET, each of which are discussed in detail below.

#### *1.4.2.1 Maximal oxygen uptake ($VO_{2Max}$)*

$VO_{2max}$ is the maximum volume of oxygen that an individual can utilise per unit time at peak exercise. $VO_{2max}$ is the gold standard measure of aerobic fitness (Shephard 1984) and reflects the combined ability of a subject to extract oxygen from the inspired air and deliver it to tissues for respiration (Wasserman et al. 2011).

It is important to distinguish $VO_{2max}$ from $VO_{2peak}$. $VO_{2max}$ is the maximum value physiologically possible for an individual to achieve at a given attempt on a protocol, however it is not achieved in all CPET attempts. $VO_{2peak}$ is the maximum volume of oxygen extracted during a CPET (when a participant has reached volitional exhaustion), *but is not necessarily a physiological maximal value* (Poole and Jones 2017).

Various criteria are used to determine whether a $VO_{2peak}$ value represents a $VO_{2max}$. The most robust and highly regarded criteria for a test being designated "maximal" is the achievement of a plateau in a graph of $VO_2$ against time, implying that despite an increase in work performed, there is no increase in oxygen consumption (Howley et al. 1995). However as this is not always observed, other criteria commonly in use include a high level of blood lactate (most commonly from finger or ear-lobe prick samples), a respiratory exchange ratio (RER) >1.15, an achievement of >95% of the age-related predicted maximum heart rate (commonly 220-age (y) (Fox et al. 1971)) or a high rating of perceived exertion on a Borg scale (Howley et al. 1995). Recent changes to CPET practice in research laboratories have recommended a $VO_{2max}$ verification phase of a CPET, which should follow a ramp incremental test. In the protocol described for example by Rossiter and colleagues, participants cycled at 20 W for 5 minutes after they performed a ramp incremental test to volitional exhaustion, before performing a second, constant load test set at 105% of their power output at the termination of the ramp incremental test ($W_{peak}$) (Rossiter et al. 2006).

Although a $VO2_{max}$ is a more robust physiological value and $VO_{2peak}$ has a larger subjective element to it, large numbers of older patients when being asked to exercise to volitional exhaustion will not meet the criteria for a $VO_{2max}$ test (Huggett et al. 2005). Indeed a large proportion of clinical labs providing a CPET service for patients report $VO_{2peak}$ values instead of $VO_{2max}$ and the validity of $VO_{2peak}$ as a measure of fitness is argued for by scientists from the Wassermann lab (Green and Askew 2018; Azevedo et al. 2018) who state that it is a valid measure of physical fitness and is endorsed by both the ATS ACCP guideline and the British POETS guideline (Levett et al. 2018; Weisman et al. 2003).

#### *1.4.2.2 Anaerobic threshold (AT)*

The anaerobic threshold (AT) is the point during an incremental exercise test at which there is a sustained rise in blood lactate concentration above resting levels, due to an increasing contribution of anaerobic glycolysis to metabolism (Wasserman and McIlroy 1964). The AT is a submaximal exercise measure, and is described as an exercise capacity below which continued exercise is sustainable (Older and Levett 2017). The AT is of interest for two reasons, its reliability of determination (Gaskill et al. 2001) (without a

volitional component) and its clinical significance (Older et al. 1999). Anaerobic threshold can be determined either invasively through repeated blood sampling or non-invasively through analysing expired respiratory gases (Weisman et al. 2003).

Invasive determination of the anaerobic threshold can be carried out by repeated sampling of either blood lactate or blood bicarbonate but requires either the placing of either an arterial or venous cannula, or repeated arterial or venepuncture. This is rarely carried out clinically and not included in clinical CEPT guidelines (Levett et al. 2018) but may be used in the research setting (Gaskill et al. 2001; Albouaini et al. 2007).

The majority of clinical laboratories determine the AT through the non-invasive analysis of expired respiratory gases. This is possible due to the Henderson-Hasselbach equation, which when applied to the bicarbonate buffering system, dictates that a decrease in the pH of the blood, due to excess acid production (in this case lactic acid), leads to buffering by bicarbonate in the blood leading to the production of carbon

dioxide Figure 6 Bicarbonate buffering system as described by the Henderson- Hasselbach equation.

$$H^+ + HCO_3^- \leftrightharpoons H_2CO_3 \leftrightharpoons CO_2 + H_2O$$

*Figure 6 Bicarbonate buffering system as described by the Henderson- Hasselbach equation*

This excess carbon dioxide is exhaled and detected by the gas analyser during the CPET. Thus non-invasive determination of the AT is dependent on detecting this "excess" $CO_2$ (Beaver et al. 1986).

Despite this theoretical underpinning of non-invasive determination of AT, some authors have argued against describing AT's determined non-invasively as true ATs and refer to this as the ventilatory threshold (VT), insisting that a true measurement of the AT must involve invasive determination of blood lactate (Pallaré et al. 2016).

Despite this argument, methods of determining the AT non-invasively have been described in both the ATS/ACCP and POETS CPET guidelines (Weisman et al. 2003; Levett et al.

2018). These both recommend picking the AT as the point at which there is an inflection in the gradient of $VO_2/VCO_2$, a change in the VE graph and an increase in the PET $O_2$ graph as shown below (Figure 7). AT determined in this way is reported as the $VO_2$ at which this inflection occurs.

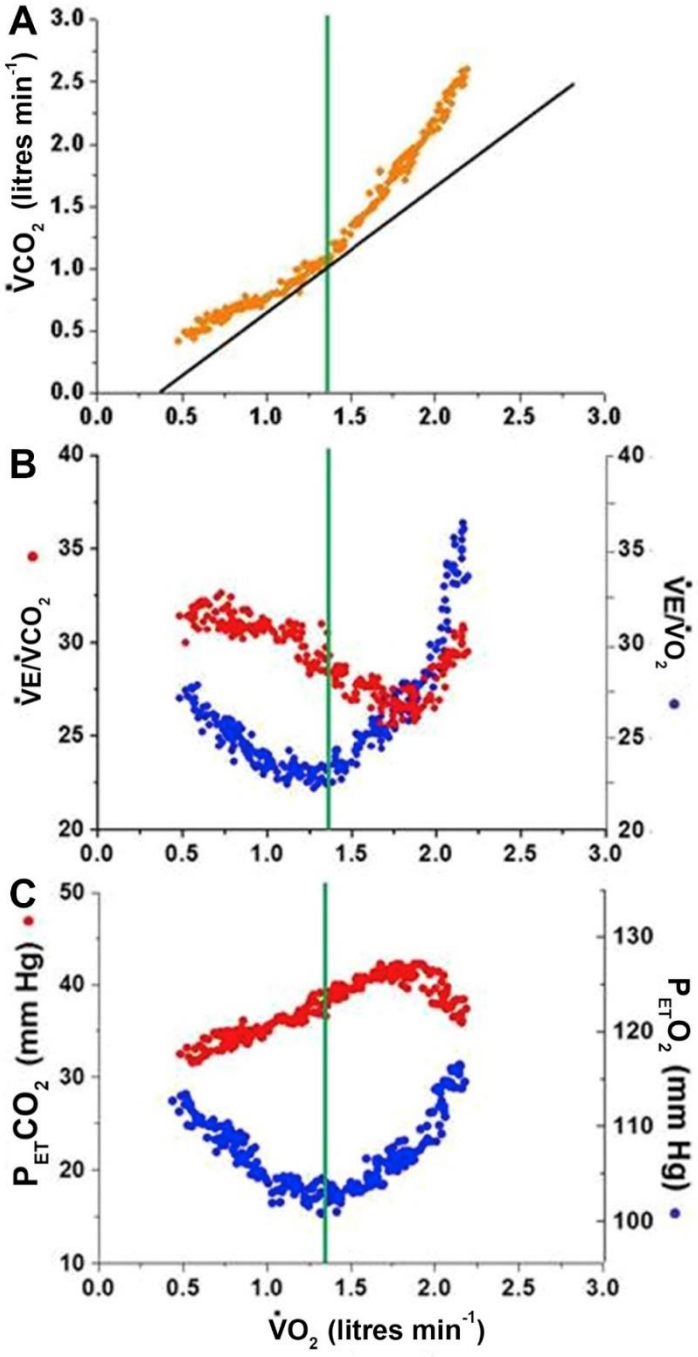


*Figure 7. Determination of anaerobic threshold (AT)*

*Shown as the green line based on changes in (A) the V slope, (B) the VE/VO2 graph and (C)End -tidal $PO_2$ graph. Image taken from Levett et al., 2018. Br J Anaest.*

The recommendation is that determination in this regard should be carried out by two trained, experienced assessors and is highly repeatable (Kaczmarek et al. 2019; Parkes and Moore 2017).

Older first reported a practical clinical use of AT in an observational cohort study of 187 older patients who were having major abdominal surgery who had performed a CPET prior to their operation (Older et al. 1993). In this cohort he observed that mortality, specifically due to cardiac complications was significantly higher in those patients who had an AT of <11ml/Kg/min than in those who had an AT greater than this (8% *vs.* <1%). He subsequently changed practice in his institution and mandated CPET prior to major abdominal surgery in patients over the age of 60y and used the AT determined on this test to triage the patients to different levels of care (Older et al. 1999). All 548 patients in this study with an AT <11ml/kg/min were admitted preoperatively to an intensive care unit (ITU) to be preoptimized prior to surgery (utilising a pulmonary artery catheter to optimise fluid status and haemodynamics) and then returned to the ITU postoperatively. Patients with an AT >11ml/kg/min went to a standard ward bed postoperatively to

recover, unless they had displayed any evidence of myocardial ischaemia on their CPET, in which case they went to a high dependency unit (which provided a higher level of medical care, but no option for organ support as found in an ITU). This policy led to good triage of patients, with no cardiovascular deaths occurring in those with an AT>11ml/kg/min who were sent to the standard ward and a mortality of 4.6% in those deemed high-risk and given ITU support (where high-risk mortality had been 8% in his 1993 cohort) (Older et al. 1999).

Although it is important to note that this was an observational study, conducted years after the authors' earlier study and thus this reduction in mortality may have been contributed to by unmeasured factors, such as improvement in other aspects of clinical care. Nether the less, the use of preoperatively measured AT to stratify risk appeared to allow good triage of patients' access to ITU, a limited and expensive resource.

The ability of preoperatively measured AT to predict mortality from major surgery has been subsequently demonstrated in further cohort studies: a cohort of 847 patients having major abdominal surgery showed AT<11ml/kg/min to have a relative risk of in-hospital death of 6.8 (95% CI 1.6-29.5) (Wilson et

al. 2010). The 90-day mortality of this cohort was also analysed which again demonstrated a significant survival advantage in patients with AT>11ml/kg/min persisting to 90 days.

In addition to the relationship between AT and postoperative mortality, it's relationship with postoperative complications has also been studied. One multi-centre cohort study of 703 patients having major colorectal surgery demonstrated that an AT ≤11.1 ml/kg/min (this cut-point was derived from the authors' own ROC curve analysis, hence the minor difference from the threshold of <11ml/kg/min quoted in the majority of other studies) to have an odds ratio of 7.6 (95%CI 4.4 – 12.9) for any postoperative complication (West et al. 2016).

Due to the perceived importance of AT perioperatively, it has been used as the primary outcome measure in many of the recent trials of exercise interventions preceding surgery (Dunne et al. 2016; Tew et al. 2017). In earlier preoperative training studies, an increase of 2ml/kg/min was considered the minimum clinically important difference (Kothmann et al. 2009) however more recent studies have defined this as 1.5ml/kg/min (Dunne et al. 2016; Tew et al. 2017). The strategic aim of such prehabilitation interventions is that by

improving AT preoperatively, this may lead to a reduction in postoperative morbidity and mortality. There is however currently no evidence for this, although two large multicentre RCTs are currently recruiting patients to test this hypothesis (ISRCTN82233115 and NCT03509428).

The clinical application of AT is not limited to patients having major surgery. A large cohort study comprising 1639 healthy Finnish men with a median follow up of 26 years demonstrated a linear, inverse relationship between AT and both fatal cardiovascular events and all-cause mortality (Kunutsor et al. 2017). A low AT is has also been found to be more predictive of early death than maximal measures in patients with heart failure (Gitt et al. 2002).

#### *1.4.2.3 Maximum wattage ($W_{peak}$)*

Another variable that can be measured during a CPET is the peak power output at the termination of the test (Levett et al. 2018). During an incremental exercise test this represents a measure of exercise tolerance (Whipp and Ward 2009) as the $W_{peak}$ will be directly associated with test duration. Improvements in this outcome are often reported to demonstrate effectiveness in an intervention study, however it

is important to note that $W_{peak}$ is directly related to the ramp selected (Morton 2011), thus it is vital to maintain the same ramp protocol for tests before and after an intervention. Exercise capacity has also previously been shown to be a predictor of mortality in a large cohort study of 6213 men referred for CPET in America (Myers et al. 2002).

### 1.4.3 Measurement of vascular health

As previously described in section 1.2.3, increasing attention is being paid to measuring deficits in the peripheral vasculature which may precede the development of overt atherosclerosis and cardiovascular disease by many years (Widmer and Lerman 2014). Vasculature deficits may be measured in either large conduit vessels (often described as macrovascular dysfunction (Corretti et al. 2002b) ) or in smaller vessels (commonly termed microvascular dysfunction (Lindner 2018)), with impairments at both levels associated with negative health outcomes (De Boer et al. 2011; Inaba et al. 2010).

*1.4.3.1 Measuring macrovascular dysfunction*

The gold standard measurement of the function of large arteries is flow-mediated (vaso)dilation (FMD) of the brachial artery (Corretti et al. 2002b). FMD is a measure of endothelial-dependent dilatation in an artery following the release of a downstream occlusive stimulus. This sudden reperfusion leads to an increase in shear stress on the endothelium, leading to Nitric Oxide release and subsequent dilatation of the artery (Raitakari and Celermajer 2000; Corretti et al. 2002b). It is calculated as the percentage increase in the diameter of the brachial artery from rest, after the release of the downstream occlusion (Harris et al. 2010).

The relative inability of an artery to dilate in this manner has previously been linked with endothelial health and cardiovascular risk (Charakida et al. 2010) and in cohort studies is linked with the subsequent development of hypertension (Rossi et al. 2004) and in a meta-analysis of 8300 subjects has been demonstrated to be predictive of cardiovascular events (Green et al. 2011).

FMD has been previously shown to improve with some exercise interventions such as chronic training with moderate

continuous aerobic exercise (Meyer et al. 2006) and acutely following a bout of high intensity interval exercise (Currie et al. 2012) and with drug treatment of diabetes (Stojanović et al. 2016), which may lead to an improvement in cardiovascular risk.

#### *1.4.3.2 Limb Blood flow*

Limb blood flow is another measure of macrovascular function and is easily measured using a combination of 2D and Doppler ultrasonography (duplex) and can give results with a high degree of certainty (Hussain 1997). Doppler ultrasound utilises the doppler effect, first described by Christian Andreas Doppler in the mid-19$^{th}$ Century in relation to the shift in the frequency of light emitted by distant stars either moving towards or away from our galaxy (Coman 2005). The same principle is applied to Doppler ultrasound, where the imaging system uses the shift in frequency of the ultrasound waves reflected back from moving blood flow in order to calculate it's velocity, which when combined with measurement of arterial diameter using B-mode ultrasound allows the calculation of blood flow (Oglat et al. 2018). One key technical point in the use of duplex ultrasound for the measurement of blood velocity, is that the angle of insonation must remain less than 60$^{o}$ by convention,

in order to minimize any error in measurement (McNaughton and Abu-Yousef 2011). This is due to the presence of the cosine of the angle of insonation being in the formula used by ultrasound machines. For angles of insonation greater than $60^{o}$, there are proportionally larger changes in the cosine of the angle; for angles of $60^{o}$ to $90^{o}$, the cosine of this angle, decreases from 0.5 to 0, whereas for angles of $0^{o}$ to $60^{o}$, the cosine of this angle only decreases from 1 to 0.5. Thus at angles of insonation greater than $60^{o}$, any small variation in probe placement or the position of the Doppler gate on the B-mode image is disproportionately magnified (McNaughton and Abu-Yousef 2011).

There are many factors determining resting blood flow to a limb and also its response to an exercise stimulus. Resting and post-exercise limb blood flow has been previously shown to reduce with increasing age (Parker et al. 2008; Dinenno et al. 1999) and to increase in response to resistance training (Phillips et al. 2012), only to then decrease with de-training (Stebbings et al. 2013). Previous work has shown that habitually more active older adults are able to maintain greater post-exercise leg blood flow when compared to those who are less active (Olive et al. 2002), but that leg blood flow even

when habitually exercising regularly does steadily reduce with advancing age (Proctor et al. 1998).

Interestingly, it has been shown that upper limb aerobic exercise can remotely improve lower limb exercise tolerance (Nawaz et al. 2001).

Reduced resting limb blood flow has previously been correlated with cardiovascular disease events in diabetics (Yoshimura et al. 2006) and it has been hypothesised that a reduction in limb blood flow may be a step in the development of insulin resistance as a reduction in blood flow to the skeletal muscle may lead to less peripheral glucose uptake (Lind and Lithell 1993; Julius et al. 1992).

#### *1.4.3.3 Muscle microvascular blood flow*

It has long been known that microvascular blood flow of a muscle bed can vary independently of the macrovascular flow to a limb due to independent control via the terminal arterioles (Honig et al. 1982; Sweeney and Sarelius 1989). This microvascular blood flow has purported roles in the pathogenesis of insulin resistance (De Boer et al. 2011).

Muscle capillarisation has also been shown to have important roles in the metabolism of skeletal muscle, with differences in in capillary density at baseline before a RET intervention being a major determinant of hypertrophy in response to RET in older adults (Snijders et al. 2017).

Clinically it has been shown that muscle microvascular blood flow is significantly reduced acutely in patients with severe sepsis (Neviere et al. 1996) and in the context of insulin resistance and advancing age (Keske et al. 2016; Hildebrandt et al. 2017). In relation to sepsis this reduction is likely due to a profound alteration to normal microcirculatory control mechanisms related to the systemic inflammatory response (Neviere et al. 1996), however the mechanism with insulin resistance and ageing is less clear (Lindner 2018).

Despite these deficits in microvascular blood flow in a number of clinical conditions, previous work has shown that microvascular blood flow can be improved by RET, even in older individuals (Phillips et al. 2015).

Although microvascular blood flow in muscle can be measured through photoplethysmography (Allen 2007; Challa et al. 2017), or Xenon-labelled tomography (Mesquita et al. 2011)most measurements in the research setting are made using contrast enhanced ultrasound (CEUS) (Greis 2011; Hildebrandt et al. 2017; Mitchell et al. 2013; Dunford et al. 2018).

The principle of CEUS is to administer a contrast agent intravenously which then facilitates measurement of microvascular blood flow in a tissue bed by ultrasound. Although there are a number of contrast agents available for CEUS, the common feature between all commercially available contrast agents is that they take the form of a bubble (Paefgen et al. 2015; Dietrich et al. 2018), which may range in size from nanometres to micrometres. Early studies using CEUS for the measurement of muscle microvascular flow used the contrast agent Definity®, however it's use was restricted in 2008 by the Food and Drug Administration board of the USA due to potential toxicity concerns (Appis et al. 2015) and led to supply issues in the UK (Mitchell et al. 2013).

Since this supply issue arose many groups across Europe have been using Sonovue® (Ma et al. 2012; Mertz et al. 2018; Hildebrandt et al. 2017) or Optison (GE Healthcare) (Lindner et al. 2019; Vincent et al. 2002) as their contrast agent of choice.

Measuring muscle microvascular blood flow by CEUS is carried out by continuous recording of an ultrasound image of the muscle in question after a period of infusion of the contrast agent that allows a systemic steady state concentration to be achieved. At the start of a measurement, a brief high Mechanical Index "flash" of ultrasound is delivered from the ultrasound probe, which destroys the contrast agent bubbles under the probe (Sjøberg et al. 2011). A recording is then made of the bubbles being replenished in the studied tissue bed, which allows quantification of microvascular blood flow (Mitchell et al. 2013).

### 1.4.4 Measurement of body composition

A measurement of body composition is often performed as it gives more information than simple measurement of height and weight and calculation of Body Mass Index (BMI) (Borga et al. 2018). The key outcomes from an assessment of body composition are measures of fat mass and lean mass as both

reduced lean mass and increased fat mass are associated with a higher risk of mortality (Lee et al. 2018b).

Body composition can be measured using a variety of methods. The most basic of these are a simple measurement of body weight and height to calculate a BMI and to compare this against population reference standards (Ranasinghe et al. 2013). In relation to disease risk these simple measures can be enhanced slightly by the addition of circumferences as waist circumference (Bigaard et al. 2003), waist: hip ratio (Dalton et al. 2003) and neck circumference (Ben-Noun and Laor 2003) have all been associated with CVD risk. Other methods of body composition measurement include skinfold thickness measurement (Duren et al. 2008) and bioelectrical impedance analysis (Wagner and Heyward 1999), both of which generate estimates of percentage body fat from regression equations (Durnin and Womersley 1974; Lukaski et al. 1986) and require little equipment and can be carried out outside the laboratory setting.

It is in the laboratory setting that the most accurate measurements of body composition (fat mass and lean mass) can be made using either Dual-Energy X-ray Absorptiometry

(DXA) (Lander Svendsen et al. 1993) or Magnetic Resonance Imaging (Ross et al. 2006). MRI is regarded as the gold standard for body composition assessment, however the technique is expensive and requires a high degree of expertise in order to perform analyse the scans (Hauptfleisch et al. 2013). Conversely, DXA is relatively easy to perform with most machines linked to automated software providing data for fat mass and fat free mass for both whole-body and regions of interest. Despite this, the precision of DXA has been questioned and although multiple studies have demonstrated that DXA measurements correlate very well with those from MRI (Cheung et al. 2016; Kim et al. 2002), others have found this not to be the case (Maden-Wilkinson et al. 2013), with age-related losses of muscle mass seemingly underreported by DXA (vs. MRI) and its ability to detect changes in body composition over time being recently questioned (Maden-Wilkinson et al. 2013). In addition, DXA measures can be influenced by factors such as subject hydration (Horber et al. 1992) and, although low, the radiation dose emitted by DXA is an additional consideration (Plank 2005).

### 1.4.5 Measurement of insulin resistance

Several methods of measuring insulin resistance (or conversely insulin sensitivity) exist which may range from a simple measurement of fasting serum glucose and insulin concentrations, to frequently sampled oral or I.V glucose tolerance tests, to more complex but gold-standard insulin clamp techniques (Ferrannini and Mari 1998).

#### *1.4.5.1 Insulin clamps*

The euglycaemic, hyperinsulinaemic clamp method was first described in 1979 (DeFronzo et al. 1979) and is widely regarded as the gold standard for the assessment of insulin resistance (Kim 2009). This time-consuming method requires a constant infusion of insulin to supress endogenous hepatic glucose production whilst a variable rate of glucose infusion is administered to maintain a normal serum glucose concentration until a steady state is arrived at, where the rate of glucose delivery is equal to the rate of glucose uptake, thus allowing quantification of how much glucose is utilised for a given concentration of insulin (Muniyappa and Madan 2018).

This technique, whilst being able to very accurately generate a measure of insulin sensitivity, is labour intensive and expensive and requiring a large number of blood samples

(often every 5 minutes for several hours) (Muniyappa and Madan 2018).

#### *1.4.5.2 Oral glucose tolerance tests (OGTT)*

The standard, World Health Organisation (WHO) oral glucose tolerance test is an internationally recognised method of diagnosing type 2 diabetes mellitus (World Health Organisation 2006), of which insulin resistance is one of the main hallmarks (Taylor 2012). When performed clinically, the test simply involves measurement of fasting glucose, before the administration of 75g oral glucose. A blood sample is then taken 2 hours after the glucose is administered and a plasma glucose of 11.1mmol/l or greater is diagnostic of diabetes mellitus (World Health Organisation 2006)

An OGTT performed in this fashion gives a categorical result; diabetic, impaired glucose tolerance (2-hour glucose concentration between 7.8mmol/l and 11.0mmol/l) or normal glucose tolerance (World Health Organisation 2006; Shaw et al. 1999). These broad categories based solely on plasma glucose concentration are adequate for informing clinical decisions such as whether to begin treatment for diabetes but

provide limited scope for understanding or measuring insulin resistance.

Adding measurements of plasma insulin to the measurements of glucose during an OGTT and measuring glucose at intervals throughout the OGTT, rather than just at the start and end, can increase its power to study insulin resistance quantitatively (Hayashi et al. 2013). Further information can be obtained by extending the duration of the OGTT to 3 hours (Anderwald et al. 2011). From raw data points of plasma glucose and plasma insulin concentrations, areas under each concentration curve can be determined which give an indication of glucose tolerance and insulin resistance (Allison et al. 1995).

An intravenous glucose may be performed in the same fashion as an OGTT, however are used less often due to thrombophlebitis at the injection site (Nadon et al. 1964)

#### *1.4.5.3 Indices of insulin sensitivity derived from OGTT*

Several indices of insulin sensitivity can be calculated from the values obtained for both fasting plasma glucose and insulin concentrations and from those obtained from an OGTT although none are in routine use in clinical practice and are

primarily research tools. One of the best known and most widely used indices calculated from fasted samples only is the homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al. 1985). This measure has been robustly validated against measures such as the hyperinsulinaemic euglycaemic clamp and provides a useful static measure of insulin resistance (Wallace et al. 2004). HOMA-IR is calculated as:

$$\frac{[Fasting\ insulin(mUL^{-1})] \times [Fasting\ glucose(mmolL^{-1})]}{22.5}$$

However, there are no defined normal reference ranges in common use for HOMA-IR.

There are also several dynamic indices of insulin sensitivity derived from OGTT data which have been validated (Patarrão et al. 2014), of which the best known are: 1) the Matsuda index (Matsuda and DeFronzo 1999) which reflects combined peripheral and hepatic insulin sensitivity, and 2) the Cederholm index (Cederholm and Wibell 1990) which estimates mainly peripheral sensitivity (Patarrão et al. 2014). More information on these indices is provided in section 4.9.3.

## 1.5 Impact for surgery

A large number of conditions which benefit from surgical management increase in incidence with increasing age. However the rates of elective surgical procedures performed declines with age in the UK (Royal College of Surgeons 2012). Older patients have a tendency to be less fit and are at increased risk of postoperative complications and so are more likely to be turned down for surgery (Association of Anaesthetists of Great Britain and Ireland 2014).

Exercise and other non-pharmacological interventions have the potential to reverse some of the decline in cardiovascular fitness and other physiological components associated with ageing and improve the "fitness for surgery" of older adults and potentially allow increased access to surgical care for those most in need of it.

### 1.5.1 Prehabilitation

"ERAS"- enhanced recovery after surgery has developed since the early 2000s in an attempt to decrease post-operative length of stay and convalescence after major surgery (Khan et

al. 2009). In brief, ERAS includes a variety of interventions such as reduction in perioperative fasting, early mobilisation and an early return to enteral nutrition postoperatively (Khan et al. 2009). ERAS only seeks to minimise the post-operative catabolism and consequent loss of function and does nothing to improve the condition of patients before surgery.

More recently interest has been shown in the potential of exercise intervention before surgery to improve patient fitness, with the aim of improving outcomes even further. Several small studies have been conducted to date with promising results. A recent systematic review and meta-analysis of exercise prehabilitation prior to surgery suggested it may lead to a significant reduction in postoperative complications after abdominal surgery (Moran et al. 2016a). However the majority of studies in this review used exercise interventions with a significant time requirement, with most requiring training 6 days a week and some sessions requiring up to an hour of different exercises in each session (Gillis et al. 2015).

The time requirement of the prescribed exercise intervention is one factor that does need to be considered when deciding

on how best to implement a programme of prehabilitation, since "lack of time" is consistently rated by both young and older groups of adults as a barrier to exercise (Justine et al. 2013).

When designing a prehabilitation programme, the length of the time period between entering the programme and the time of surgery also needs to be considered. In the setting of cancer surgery for example there exist time pressures not only from the biological perspective of not wishing to delay treatment and risk progression of the disease, but also from administrative time targets. Thus any prehabilitation intervention needs to fit in with clinical targets such as the 31-day decision to treat and 62-day from referral targets in the UK NHS for cancer surgery (Cancer Research UK 2015).

Improvement in preoperative fitness has been measured using different techniques: the 6-minute walk test (Carli et al. 2010) and respiratory function (Dronkers et al. 2010, 2008) and CPET parameters (Kaibori et al. 2013) have all been used. Measures such as the 6 minute walk test are clearly attractive because of their simplicity, requiring minimal equipment and being quick and simple to perform and interpret (Guyatt et al.

1985). Despite this, it is not routinely performed in a preoperative setting.

The use of preoperative CPET is becoming more common in the UK, especially in higher risk patients (Moran et al. 2016b). However it is still not available in many UK institutions due to the specialist equipment and staffing required and it has not yet been recommended by NICE as a standard of practice (NICE 2016).

CPET is however an attractive measure of preoperative fitness as having major surgery may be likened to a prolonged bout of endurance exercise as patients need to spontaneously increase their cardiac output in response to the physiological demands of the procedure (Aitkenhead et al. 2013). Assessment of variables at CPET may give an insight into how a patient may respond to an operative intervention (Moran et al. 2016b), much in the same way it can be used to quantify an athletes fitness prior to a training programme or an event.

One other potential marker of fitness for surgery is arterial blood pressure. Blood pressure is routinely measured both in

primary care before referral for consideration for elective surgery and also in surgical pre-assessment clinics. Recent joint guidelines from the Association of Anaesthetists of Great Britain and Ireland and the British Hypertension Society recommend a delay in referral to allow blood pressure control for patients with stage 2 hypertension or greater (>160/100 mmHg)(Hartle et al. 2016).

However, the evidence that preoperative hypertension actually increases morbidity or mortality is lacking.

Obesity, however, unlike hypertension is well known to have a negative impact on postoperative outcome, and is associated with an increase in post-operative infections and poorer return of function (Tjeertes et al. 2015; Vincent et al. 2012). Thus increasing numbers of NHS hospitals are restricting access to elective surgery for benign conditions until obese patients have lost weight (Pillutla et al. 2018).

Similar to that known for CRF, preoperative insulin resistance has been consistently linked to poor outcomes from elective surgery (Sato et al. 2010; Hofferberth et al. 2012). That this

may be worsened by preoperative fasting (Tewari et al. 2015) is why standard ERAS includes the use of preoperative carbohydrate drinks (Bilku et al. 2014).

It is well established that a period of exercise training may improve insulin sensitivity, potentially mediated through weight loss (Keshel and Coker 2015), however it is unknown whether this will translate to improved surgical outcomes.

Finally, although sections 1.2.3 and 1.4.3.3 described microvascular blood flow through skeletal muscle, splanchnic microvascular blood flow is potentially of more direct relevance to GI surgical outcomes than muscle microvascular blood flow (Takala 1997), although this is less easily measured non-invasively. Poor microvascular blood flow to the gut has been associated with higher rates of anastomotic failure in both the oesophagus (Miyazaki et al. 2002) and the rectum (Vignali et al. 2000). Although there are no studies comparing muscle microvascular blood flow with visceral blood flow at rest, it could follow that improvements in one could be associated with the other.

One currently widely employed strategy to improve perioperative insulin resistance, the provision of carbohydrate drinks prior to surgery during preoperative fasting, and this has been consistently shown to improve outcomes (Awad et al. 2013). Following this, it is not unreasonable to predict that preoperative improvement in insulin resistance may bring about improvements in surgical outcomes.

## 1.6 Potential time-efficient interventions as countermeasures to age-associated physiological deficits

### 1.6.1 High intensity interval training

High intensity interval training (HIIT), described since the mid part of last century (Müller 1953), involves brief periods of intense exertion interspersed with periods of either low intensity exercise or rest. Although used to improve athletic performance in elite populations for many years (Fiskerstrand and Seiler 2004; Magel et al. 1975) , HIIT has become very popular in commercial "exercise-for-health" circles in the 21st century and is now the mainstay of a variety of fitness brands

and workout programmes (The Body Coach 2017; British Military Fitness 2017; Insanity 2017).

The principle underlying HIIT is that to overload of the body with a high-intensity stimulus will lead to greater physiological adaptions than traditional moderate-intensity steady-state training (Upadhyay and Sandhu 2016; Gibala et al. 2008).

Other potential benefits of HIIT (when compared to lower intensity steady-state training) include potentially increased compliance of participants due to shorter time requirements (Jung et al. 2015) and its ability to quickly improve both markers of aerobic and cardiorespiratory health (Gibala et al. 2012). The most recent systematic review and meta-analysis of 65 studies utilising HIIT concluded that HIIT has the ability to improve $VO_2$ max in both long term and short term training durations (Batacan et al. 2017).

Although not all studies have demonstrated superiority of HIIT to moderate steady-state exercise (Fisher et al. 2015; Foster et al. 2015) in terms of absolute improvements in fitness, the time- efficient nature of many (but not all) HIIT protocols

compared to moderate steady-state exercise is evident (Gillen and Gibala 2014).

HIIT has also been shown to be effective at improving the CRF of older adults in a short time-frame (in keeping with UK cancer surgery target-times) in experimental pilot studies (Boereboom et al. 2016b) but not yet in a randomised controlled trial (RCT). This pilot work also showed the potential for HIIT to improve blood pressure (systolic only) in these older adults. Similarly, a few trials in younger subjects have also shown HIIT to have to the potential to reduce blood pressure (Mohr et al. 2014; Ciolac 2012; Molmen-Hansen et al. 2012).

HIIT has recently been shown to be acceptable as a prehabilitation strategy for patients having a liver resection (median age 62 y) in an RCT, resulting in significant improvements in cardiovascular fitness (Dunne et al. 2016). In agreement with this, as yet unpublished work from our research group has shown similar findings in co-morbid octogenarians and those with urological cancers.

#### *1.6.1.1 HIIT protocols*

All HIIT regimes, despite having a central characteristic in common; the interspersion of brief periods of intense exertion

with periods of either low intensity exercise or rest, may vary in many different ways not limited to:

i) the intensity of the exercise during the intervals,

ii) the duration of the exercise intervals,

iii) the duration of the low-intensity intervals, and

iv) the number of intervals per session.

Due to the large variation between HIIT protocols some may be more demanding than others with some potentially too demanding for the average sedentary older adult (Hardcastle et al. 2014).

One of the first HIIT protocols described in the literature, Wingate HIIT, came from the development of the Wingate anaerobic test in the late 1970s (Bar-Or 1987). Wingate HIIT involves very short supra-maximal "all-out" sprint intervals (Gillen and Gibala 2014). These protocols have been consistently demonstrated to be effective at improving CRF (Vollard et al. 2017), however many would argue that that these "all-out" sprints may not be palatable to sedentary

(Hardcastle et al. 2014) or older adults with the intensity of Wingate exertions often in excess of 300% $W_{peak}$ (Astorino and Thum 2016). In addition, the time-efficiency of Wingate HIIT is not as great as many may envisage. Although each high-intensity bout is very short (~30 secs), the required rest period in between each high-intensity exertion is relatively long (4-5 mins (Burgomaster et al. 2008)), such that a standard Wingate session may last as long as 30 mins (Vollaard and Metcalfe 2017).

Following on from these supramaximal sprint protocols came the development of lower-intensity HIIT protocols. Although the intensity of the intervals in these protocols was much lower and as such more manageable, many of these protocols included "high"-intensity efforts that were a few minutes in duration (Talanian et al. 2007), with similar or even slightly longer recovery periods (Perry et al. 2008), again challenging the time-efficiency aspect of this training. Eventually based on the work of Ready et al in the 1980's lower-volume, reduced-intensity (compared to Wingate) HIIT protocols began to be used as common-place in exercise interventions in both healthy volunteers and patients groups (Little et al. 2010; Ready et al. 1981).

Based on these protocols (which were commonly 10 x 1-minute intervals (with 90-second rest periods) at ~100% $W_{peak}$, and on work demonstrating the relative importance of intensity over interval number (Phillips et al. 2017a) our research group developed our 5 x 1-minute HIIT protocol, which employs 5, 1 minute training intervals set at an intensity of ~120% of the participants peak power output at termination of a ramp incremental cardiopulmonary exercise test, interspersed with 90-second rest periods (Phillips et al. 2017a)) (Figure 8).

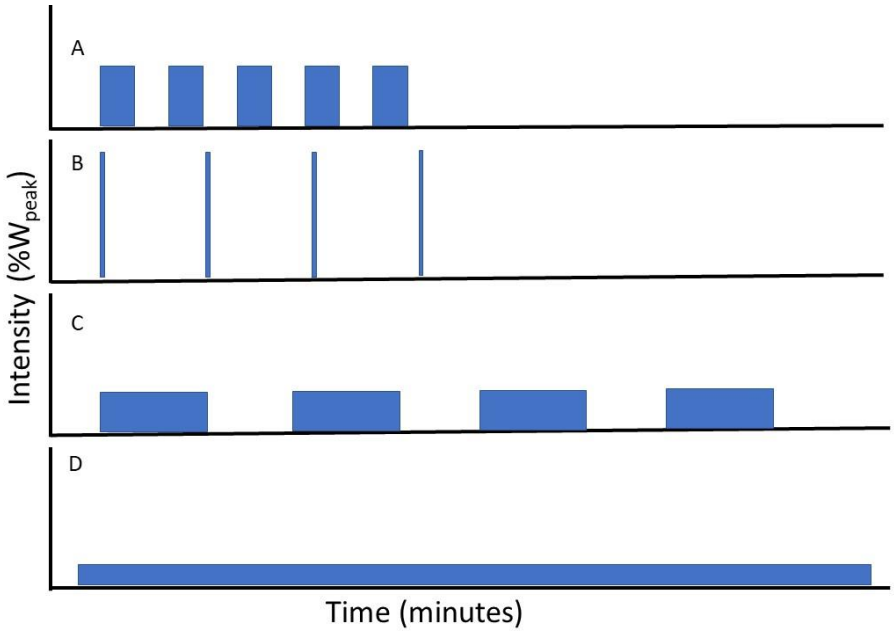


*Figure 8 A comparison between various High-intensity interval training protocols; (A) Our reduced exertion 5x1 minute protocol, (B) traditional Wingate training, (C) a typical 4x4 minute protocol and (D) moderate continuous aerobic exercise.*

After the development of this protocol for use in younger adults (Blackwell et al. 2017; Phillips et al. 2017a), it has subsequently been used successfully in multiple cohorts of older adults over a 4-week training period (Boereboom et al. 2016b) and has also been piloted in cancer patients (Boereboom et al. 2019). These studies have demonstrated an ability to improve measures of cardiorespiratory fitness and blood pressure over 4-weeks. However, to date this protocol has not been evaluated over either shorter or longer time periods.

### 1.6.2 Isometric exercise

Isometric exercise is the use of a muscle or muscle group to exert a sustained force against an unmoving load without any change in that muscle group's length (Wiley et al. 1992). This is in contrast to isotonic exercise where a force is exerted in order to move a load involving change in the muscle groups' length.

Isometric exercise has been shown to have a profound effect on blood pressure, leading to an initial hypertensive response during and immediately after exercise (Wiley et al. 1992), which quickly returns to normal values (de Araújo et al. 2011).

Interestingly however, chronic isometric exercise training can lead to significant reductions in resting blood pressure after relatively short exercise programmes (Inder et al. 2016; Kelley and Kelley 2010).

The mechanism by which this training effect on blood pressure occurs is currently unknown despite work done to investigate its effect on sympathetic activity (Taylor et al. 2003) and endothelial function (McGowan et al. 2007b). These studies found that IHG training was associated reduction in sympathetic nervous system activity through an unknown mechanism, which could in part be responsible for its hypotensive effects. The study by McGowan and colleagues demonstrated that changes in endothelial function from IHG appeared to be limited only to the arteries of the trained limb, making a systemic change in endothelial function an unlikely candidate as the mechanism behind IHG mediated reductions in BP (McGowan et al. 2007b).

Despite this lack of mechanistic understanding, a 2016 meta-analysis of 302 participants (Inder et al. 2016) showed significant mean reductions in systolic (-5.2 mmHg) and diastolic (-3.91 mmHg) BP from isometric exercise training

programmes of 2 weeks or more in duration. However, the majority of trials included in this analysis involved isometric exercise of the leg muscles of younger subjects, many of whom were not hypertensive. In a subgroup analysis, hypertensive and older participants achieved larger reductions in MAP, however reductions in systolic BP were not significant.

Various exercise protocols exist for isometric exercise training and have been studied previously. These primarily vary in the number of sessions per week (between 3 and 5) and the relative intensity of exercise (Wiles et al. 2010; Devereux et al. 2011; Gill et al. 2015) (either measured by % of maximum voluntary force, % Max heart rate or % maximal EMG signal.) An optimal number of contractions per session and the duration of each contraction has also not been agreed. There are also significant differences between study protocols with regards to the limb trained (arms vs. legs) and also whether the training is bilateral (Baross et al. 2013; Devereux et al. 2011), unilateral (Hess et al. 2016) or alternating limb (Taylor et al. 2003).

Of all these possibilities sustained isometric handgrip (IHG) contractions against a handgrip dynamometer, has begun to

emerge in the literature as a convenient and effective intervention to elicit BP reductions (Millar et al. 2008). Potential advantages of handgrip over large muscle (legs) isometric exercise is less equipment, portability, a reduction in the amount of discomfort experienced and the potential for its use as a home-based intervention.

Very few randomised controlled trials of IHG have so far been published although there have been several published systematic reviews. A 2010 systematic review included 3 trials comprising a total of 81 participants (42 in the intervention arms) and meta-analysis demonstrated a 13.4 mmHg (95% CI: 15.3- 11.0) reduction in systolic BP and a 7.8 mmHg (95% CI: 16.5- 3.0) reduction in diastolic BP. However, the three trials included had participants of all ages (one trial included subjects as young as 20 years old), included a variety of IHG protocols (some unilateral, some bilateral) and lasted for between 8 and 10-weeks.

A 2017 systematic review of IHG studies in medicated hypertensive patients only included 3 further RCTs, with study participant mean ages from 52 years to 65 years. There were

conflicting results regarding blood pressure precluding meta-analysis (Farah et al. 2017).

A further systematic review published in 2017, of all RCTs in adults of all ages performing IHG included 7 studies (Jin et al. 2017). This review included studies with mean participant ages of 20 years to 66 years and demonstrated a mean reduction in systolic BP of 8.3 mmHg (95% CI: 11.2 – 5.5) and a mean reduction of 3.8 mmHg (95% CI: 6.1- 1.7) in diastolic BP. The most common length of the training protocol in these studies was 8-weeks and, whilst all protocols used 4 x 2-minute contractions at ~30% of the subjects maximum voluntary contraction (MVC), there were differences in the number of limbs used (some used alternating limb protocols whilst others used unilateral contractions).

Most studies investigating the beneficial effects of IHG have examined the effect of IHG on resting BP but, some of the most recent studies have begun to report ambulatory blood pressure results. One uncontrolled study, only published recently in abstract form showed a significant reduction in ambulatory BP with 12 weeks IHG in a cohort of mild hypertensive patients (Carlson et al. 2017). However, three subsequent RCTs have

failed to demonstrate any reduction in ambulatory blood pressure. One, with a mean age of 59 published in 2018 reported a significant reduction in office BP, but no effect on ambulatory BP (Farah et al. 2018), similar to another in 2018 with a mean age of 33 (Goessler et al. 2018). Finally an RCT of patients with a mean age of 60 did not show a reduction in either office or ambulatory BP from IHG (Pagonas et al. 2017).

In addition, very few studies have examined the effect of IHG on other health related variables such as CRF or vascular health. One study from our research group evaluated the effect of IHG on the CRF of healthy middle-aged adults and found no benefit in either $VO_{2\ peak}$ or AT (Blackwell et al. 2017).

The effect of IHG on measures of vascular health such as FMD have previously been examined as secondary endpoints in some of the trials with BP as a primary endpoint. Two trials have shown an improvement in FMD following IHG (Badrov et al. 2016; McGowan et al. 2007b), however in these trials the changes in FMD were only found in the arm which performed the IHG training, with no effect in the untrained arm, potentially suggesting a local effect rather than a systemic improvement in vascular health.

In summary, IHG has previously shown good potential in RCTs as an intervention to improve some measures of health, however data on its effects in older adults is lacking.

### 1.6.3 Remote ischaemic preconditioning

Ischaemic preconditioning is the well-described phenomenon of the beneficial effect a short period of ischaemia on a tissue, which prepares it to cope with a subsequent period of ischaemia (Sukkar et al. 2016). Remote ischaemic preconditioning (RIPC) builds on this principle, making one remote area of tissue ischaemic for a short period of time in order to benefit another tissue remote from the first during a subsequent period of ischaemia (Xie et al. 2018). Ischaemic preconditioning was first described in the late 1980's using a canine model where it was noted that short periods of myocardial ischaemia appeared to protect the heart and lead to less infarction from a subsequent longer period of ischaemia (Murry et al. 1986). This phenomenon was subsequently investigated in human hearts in a small RCT of 14 patients having coronary artery bypass grafting, in which a short period of ischaemic preconditioning in the intervention group led to significantly higher levels of Adenosine Tri-phosphate (ATP) in

biopsies of their myocardium taken after a subsequent longer period of ischaemia (Yellon et al. 1993). The investigators hypothesised that this increase in cellular ATP in the myocardiocytes of the intervention group demonstrated that they were more resistant to the prolonged ischaemia. The field then progressed to studying the potential of RIPC in cardiac surgery (as subjecting a limb to ischaemia is technically easier and safer than inducing myocardial ischaemia), with the first RCT published in 2006 (Cheung et al. 2006). This trial was carried out on paediatric cardiac surgical patients, with a single leg being rendered ischaemic using a BP cuff for 4, 5-minute periods, with 5 minutes of reperfusion time between each period of ischaemia. The intervention group had significantly lower serum troponin I and a significantly lower inotropic requirement postoperatively, leading the authors to conclude it to be a successful intervention (Cheung et al. 2006).

Currently the mechanistic underpinning of these adaptations is not fully understood, however they are thought to be due to a combination of cell signalling pathways including a variety of kinases and myocardial cell surface receptors (Hausenloy and Yellon 2009).

Remote ischaemic preconditioning is being studied for potential application in a variety of clinical conditions, but as alluded to so far it is has produced most interest in cardiac surgery (Sukkar et al. 2016). In this field, several RCTs have been conducted, with meta-analyses reaching different conclusions on many secondary endpoints including MI, acute renal failure and reductions in length of stay (Xie et al. 2018; Sardar et al. 2016). However there us agreement that remote ischaemic preconditioning does not improve overall survival (Sardar et al. 2016). Interest has also been taken in the observation in limited clinical trials that RIPC could prevent contrast induced nephropathy in high risk patients receiving iodinated contrast agents (Er et al. 2012).

Outside of clinical practice, ischaemic preconditioning is also under investigation in the setting of exercise science. One RCT showed that a single session of RIPC consisting of 4 episodes of 5 minutes ischaemia to an arm interspersed with 5 minute reperfusion times could significantly improve the maximal swimming performance of elite athletes (Jean-St-Michel et al. 2011). Performance benefits have also been reported from local IPC of the legs before sprint cycling in trained cyclists (Patterson et al. 2015; Cruz et al. 2015) and also in trained

runners during a 5km time trial (Bailey et al. 2012). However these significant benefits were not seen in another randomised controlled trial of IPC in runners over a 30m sprint (Gibson et al. 2013). The use of IPC to improve exercise performance was examined in a 2019 systematic review, which demonstrated an overall benefit to aerobic exercise performance, however few studies examined measures of CRF (Caru et al. 2019).

One further clinical application of RIPC is the observation in limited number of case studies that chronic RIPC may have a blood pressure lowering effect both in normotensive and pre-hypertensive patients (Madias 2011; Madias and Koulouridis 2014; Madias 2015a, 2015b). These effects were not borne out in an RCT of healthy young volunteers (mean age 28) where there was no significant change in either systolic or diastolic blood pressure (Kimura et al. 2007). There is no RCT to date examining the effect of chronic RIPC on older subjects.

A systematic review conducted in 2016 of the potential cardiovascular benefits of repeated RIPC concluded that there was limited evidence for a potential impact of RIPC on BP (Epps et al. 2016).

Few studies have investigated the effects of RIPC on vascular health. Two clinical trials have investigated the effect of IPC on FMD, where it was significantly improved after either daily IPC for 7 days or 3 times a week for 2-8 weeks (Jones et al. 2014a, 2014b). Of note both trials examined the effect of local IPC to the limb in which FMD was subsequently measured and thus neither can comment on the potential for remote improvements in FMD from the IPC site.

Animal work has begun to show potential utility for RIPC in abdominal surgery with rat intestine having been demonstrated to be protected by RIPC prior to an ischaemic insult (Erling et al. 2013).

In summary, RIPC has shown early potential as an intervention to improve some measures of health, however, has yet to be investigated thoroughly in older adults.

## 1.7 Hypothesis and aims

This chapter has described several key health-related deficits which are associated with ageing and has highlighted the relevance of these both to the ageing adult. The chapter then

went on to discuss the rationale behind three time-efficient interventions which could have utility to improve some of these deficits, but which require further evaluation in older adults.

The main hypothesis of this thesis is that 6 weeks of either HIIT, IHG or RIPC may lead to significant reductions in resting blood pressure in a population of adults aged 65-85. Secondary hypotheses are that these interventions will also lead to improvements in insulin sensitivity, cardiorespiratory fitness, body composition and macro and microvascular function. This thesis aims to test this hypothesis .

The objectives of this thesis are to:

1. Perform a systematic review of non-pharmacological interventions to reduce blood pressure in older adults.
2. Design and carry out a randomised controlled trial of 6-weeks of HIIT, IHG and RIPC *vs.* Control in a group of healthy older adults, measuring their effect on the deficits outlined in the introduction above.
3. Map the time-course of physical adaptations to a standardised HIIT protocol in healthy older adults.

## 2 Exercise and other non-pharmacological strategies to reduce blood pressure in older adults: a systematic review and meta-analysis

## 2.1 Chapter focus

This chapter contains a systematic review and meta-analysis of randomised controlled trials reporting the effect of exercise and other non-pharmacological interventions to reduce blood pressure in older adults.

## 2.2 Introduction

In the UK, the prevalence of hypertension is estimated at 31% for men and 28% for women; unchanged for a decade (Knott and Mindell 2012). Hypertension increases risk of cardiovascular events and this risk is lowered by reducing blood pressure (BP). A meta-analysis involving 464,000 patients showed both ischaemic heart disease and cerebrovascular events can be significantly reduced by a 10 mmHg reduction in systolic blood pressure (SBP) or 5 mmHg reduction in diastolic BP (DBP) (Law et al. 2009). Treating hypertension is expensive but is offset by reduction in the need for care following cardio-cerebro-vascular events. Reducing the BP of the population in general may save up to £850 million over 10 years (NHS England 2017; Moran et al. 2015; Public Health England 2014).

Hypertension is managed pharmacologically for the most part (NICE 2011; Mancia et al. 2013; Ross and Macleod 2005). Antihypertensives can be effective in lowering BP (Baguet et al. 2007), but compliance remains an issue as many patients dislike taking medication for asymptomatic disease or experience problematic side effects (Misselbrook and Armstrong 2001; Ross et al. 2001).

Lifestyle modification is often the first line in management in treatment guidelines (NICE 2011; Nice 2013; Mancia et al. 2013; Weber et al. 2014). Whilst they are financially attractive (Harada et al. 2001) and effective within studies (Cornelissen and Smart 2013; Cornelissen and Fagard 2005; Semlitsch et al. 2013; Kelley et al. 2001; Huang et al. 2013; He et al. 2013; Alam and Johnson 1999; Taylor et al. 2011; Bouaziz et al. 2017; Halbert et al. 1997; Lee et al. 2010b; Rogers et al. 2009; Lee et al. 2010c), lifestyle interventions, including sodium intake reduction and various forms of exercise training may suffer from poor compliance outside the setting of randomised controlled trials (RCTs) (Hamer 2010).

There is a well described association between advancing age and hypertension, with prevalence increasing from 8% of men and 2% of women in the 16-24 age range to 66% of men and 78% of women aged 75 and over (Knott and Mindell 2012). However, the majority of studies evaluating lifestyle and exercise interventions have been carried out in younger adults and not older people. Clinical guidelines and evidence in older adults is lacking, with a recent systematic review of cardiovascular disease prevention concluding that guidelines

are often vague in their coverage of older people and often based on limited evidence (Jansen et al. 2015). No review to date has evaluated the evidence from lifestyle modification RCTs involving participants with a mean age of 65 or over (the Organisation for Economic Co-operation and Development's definition of older (OECD 2016)), where interventions suitable for younger adults may be difficult for older adults to adhere to because of mobility impairment and other challenges (Knoepfli-Lenzin et al. 2010). We aim to address this gap in evidence in by conducting a systematic review of the existing literature and meta-analysis.

## 2.3 Methods

### 2.3.1 Study design

This systematic review was registered prospectively with PROSPERO (registration number CRD42017059443) and was carried out in accordance with the PRISMA statement (Moher et al. 2009). Only RCTs evaluating a physical activity or lifestyle modification intervention were included. Other inclusion criteria were mean participant age of 65 years or older (OECD 2016), interventions lasting 2 weeks or more, and trials where resting BP was reported before and after

intervention. Trials involving a drug treatment or nutraceutical supplement were excluded.

## 2.4 Literature search

Literature searches were carried out by a trained Clinical Research Librarian using the following databases: MEDLINE, EMBASE, CINAHL, AMED, and PubMed (all inception to 3rd March 2017). No language or date restriction was applied to the searches. The Cochrane library of systematic reviews was searched for relevant reviews and Clinicaltrials.gov was searched for unpublished studies. Previous systematic reviews of related topics were also searched for relevant studies. References of identified potentially relevant studies were hand-searched for further studies. Finally, all studies citing the identified potentially relevant primary studies were screened for inclusion using Google Scholar. Example search strategies can be found in Appendix 1.

Abstracts were screened by one author (PH) with the aid of Rayyan systematic review software (2016, Qatar Computing Research Institute, Doha, Qatar) (Ouzzani et al. 2016). Full text versions of potentially relevant primary studies were then

independently screened against the inclusion and exclusion criteria by two authors (PH and BD) and agreement to inclusion reached by consensus.

## 2.5 Data extraction

Study characteristics were extracted by one author (PH) with outcome data independently extracted and verified by two authors (PH and JB). Risk of bias for included studies was assessed using the Cochrane Collaboration tool for assessing risk of bias independently by two authors (PH and BD) with any disagreement resolved by consensus. When BP was only reported in graphical form, data were extracted using the online tool WebPlotDigitizer (Version 3.12, Austin, Texas, USA) (Rohatgi 2019).

## 2.6 Statistical analysis

To facilitate meta-analysis of change variables when standard deviations of change were not reported, standard deviations were imputed using methods described in the Cochrane handbook (The Cochrane Collaboration 2008). These standard deviations were calculated using a correlation coefficient of change of 0.8 (the mean correlation coefficient of change from

those studies which reported a standard deviation of change). Outcomes were aggregated using a random-effects model. Changes in blood pressure (systolic and diastolic) are presented as differences in means with 95% confidence intervals (CI) with mmHg as the units. We regarded changes of 10 mmHg in SBP and 5 mmHg in DBP as clinically significant (Law et al. 2009). We used the $I^2$ statistic to quantify statistical heterogeneity, with values above 50% as evidence of statistical heterogeneity. We assessed publication bias qualitatively using Funnel plots and quantitatively using Egger's linear regression test ($p<0.05$). We investigated heterogeneity using a random effects, restricted maximum likelihood meta-regression. Covariates included duration of intervention and whether studies included participants with hypertension (BP 140/90) or not. We report the between-study heterogeneity explained by the model ($R^2$ analogue) with a corresponding p value. The Knapp Hartung modification was used as the variance estimator. To assess the quality of evidence, the GRADE approach (The Cochrane Collaboration 2008) was used with evidence downgraded to moderate, low or very low quality owing to concerns over unexplained heterogeneity, indirectness of evidence, possible publication bias, imprecision in effect estimates and concerns over risk of

bias. All calculations were carried out using STATA 14 (StataCorp, Texas USA).

## 2.7 Results

### 2.7.1 Search results

A total of 719 abstracts were screened for inclusion, 666 from the initial literature search, 32 from the reference lists of other identified studies and 21 from other systematic reviews. No relevant unpublished studies were identified on Clinicaltrials.gov. Of the 719 abstracts screened 639 were excluded as not being relevant, leaving 80 studies for full-text review. Of 80 studies, 27 were excluded leaving 53 studies (Applegate et al. 1992; Barone et al. 2009; Blumenthal et al. 1989; Bouchonville et al. 2014; Braith et al. 1994; Broman et al. 2006; Chomiuk et al. 2013; Cononie et al. 1991; Dimeo et al. 2012; Dusek et al. 2008; Faulkner et al. 2013; Finucane et al. 2010; Gerage et al. 2013; Gonçalves et al. 2014; Hamdorf and Penhall 1999; Huang et al. 2006; Jessup et al. 1998; Kallinen et al. 2002; Lee et al. 2007; Li et al. 2003, 2005; Lim et al. 2015; Lovell et al. 2009; Madden et al. 2010; Millar et al. 2008; Miura et al. 2015; Mota et al. 2013; Nestel et al. 1993; Niederseer et al. 2011; Nishijima et al. 2007; Ohkubo

et al. 2001; Okumiya et al. 1996; Pagonas et al. 2014; Patil et al. 2015; Posner et al. 1992; Puggaard et al. 2000; Simons and Andel 2006; Sousa et al. 2013; Stachenfeld et al. 1998; Sunami et al. 1999; Taylor et al. 2003; Teng et al. 2007; Thomas et al. 2005; Valente et al. 2011; Venturelli et al. 2015; Vincent et al. 2003; Wang et al. 2012; Westhoff et al. 2007, 2008; Whelton et al. 1998; Wood et al. 2001; Yassine et al. 2009; Young et al. 1999) for inclusion in the qualitative/quantitative analysis (Figure 9).

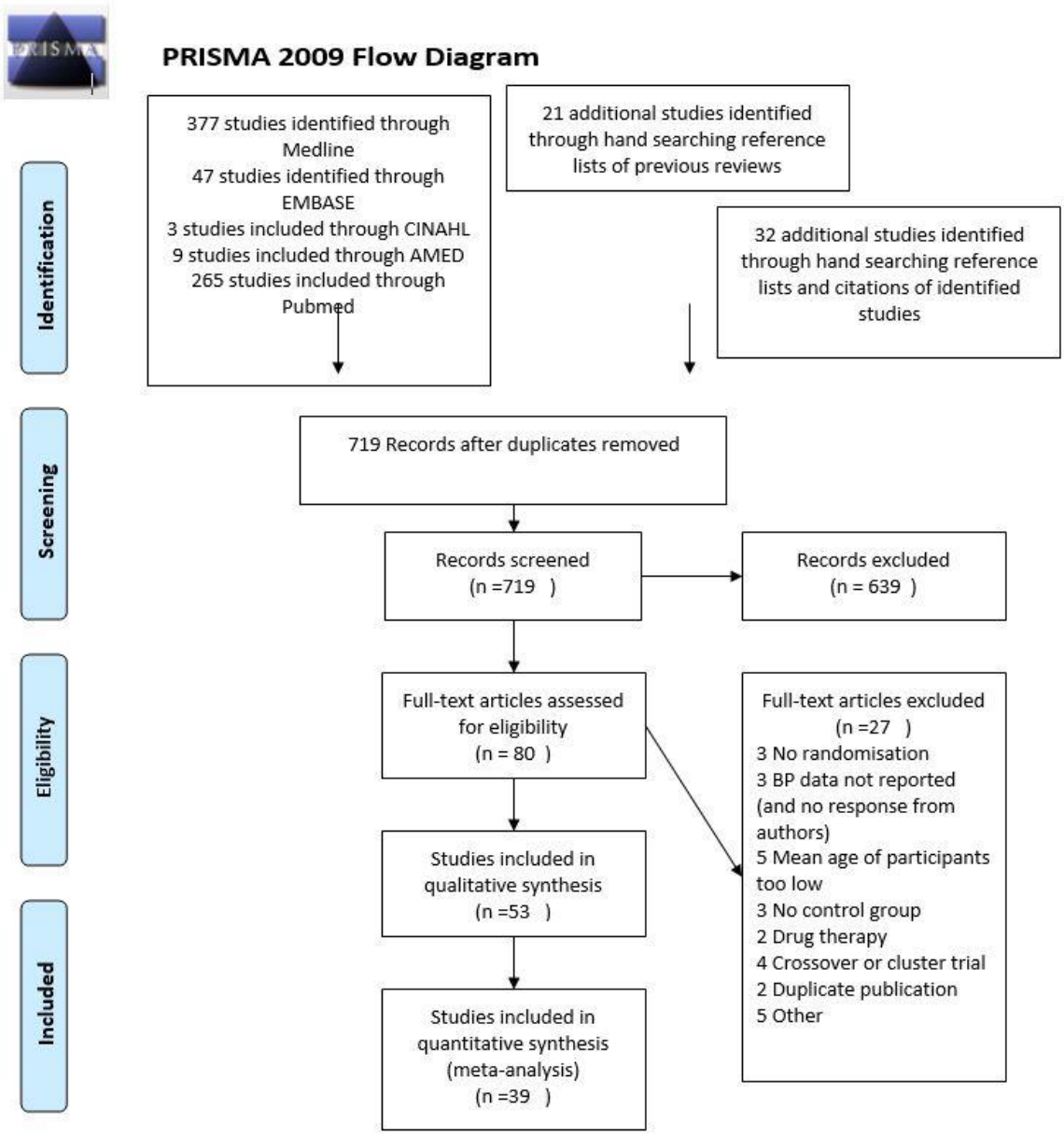


*Figure 9 PRISMA flow diagram of the systematic review*

### 2.7.2 Study characteristics

The characteristics of the included studies are shown in Table 1 and Table 2. The earliest study meeting inclusion criteria was published in 1989 and the latest in 2016. All studies were published journal articles. The majority of interventions

studied can be broadly categorised into aerobic exercise training (AET), dynamic resistance exercise training (RET) (forms of exercise involving moving external loads through a defined range of motion i.e. weight training), combined aerobic and dynamic resistance exercise training (COM) (Tambalis et al. 2009) and isometric exercise training (IET) (a form of resistance exercise training during which the muscle being exercised exerts a constant force against a fixed load without changing length). Both studies employing IET in this review used a sustained handgrip contraction around a dynamometer as their intervention. Other studies described yoga, dietary interventions, relaxation therapy, music therapy and traditional Chinese alternative therapies (e.g. cobblestone mat walking).

*Table 1 Characteristics of included studies*

| Study | N | %Male | Mean age | BP of participants | Other participant characteristics | Method of Blood pressure measurement |
|---|---|---|---|---|---|---|
| Applegate 1992 | 47 | 45 | 65 | Mild diastolic hypertension (DBP 85-100 mmHg) | Overweight (115% ideal body weight) | Rested seated |
| Barone 2009 | 101 | 49 | 65 | Prehypertension or mild hypertension (SBP 130-159 or DBP 80-99) | | Rested (position not specified) |
| Blumenthal 1989 | 101 | 50 | 67 | Not specified | free from coronary artery disease | Rested seated |
| Bouchonville 2014 | 107 | 37 | 70 | Not specified | Obese (BMI>30) | Rested supine |
| Braith 1994 | 44 | Not specified | 66 | Not specified | Sedentary | Rested seated |
| Broman 2006 | 29 | 0 | 69 | Normotensive | | Rested seated |
| Chomiuk 2013 | 68 | 12 | 71 | Not specified | | 24Hr Ambulatory |
| Cononie 1991 | 56 | 45 | 72 | Hypertensive and normotensive | free from cardiovascular, respiratory disease or diabetes | Rested seated |
| Dimeo 2012 | 50 | 44 | 65 | Hypertensive (BP > 140/90) | Must be on at least 3 antihypertensives | 24 Hr ambulatory and rested seated |

| | | | | | | |
|---|---|---|---|---|---|---|
| Dusek 2008 | 122 | 45 | 67 | Isolated systolic hypertension (SBP 140-159, DBP <90) | Taking 2 antihypertensives | Rested seated |
| Faulkner 2013 | 68 | 68 | | Not specified | TIA - diagnosed within 7 days of randomisation | Rested (position not specified) |
| Finucane 2010 | 100 | 56 | 71 | Not specified | Nondiabetic and no ischaemic heart disease | Rested seated |
| Gerage 2012 | 29 | 0 | 66 | Normotensive (BP <140/90) | Sedentary | Rested seated |
| Goncalves 2014 | 17 | 0 | 66 | Hypertensive (BP>160/100) | Sedentary | Rested seated |
| Hamdorf 1999 | 38 | 0 | 83 | Not specified | Sedentary | Rested seated |
| Huang 2006 | 52 | Not specified | 84 | Not specified | Sedentary | Rested seated |
| Jessup 1998 | 21 | 50 | 69 | Normotensive | Sedentary | Rested seated and 24Hr ambulatory |
| Kallinen 2002 | 42 | 0 | 76-78 | Not specified | | Rested seated |
| Lee 2007 | 202 | 58 | 71 | Hypertensive (SBP 140-179) | | Rested seated |
| Li 2003 | 40 | 78 | 73 | Not specified | Sedentary | Rested seated |

| | | | | | | |
|---|---|---|---|---|---|---|
| Li 2005 | 108 | 32 | 78 | Not specified | Sedentary | Rested seated |
| Lim 2015 | 20 | 0 | 71 | Not specified | | Rested seated |
| Lovell 2009 | 24 | 100 | 74 | BP <150/90 | free from cardiovascular disease | Rested (position not specified) |
| Madden 2010 | 36 | 58 | 71 | Hypertensive (BP>130/80) or on antihypertensives | Must have T2DM and hyperlipidaemia | Rested (position not specified) |
| Millar 2008 | 49 | 43 | 66 | Normotensive | | Rested (position not specified) |
| Miura 2015 (hypertensives) | 106 | 0 | 72 | Hypertensive (BP140-149/ 90-99) | | Rested supine |
| Miura 2015 (normotensives) | 115 | 0 | 72 | Normotensive (BP<140/90) | | Rested supine |
| Mota 2013 | 64 | 0 | 67 | Hypertensive- medicated | Sedentary | Rested seated |
| Niederseer 2011 | 42 | 52 | 67 | Not specified | | Rested seated |
| Nishijima 2007 | 561 | 44 | 67 | Both normotensive and hypertensive | BMI 24.2- 34.9 plus two or more risk factors for cardiovascular disease | Rested seated |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ohkubo 2001 | 39 | 49 | 67 | Both normotensive and hypertensive | SBP >120 | not specified |
| Okumiya 1996 | 42 | 43 | 79 | Not specified | no cardiovascular disease | Rested seated |
| Pagonas 2014 | 72 | 43 | 67 | Hypertensive BP> 140/90 or on antihypertensive medication | | 24Hr Ambulatory - reported as day and night-time. |
| Patil 2015 | 60 | 100 | 69 | Isolated systolic hypertension (pulse pressure > 60, SBP <160. DBP <100) | no other cardiovascular risk factors | Rested seated |
| Posner 1992 | 247 | 38 | 69 | BP <165/90 | Sedentary | Rested seated |
| Puggard 2000 | 55 | 0 | 85 | Not specified | | resting supine |
| Simons 2006 | 64 | 30 | 84 | normotensive | sedentary and not diabetic | Rested seated |
| Sousa 2013 | 59 | 100 | 69 | Not specified | sedentary | Rested seated |
| Stachenfeld 1998 | 17 | 0 | 72 | normotensive | no cardiovascular disease | Rested supine |
| Sunami 1999 | 40 | 50 | 67 | normotensive | | not specified |
| Taylor 2003 | 17 | 59 | 67 | Hypertensive (BP>140/85) | | Rested seated |
| Teng 2007 | 30 | 27 | 81 | Hypertensive | | Rested seated |
| Thomas 2005 | 207 | 55 | 69 | Normotensive | | Rested seated |

| | | | | | | |
|---|---|---|---|---|---|---|
| Valente 2011 | 27 | 41 | 67 | Not specified | Obese (BMI 25-40) sedentary | Rested seated |
| Venturelli 2015 | 40 | 50 | 68 | Hypertensive BP 140-159/90-99 | | not specified |
| Vincent 2003 | 62 | 45 | 68 | BP < 160/100 | | Rested seated |
| Wang 2012 | 361 | 32 | 77 | Not specified | sedentary | not specified |
| Westhoff 2007 | 54 | 48 | 68 | isolated systolic hypertension (SBP>140 DBP <90) | | 24Hr Ambulatory |
| Westhoff 2008 | 24 | 46 | 67 | Hypertensive (SBP >140 and/or taking antihypertensives) | | not specified |
| Whelton 1998 | 975 | Not specified | 66 | Hypertensive (BP <145/85 whilst taking antihypertensives) | | Rested seated |
| Wood 2001 | 36 | 47 | 68 | Not specified | | not specified |
| Yassine 2009 | 24 | 36 | 66 | Not specified | Obese (BMI30-40) and sedentary | Rested seated |
| Young 1999 | 62 | 21 | 67 | Hypertensive (BP130-159/<95) | not taking antihypertensives | Rested seated |

*Table 2 Characteristics of interventions in included studies*

| Study | Intervention groups | Details of interventions | Frequency of interventions | Duration of intervention | Controls | Details of control |
|---|---|---|---|---|---|---|
| Applegate 1992 | Combined aerobic exercise, diet induced weight loss of 4.5Kg and low sodium diet | 30mins slow walking | 4x per week | 6 months | Non-intervention controls | |
| Barone 2009 | Combined aerobic and resistance exercise | 45mins aerobic exercise at 60-90% Max heart rate and 7 resistance exercises, 2 sets of each of 12-15 reps at 50%1RM | 3x per week | 6 months | Non-intervention controls | |
| Blumenthal 1989 | Aerobic exercise | 60 mins comprising 10mins warm up, 30 mins cycling, 15mins brisk walking/jogging | 3x per week | 4 months | Non-intervention controls | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | and 5mins cool down | | | | |
| | Yoga | 60 mins | 2x per week | | | |
| | Combined aerobic and resistance exercise | 90mins broken down into 30 mins aerobic exercise (cycling, treadmill or stair climbing) aiming for 70-85% Max heat rate, 30 minutes resistance exercise (9 exercises for 1-2 sets at 6-8 reps of 70-85% 1RM) | 3x per week | 12 months | Non-intervention controls | |
| Bouchonville 2014 | Diet induced weight loss | Various low impact exercises- 40minutes at 65-70% HRmax | NA | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Combination of Exercise intervention and dietary intervention | as above | 3x per week | | | |
| Braith 1994 | Moderate intensity aerobic exercise | 45 mins walking at 50% Max heart rate increasing to 70% | 3x per week | 6 months | Non-intervention controls | |
| | High intensity aerobic exercise | 35mins walking uphill at 50 % max heart rate increasing to 85% | 3x per week | | | |
| Broman 2006 | Deep water running | 30mins interval training 3x 10mins with 2mins rest | 2x per week | 2 months | Non-intervention controls | |
| Chomiuk 2013 | Nordic Walking | 30mins Nordic walking | 3x per week | 6 weeks | Non-intervention controls | |
| Cononie 1991 | Resistance training | 10 exercises | 3x per week | 6 months | Non-intervention controls | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Aerobic exercise | 20-30minute walk at 50% VO2max increasing to interval training (jogging, brisk walking or uphill walking for 35-45 mins at 75%-85% VO2 Max) | 3x per week | | | |
| Dimeo 2012 | Aerobic exercise | Treadmill walking - interval training to obtain a lactate of 1.5-2.5 | 3x per week | 2-3months | Non-intervention controls | |
| Dusek 2008 | Relaxation therapy | 20 mins daily plus one supervised 60-minute session per week | daily | 2 months* Only first part of study included as protocol afterwards involved medication changes) | Lifestyle advice | |
| Faulkner 2013 | Combined aerobic and | 30 mins aerobic exercise (cycling and walking) at | 2x per week | 2 months | Non-intervention controls | |

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|---|---|---|---|---|---|---|
| | resistance exercise | 50-85% HR Max, 60 mins of resistance exercise | | | | |
| Finucane 2010 | Aerobic exercise | 60mins cycling at between 50-70% Wmax | 3x per week | 3 months | Non-intervention controls | |
| Gerage 2012 | Resistance training | 8 exercises, 2 sets of 10-15 reps | 3x per week | 3 months | Stretching programme | 25-30 mins 2x per week |
| Goncalves 2014 | Resistance training | 8 exercises, 2 sets of 15 reps | 3x per week | 3 months | Stretching programme | 2x per month |
| Hamdorf 1999 | Aerobic exercise | Walking (increasing from 5 minutes to 25 minutes by 1 minute per week) | 2x per week | 6 months | Non-intervention controls | |
| Huang 2006 | Moderate intensity aerobic exercise | Various low impact exercises-40minutes at 65-70% HRmax | 3x per week | 10 weeks | Stretching programme | Not stated |
| | High intensity aerobic exercise | Various low impact exercises 40 mins at 85-90%HR max | 3x per week | | | |

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|---|---|---|---|---|---|---|
| Jessup 1998 | Aerobic exercise | 25- 45 mins treadmill walking and stair climbing progressing from 50%HR Max to 85% HR Max | 3x per week | 4 months | Non-intervention controls | |
| Kallinen 2002 | Aerobic exercise | 1hr walking 2x per week and 1hr step aerobics 1x per week | 3x per week | 18 weeks | Non-intervention controls | |
| | Resistance training | 60 mins | 3x per week | | | |
| Lee 2007 | Walking (not directly observed) | Community walking intervention delivered by a nurse with verbal encouragement to increase walking. Monitored by a pedometer | | 6 months | Non-intervention controls | |
| Li 2003 | Cobblestone mat walking | 12-25 mins walking on a cobblestone mat | 3x per week | 2 months | Non-intervention controls | |

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|---|---|---|---|---|---|---|
| Li 2005 | Cobblestone mat walking | 6-30 mins walking on a cobblestone mat | 3x per week | 4 months | Walking | 6-30 mins walking 3x per week |
| Lim 2015 | Combined aerobic, resistance and Korean traditional dancing | 45 mins at 50-70% HR Max | 3x per week | 3 months | Non-intervention controls | |
| Lovell 2009 | Resistance training | 25 mins 1 exercise (incline squat) 3 sets of 6-10 reps increasing from 50-90% 1RM | 3x per week | 4 months | Non-intervention controls | |
| Madden 2010 | Aerobic exercise | 60 mins cycling or treadmill increasing from 50%HR Max to 85% HR max | 3x per week | 3 months | Core and strength training | 3x per week |
| Millar 2008 | Isometric handgrip | 4 sets of 2minute isometric contractions, alternating | 3x per week | 2 months | Non-intervention controls | |

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|---|---|---|---|---|---|---|
| | | hands, 30-40%MVC | | | | |
| Miura 2015 (hypertensives) | Combined aerobic and resistance training | Circuit training 90 mins | 2x per week | 3 months | Non-intervention controls | |
| Miura 2015 (normotensives) | Combined aerobic and resistance training | Circuit training 90 mins | 2x per week | 3 months | Non-intervention controls | |
| Mota 2013 | Resistance training | 40 mins increasing workload up to 80% 1RM | 3-4x per week | 4 months | Non-intervention controls | |
| Niederseer 2011 | Skiing | Mean 3.5 Hrs skiing per day for 29 days over study period | NA | 3 Months | Non-intervention controls | |
| Nishijima 2007 | Combined aerobic and resistance training | 60-90 mins including cycling 20-40 mins from 40-70% VO2 peak | 2-4x per week | 6 months | Lifestyle advice | |
| Ohkubo 2001 | Combined aerobic and resistance training | 120 mins in total consisting of cycling 10-25 mins between | 3x per week | 25 weeks | Lectures | 2x per month |

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|---|---|---|---|---|---|---|
| | | 25% and 60^ HR reserve and 5 resistance exercises | | | | |
| Okumiya 1996 | Combined aerobic and resistance training | 60 mins of light exercise including walking, balance training, dodgeball, stretching and bodyweight resistance training | 2x per week | 6 months | Non-intervention controls | |
| Pagonas 2014 | Aerobic exercise | 30-36 mins walking interval training to a target lactate concentration of 1.5-2.5 | 3x per week | 2-3months | Non-intervention controls | |
| Patil 2015 | Yoga | 60 mins | 6x per week | 3 months | Brisk walking | 60mins 6x per week |
| Posner 1992 | Aerobic exercise | 40 mins cycling at 70%VO2 peak | 3x per week | 4 months | Group talks | 1x per week |

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|---|---|---|---|---|---|---|
| Puggard 2000 | Combined aerobic and resistance training | 60 mins walking and various strength exercises to achieve 69%HR Max | 1x per week supervised plus between 1-6x per week home solo training) | 8 months | Non-intervention controls | |
| Simons 2006 | Aerobic exercise | Walking | 2x per week | 4 months | Non-intervention controls | |
| | Resistance training | 6 exercises, 1 set of 10 reps | 2x per week | | | |
| Sousa 2013 | Aerobic exercise | 30 mins walking, jogging dancing or swimming and 10mins bodyweight resistance exercises | 3x per week | 9 months | Non-intervention controls | |
| | combined aerobic and resistance training | 7 exercises 3 sets of 8-12 reps increasing from 65%1RM to 75%1RM plus 1x per week plus the aerobic intervention 2x per week | 1xper week resistance, 2x per week aerobic | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Stachenfeld 1998 | Aerobic exercise | Either trampolining or treadmill walking increasing from 20mins to 50 mins at 60%Max Hr increasing to 75% Max Heart rate | 3-4x per week | 4-6 months | group stretching and yoga | 60mins 3-4x per week |
| Sunami 1999 | Aerobic exercise | 60 mins cycling at 50%VO2 max | 2-4x per week | 5 months | Non-intervention controls | |
| Taylor 2003 | Isometric handgrip | 4 x 2minute contractions at 30%MVC, alternating hands 1min rest between contractions | 3x per week | 10 weeks | Non-intervention controls | |
| Teng 2007 | Music therapy | 25 minutes listening to classical music | daily | 1 month | Non-intervention controls | |
| Thomas 2005 | Thai Chi | 60 mins Yang style Thai Chi | 3x per week | 1 year | Non-intervention controls | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Resistance training | 60 mins for 30 reps of each of 7 exercises | 3x per week | | | |
| Valente 2011 | Dietary education | 30 mins DASH based dietary education, encouragement to exercise for 30mins per day aiming for 10% wt. loss | 1x per week | 10 weeks | Dietary education Plus resistance exercise | 40mins, 6 exercises 4 sets 8-12 reps,3x per week |
| Venturelli 2015 | Aerobic exercise | 60mins treadmill, elliptical and stepper ergometers at 70% maximum exercise capacity | 3x per week | 3 months | Non-intervention controls | |
| | Circuit training | 60 mins 4 exercises, 60s work at 1 contraction per second) | 3x per week | | | |
| | relaxation training | 60 mins relaxation and meditation | 3x per week | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Vincent 2003 | Resistance training (high intensity) | 13 exercises 1 set of 8 reps at 80%1RM | 3x per week | 6 months | Non-intervention controls | |
| | Resistance training (low intensity) | 13 exercises 1 set of 13 reps at 50% 1RM | 3x per week | | | |
| Wang 2012 | Aerobic exercise | walking (at least 150mins/wk.) plus a variety of strength exercises | 5x per week | 1 year | Non-intervention controls | |
| Westhoff 2007 | Aerobic exercise | 30 -36min Interval training on a treadmill to reach a lactate concentration of 2-3 | 3x per week | 3 months | Non-intervention controls | |
| Westhoff 2008 | Upper limb cycling | 30 mins upper limb cycling in an interval pattern to achieve a lactate of 1.5-2.5 | 3x per week | 3 months | Non-intervention controls | |
| Whelton 1998 | Sodium restriction | <80mmol sodium per day | NA | up to 37 months | Non-intervention controls | |

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|---|---|---|---|---|---|---|
| | Weight loss | diet induced weight loss of at least 4.5Kg | | | | |
| | Sodium restriction and weight loss | Combination of the other two interventions | | | | |
| | Aerobic exercise | 21-45mins treadmill or cycling at 60-70% HRmax | 3x per week | 3 months | Non-intervention controls | |
| | resistance training | 8 exercises 1-2 sets of 8-15 reps at 75% 5RM | 3x per week | | | |
| Wood 2001 | Combined aerobic and resistance training | maximum 30mins aerobic exercise and 1 set of each exercises from other group | 3x per week | | | |
| Yassine 2009 | Aerobic exercise | 50-60 mins cycling or walking to achieve 60-85% HR Max | 5x per week | 3 months | Exercise + 500kcal restriction | The same exercise programme as the other group |

| | | | | | | |
|---|---|---|---|---|---|---|
| Young 1999 | Aerobic exercise | 60 mins at 40-60% HR Reserve | 2x per week | 3 months | Thai chi | 60 mins Yang style 2x per week |

### 2.7.3 Risk of bias

All included studies carried a high risk of bias in at least one domain (Figure 10). The majority of studies were at high risk of bias due to the innate difficulties in blinding participants to a physical activity intervention. A large number of studies did not describe their random sequence allocation or allocation concealment in sufficient detail to be judged as low risk of bias, and many did not describe blinding of their outcome assessment. Many studies were at risk of reporting bias and some may have suffered from attrition bias. Finally, nearly half of the studies had groups that had either clinically or statistically significantly different BP values at baseline, thus potentially introducing bias to their results.

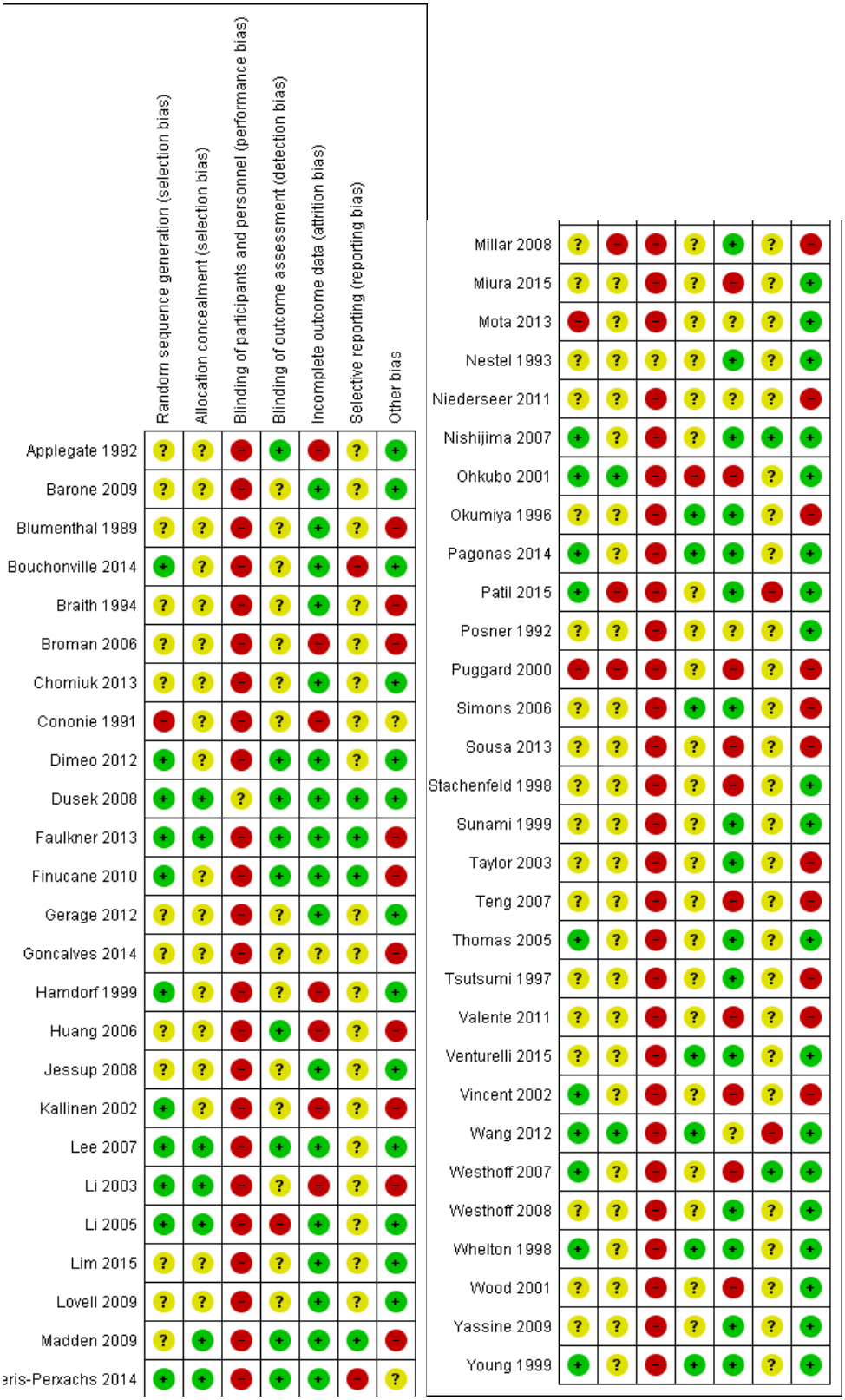


*Figure 10 Risk of bias in included studies*

Green "+"; Low risk of bias, yellow "?"; Unclear risk of bias,

Red "–"; High risk of bias

### 2.7.4 Data synthesis

It was judged that there were sufficient studies to perform independent meta-analysis for AET, RET, COM and IET interventions. Although only two studies were available for IET, as there was minimal clinical heterogeneity between the protocols it was decided to perform meta-analysis. Four studies were not included in the meta-analyses due to lack of an adequate non-intervention control group.

### 2.7.5 Aerobic exercise

In all, 26 study groups from 24 trials were analysed, comprising 925 individuals in the intervention groups and 784 control participants. AET provided a weighted mean difference in reduction in SBP of -5.09 (95% CI -7.22 to -2.97) mmHg (Figure 11) compared to control, and a difference in DBP of -2.20 (95% CI -3.08 to -1.31) mmHg (Figure 12). There was evidence of statistical heterogeneity in both analyses ($I^2$=78% and $I^2$=61% respectively). On meta-regression analysis, neither duration of intervention ($R^2$=0%; p=0.47 for SBP and $R^2$=0%; p=0.75 for DBP (Appendix 2)) nor hypertensive baseline status ($R^2$=4%; p=0.37 for SBP and $R^2$=0%; p=0.95

for DBP) could explain the between-study heterogeneity. There was no evidence of publication bias in either analysis (p=0.68 and p=0.41 respectively). The quality of evidence of both outcomes was regarded as low using GRADE criteria (downgraded owing to concerns over risk of bias and unexplained heterogeneity).

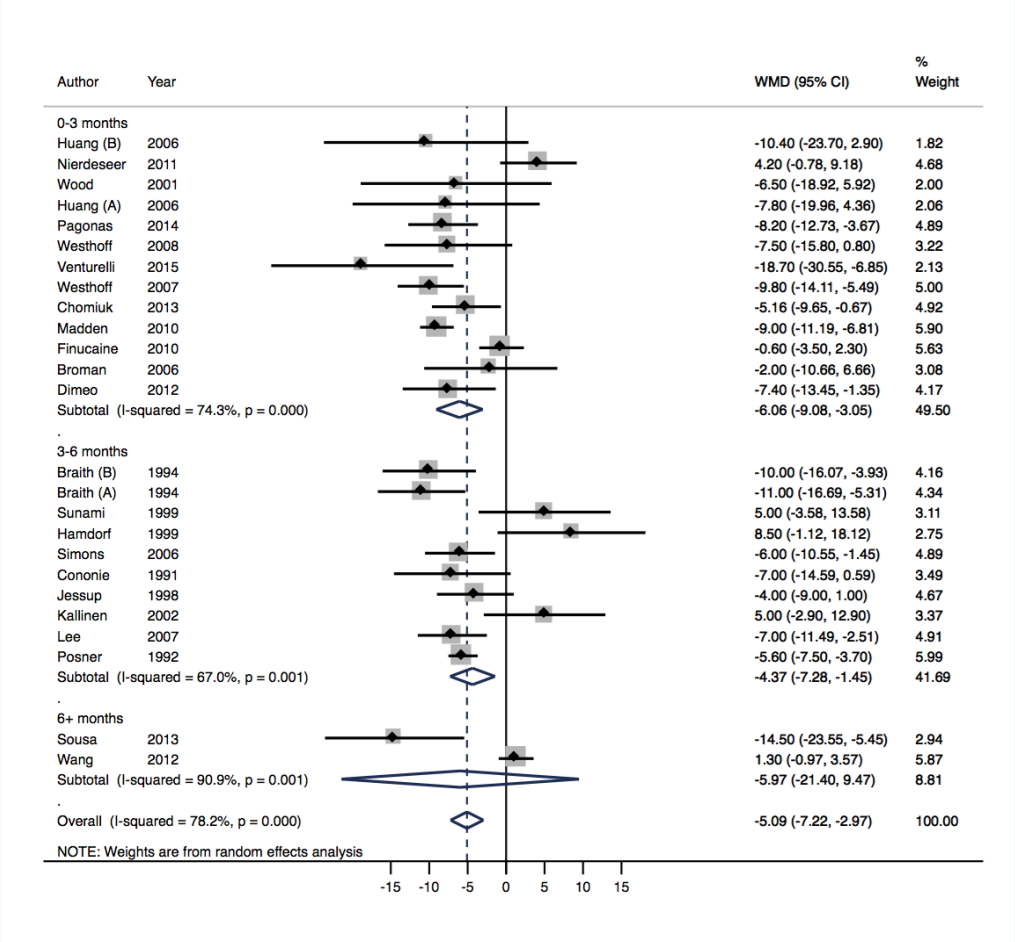


*Figure 11 Forest plot showing change in systolic blood pressure from aerobic exercise*

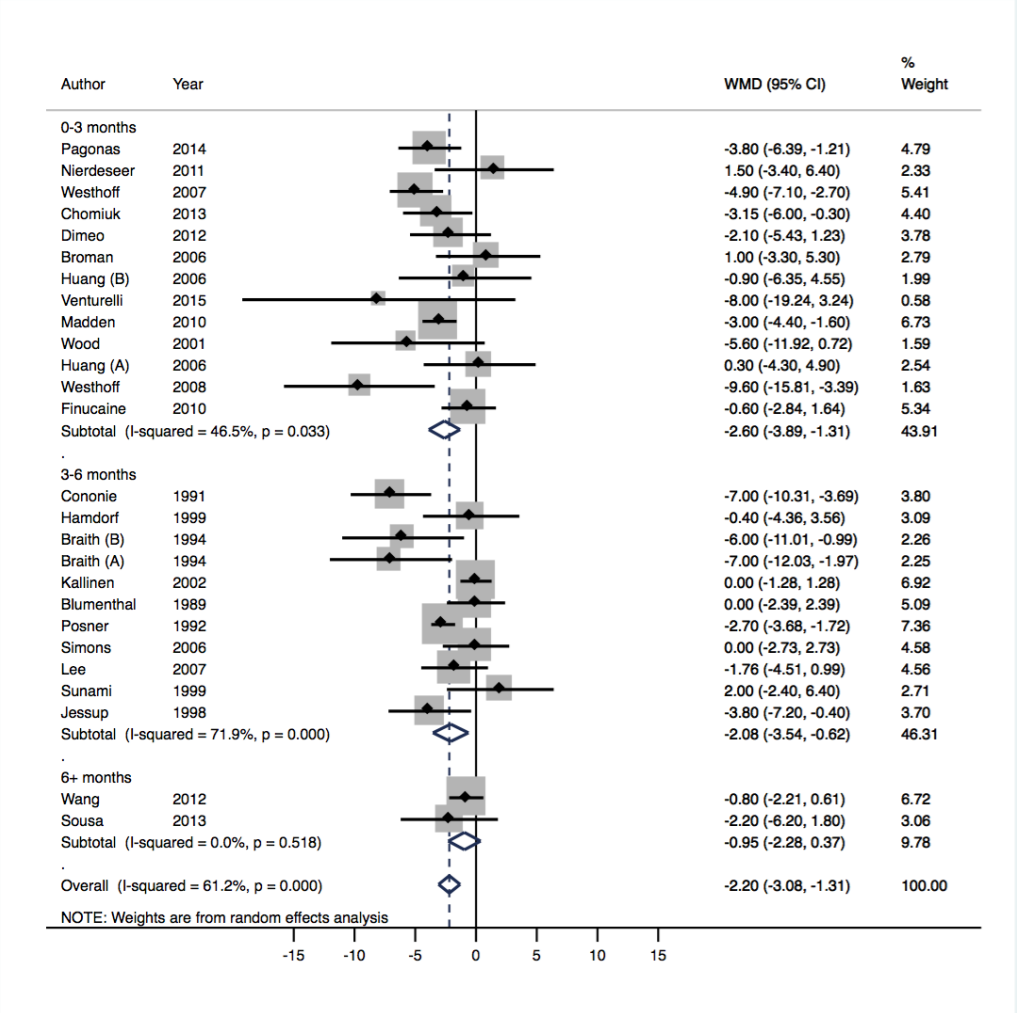


*Figure 12 Forest plot showing change in diastolic blood pressure from aerobic exercise*

## 2.7.6 Resistance exercise

For RET, 13 study groups from 12 trials were analysed, comprising 263 individuals in the intervention groups and 251 control participants. RET provided a reduction in SBP of -5.46 (95% CI -8.61 to -2.31) mmHg (Figure 13) and a reduction in DBP of -2.02 (95% CI -3.31 to -0.73) mmHg (Figure 14). There was evidence of statistical heterogeneity in both

analyses ($I^2$=71% and $I^2$=54%, respectively). On meta-regression analysis, neither duration of intervention ($R^2$=0%; p=0.81 for SBP and $R^2$=0%; p=0.72 for DBP (Appendix 2)) nor hypertensive baseline status ($R^2$=0%; p=0.69 for SBP and $R^2$=23%; p=0.19 for DBP) could explain the between-study heterogeneity. There was evidence of an asymmetrical Funnel plot for the DBP analysis (p=0.004) indicating possible publication bias. The quality of evidence was low using GRADE criteria for SBP (concerns over risk of bias and unexplained heterogeneity) and very low for DBP (concerns over risk of bias, unexplained heterogeneity and possible publication bias).

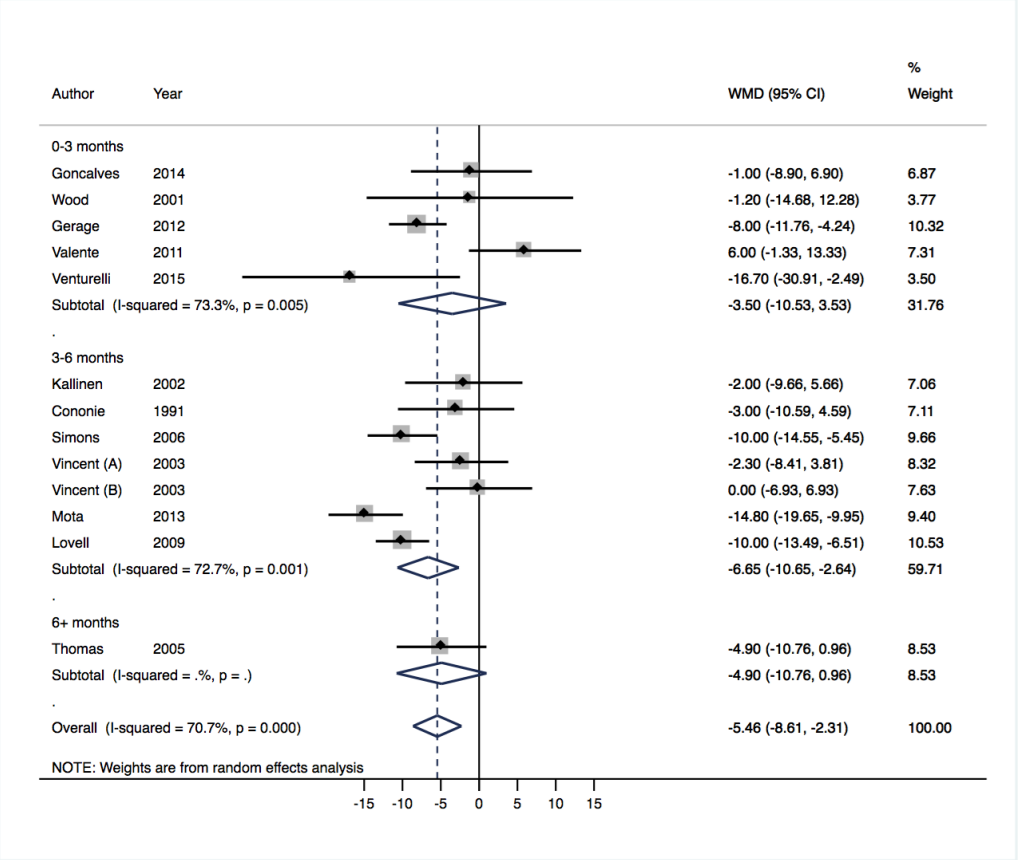


*Figure 13 Forest plot showing change in systolic blood pressure from resistance exercise*

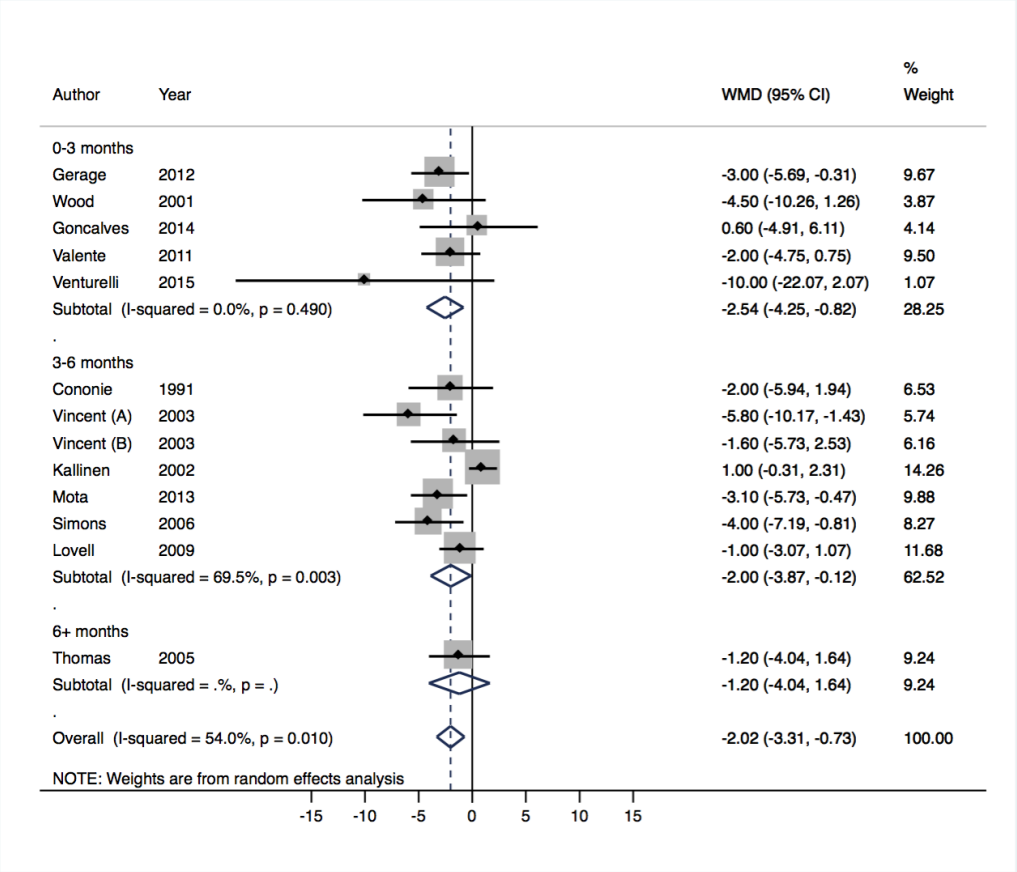


*Figure 14 Forest plot showing change in diastolic blood pressure from resistance exercise*

## 2.7.7 Combined aerobic and resistance training

For COM study groups from 12 trials were analysed, comprising 615 individuals in the intervention groups and 622 control participants. COM provided a reduction of -5.86 (95% CI -8.27 to -3.45) mmHg (Figure 15) in SBP and -3.51 (95% CI -4.43 to -2.59) mmHg in DBP (Figure 16). There was evidence of statistical heterogeneity in the SBP analysis ($I^2$=73%). On meta-regression analysis, neither duration of intervention ($R^2$=0%; p=0.65 for SBP and $R^2$=35%; p=0.18

for DBP (Appendix 2)) nor hypertensive baseline status ($R^2$=0%; p=0.65 for SBP and $R^2$=0%; p=0.37 for DBP) could explain the between-study heterogeneity. There was no evidence of publication bias for either analysis (p=0.81 and p=0.51 respectively). The quality of the evidence was low for SBP (concerns over risk of bias and unexplained heterogeneity) and moderate for DBP (concerns over risk of bias).

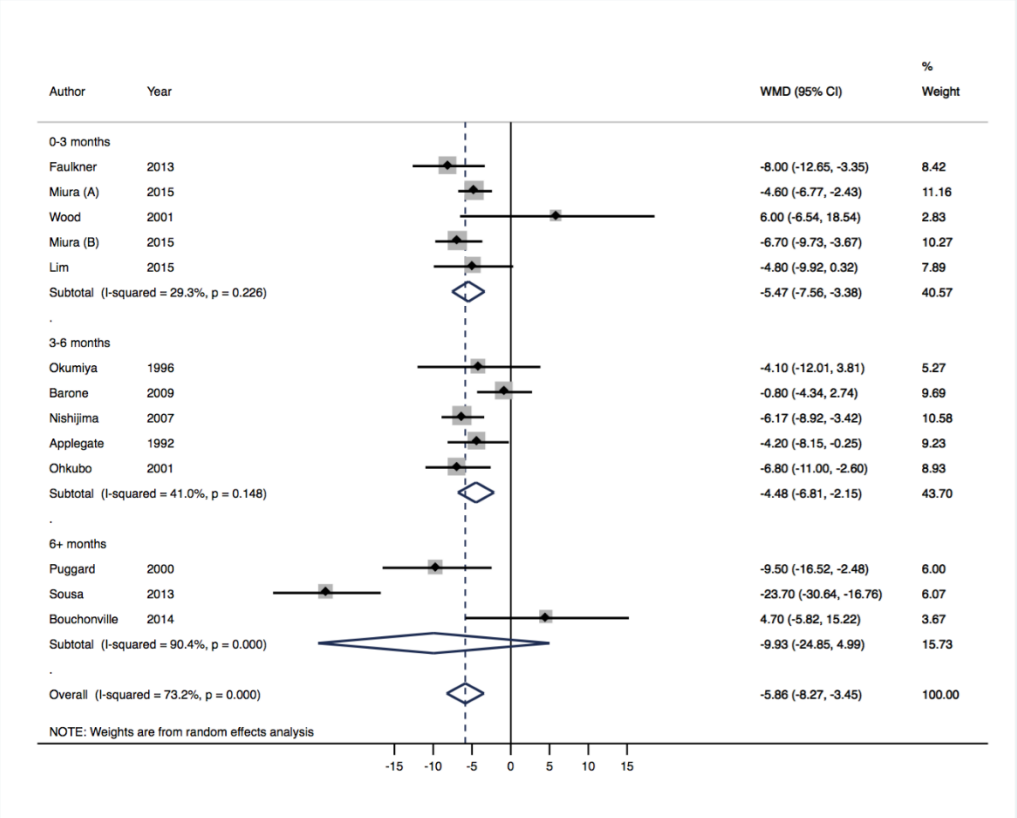


*Figure 15 Forest plot showing change in systolic blood pressure from combined aerobic and resistance exercise*

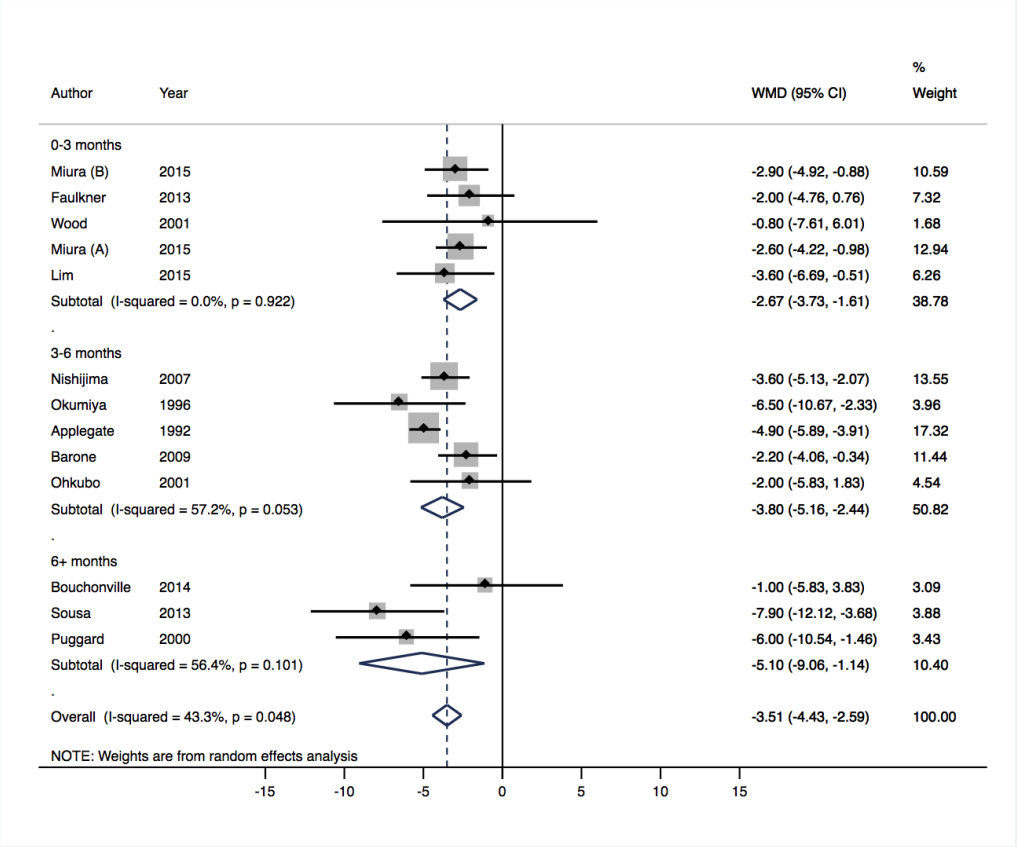


*Figure 16 Forest plot showing change in diastolic blood pressure from combined aerobic and resistance exercise*

## 2.7.8 Isometric exercise

Two studies assessing IET were analysed including 34 individuals in the intervention groups and 32 control participants. IET provided a reduction of -9.14 (95% CI -10.76 to -7.51) mmHg in SBP and -3.01 (95% CI -3.57 to -2.45) mmHg in DBP. There was no evidence of statistical heterogeneity in either analysis ($I^2$=0% for both). There were too few studies to assess publication bias. The quality of evidence was moderate for both SBP and DBP (concerns over risk of bias).

### 2.7.9 Other interventions

Three papers (Whelton et al. 1998; Bouchonville et al. 2014; Nestel et al. 1993) describe dietary interventions including either sodium restriction or calorie control induced weight loss, however there was too much clinical heterogeneity between the interventions to permit meta-analysis. Two further papers (Li et al. 2003, 2005) described cobblestone mat walking, a traditional Chinese remedy, however these were both conducted by the same author at the same institution and so meta-analysis was not carried out. Yoga (2 studies) (Patil et al. 2015; Blumenthal et al. 1989), Tai Chi (1 study) (Thomas et al. 2005), relaxation therapy (2 studies)(Dusek et al. 2008; Venturelli et al. 2015) and lifestyle advice sessions (1 study) were judged to be sufficiently different interventions to prevent meaningful meta-analysis. One study described music therapy (Teng et al. 2007).

The dietary intervention studies based on sodium restriction and calorie-controlled weight loss (3 studies) had mixed results, leading to significant reductions in both SBP and DBP in two studies but not in the third (Appendix 2).

With regard to the alternative interventions, the two studies investigating cobblestone mat walking reported non-significant and significant reductions in both SBP and DBP, respectively. Conversely, one yoga intervention study reported a significant reduction in SBP but no significant change in DBP, while another reported no significant change in DBP (SBP was not reported). Tai chi did not lead to a significant change in either SBP or DBP. Relaxation therapy, lifestyle advice sessions and music (classical) therapy were all associated with significant reductions in both SBP and DBP (Appendix 2).

## 2.8 Discussion

This systematic review has demonstrated that AET, RET or both performed together (COM), undertaken regularly over at least a three-month period can lead to reductions of 5-6 mmHg in SBP and 2-3.5 mmHg in DBP in individuals aged over 65 years. However, this did not reach our *a priori* threshold for clinical significance (10/5 mmHg) (Law et al. 2009). There was no additional benefit seen in exercise programmes lasting longer than 3 months and similar reductions in BP were seen in trials including both hypertensive and non-hypertensive individuals.

These small reductions in BP are similar to those reported by other reviews which have examined lifestyle interventions in much younger patients (Whelton et al. 2002; Semlitsch et al. 2013; Lee et al. 2010b) and are similar to those expected from the introduction of one antihypertensive agent at half-standard dose (Law et al. 2009). Thus, lifestyle intervention alone cannot be recommended as a sole treatment for hypertension but may serve as a useful adjunct to pharmacotherapy. Of note, these reductions are equivalent to those achieved in some large RCTs investigating antihypertensives (such as the PROGRESS (PROGRESS Collaborative Group 2001) and ADVANCE trials (Patel and ADVANCE collaborative group 2007)). In the ADVANCE trial this magnitude of reduction was associated with significant reductions in events, but this was not seen in the PROGRESS trial. In the setting of patients without hypertension, whose BP is below current treatment thresholds for pharmacotherapy, lifestyle interventions may be useful in further reducing (or possibly preventing increases in) BP.

There did not appear to be any additional benefit in combining AET with RET, suggesting that they may both elicit their change in BP through similar mechanisms (Cardoso et al.

2010; Diaz and Shimbo 2013). Similarly, there appears to be no significant additional benefit in extending the duration of interventions beyond 3 months as there was no interaction between intervention duration and magnitude of effect. However, as no studies reported BP follow up data in the months after intervention cessation, it is unknown whether these benefits persist or if continued intervention is required to maintain the benefit. Considering evidence from studies exploring other aspects of exercise adaptation, it may be that detraining would likely lead to benefits being lost rapidly on cessation of intervention (Zanettini et al. 1997; Moker et al. 2014).

Adherence to the interventions in the included studies varied substantially with dropout rates ranging from 0% to 26% (Vincent et al. 2003). That some dropout occurred at screening for participants who were physically incapable of participating in the interventions may reflect that some older adults find exercise interventions difficult to engage with and sustain. Further work is required to develop interventions that are accessible to those with mobility impairments and agreeable to the majority of older individuals. It is likely that the majority of subjects in the included studies were highly selected

volunteers and they may not be representative of the general population.

It is interesting that some studies in this review were published over 25 years ago with others as recently as 18 months ago whilst there are also studies currently prospectively registered on clinical trials registers. That very simple interventions are still being actively studied in older populations serves to highlight concerns about long term sustainability of some interventions, particularly dietary ones (Appel and Pickering 2004), but also the need to treat an ageing population with associated health conditions.

Of note, despite a wide-ranging search strategy intended to include all forms of lifestyle modification, this review identified very few randomised controlled trials on interventions that were not exercise based. This is despite lifestyle interventions such as sodium restriction and weight loss being recommended in various guidelines (Jansen et al. 2015).

Although the focus of this review has been on the effect of lifestyle interventions on blood pressure, many of the exercise

interventions described may have had other benefits to the participants such as an improvement in functional status (Baptista et al. 2017), which may in fact have benefitted their quality of life more than a reduction in blood pressure ever could.

Isometric exercise has the potential for large reductions in BP of up to 9 mmHg SBP. However, this figure is based on very limited evidence from only a small number of participants. Of note, of the two studies investigating IET included in this review, one included 9 patients with hypertension in the intervention group whilst the participants in the other study had a mean starting blood pressure of 122/70 mmHg. Despite several decades of research on IET, with studies on younger participants carried out since 1992 (Wiley et al. 1992), a recent systematic review of all IET was only able to include 11 trials of 302 participants (Inder et al. 2016), whilst another of only isometric handgrip could only include 7 trials and 157 participants (Jin et al. 2017). Despite this dearth of high-quality published evidence, products claiming efficacy for treatment of hypertension are available for purchase. Further high quality studies are required to investigate this novel intervention in an older population as it has several advantages

over other interventions with potential for use in those with physical mobility limitations and being very short in duration (~15 minutes per session) compared with the majority of AET and RET interventions (often 30 minutes to 1 hour per session) (Garber et al. 2011). Specifically, this time-efficiency is likely important for intervention uptake and compliance as "lack of time" is a commonly cited barrier to exercise (Booth et al. 1997). It remains unclear why such a simple and non-invasive potential therapy for hypertension has not been studied robustly in older adults to date.

### 2.8.1 Limitations

A limitation of this review, as is common to all systematic reviews, is the potential for missed studies from our search. In order to mitigate against this the search was carried out across multiple databases and an attempt to find unpublished studies was made using a clinical trials registry. Furthermore, a detailed hand search of all references of included studies was undertaken, together with another hand search of all articles citing included studies in order to minimize this risk. However, it is not inconceivable that some studies were potentially missed.

Although aiming to review the literature on a population over the age of 65 years, we may have included some patients younger than this as our inclusion criteria stipulated a mean study population age of over 65 and not 65 years as an absolute cut off. This decision was taken for a number of reasons including the paucity of papers stipulating a minimum age of 65 and also the large number of papers which did not specify their lower age limit.

A large number of trials included in this review did not provide standard deviations for changes in BP, necessitating imputation using recognised methods from the Cochrane handbook (The Cochrane Collaboration 2008). This is a common issue in trial reporting and this review has attempted to minimise any inaccuracy this may have brought about by imputing SDs from a correlation coefficient calculated from the studies who did present their change SDs. However, if physical activity were to alter the variability in change in BP then these assumptions may prove false.

Many of the meta-analyses in this review included trials with large degrees of statistical heterogeneity. A large part of this heterogeneity is most likely accounted for by the large degree

of clinical heterogeneity between described interventions and between populations BP at baseline.

The majority of trials in this review were at high risk of bias in at least one domain, which may exaggerate effect estimates in meta-analyses (Doleman et al. 2017). Particular concern must be paid to the lack of blinding of BP measurement in the majority of included studies. Furthermore, the majority of these trials relied on the use of office BP (measurements at one time point during one visit to the laboratory) as their primary endpoint, rather than ambulatory BP (which was only measured in 4 studies), which may cast doubt on the ability of the interventions to reduce BP out of the research environment (Reino-Gonzalex et al. 2017; Coats 1996).

Although the majority of studies included in this review included volunteers of both genders, no study reported changes in BP according to gender. In the context of the known gender differences in BP control, original studies are required to determine whether certain lifestyle interventions may be more effective in each gender (Maranon and Reckelhoff 2013).

None of the studies described in this review provided any long-term follow up data to demonstrate the on-going benefits to BP after a period of exercise, and further work is required to assess long-term benefits. In addition, it is unclear whether such reductions in BP can be achieved over shorter periods of time, which may have additional clinical relevance, for example for preoperative optimisation in surgical patients (Boereboom et al. 2016a).

## 2.9 Chapter conclusions

In conclusion, the best available evidence suggests that non-pharmacological lifestyle interventions involving either aerobic exercise training, dynamic resistance exercise training or a combination of the two can lead to statistically significant reductions in both SBP and DBP in older adults. However, these reductions failed to reach thresholds for clinical significance and as such cannot be recommended as antihypertensive monotherapy, in the majority of individuals. Further the studies supporting these interventions contain various limitations. Isometric exercise training, with its potential benefits in adherence in older adults, did show promise, although there is insufficient evidence for its use outside trials in older adults. More studies in the use of IET are required.

# 3 Method and time efficient strategy protocol development: A pilot study in middle aged adults

## 3.1 Chapter focus

This chapter describes a brief pilot study carried out in middle aged female participants as a means of method and protocol development and to refine experimental techniques, prior to studies carried out in older adults (who were a much more limited resource and saved for the subsequent studies).

## 3.2 Introduction

Cardiovascular disease (CVD) represents a significant global health burden, being the leading cause of death worldwide (GBD 2013 Mortality and Causes of Death Collaborators 2015). To exemplify the modifiable nature of CVD risk, known risk factors such as hypertension, dyslipidaemia, smoking, diabetes, abdominal obesity, diet and physical inactivity may all be influenced and have been held accountable for over 90% of the risk of having an acute myocardial infarction (Bonow 2002; Yusuf et al. 2004) A number of these risk factors may be modified using medication, however they are not without side effects (Finegold et al. 2014; Taylor and Thompson 2015), and compliance remains poor (Larbizabal and Deedwania 2010; Gislason et al. 2006; Peterson and McGhan 2005; Newby et al. 2006). In addition, patients are often unwilling to

take medications in the absence of symptoms (Ross et al. 2004; Munger et al. 2007).

Non-pharmacological interventions (i.e. diet and exercise) can also modulate CVD risk (Hellenius et al. 1993; Bassuk and Manson 2005). However, despite these well-publicised benefits, the uptake of physical activity remains low. One third of adults and four fifths of adolescents worldwide fail to meet physical activity guidelines (Hallal et al. 2012). Exercise referral schemes have long been promoted as a means of increasing physical activity (Fox et al. 1997), but have variable compliance and efficacy (Williams et al. 2007; Dugdill et al. 2005). Key cited barriers to physical activity include cost, childcare access and crucially, lack of time (Withall et al. 2011). These barriers make the prospect of an inexpensive, time-efficient form of physical conditioning, which can be performed at home without specialist equipment very attractive.

Repeated remote ischaemic preconditioning (RIPC) (Epps et al. 2016) and isometric handgrip training (IHG) (Inder et al. 2016) are two simple interventions which have the potential to modify CVD risk factors. RIPC involves repeatedly alternating

periods of ischaemia and reperfusion in a muscle bed in an attempt to precondition the body to withstand future periods of hypoxia. IHG involves sustained repeated contractions of the hand around a dynamometer maintaining a fixed muscle length. Single session RIPC performed immediately prior to exercise has been shown to improve athletic performance in competitive athletes (Jean-St-Michel et al. 2011) and recreationally active young individuals (Cruz et al. 2015, 2016). However, the few studies of repeated RIPC (over 7 days to 8-weeks) have had mixed effects on cardiorespiratory fitness (CRF) (Jones et al. 2014b; Banks et al. 2016; Lindsay et al. 2017), a validated predictor of future CVD risk (Kodama et al. 2009). It must however be noted that the majority of these studies were in male volunteers. The potential mechanisms behind which repeated IPC could mediate improvements in CRF are still unknown, but could involve both local effects on the muscle being preconditioned as well as remote effects via both neural pathways and humoral factors(Sharma et al. 2015). Several studies have reported the effects of repeated RIPC on resting blood pressure (BP), although as yet a consensus on its efficacy has not been reached (Epps et al. 2016). Conversely, IHG has been shown in a number of trials to reduce resting BP in both normotensive and hypertensive individuals (Inder et al. 2016). However the

only two studies to date which measured changes in CRF after a prolonged period of IHG did not show benefit (Blackwell et al. 2017; Goessler et al. 2018), however the larger of these studies was of unsupervised IHG with a reported adherence as low as 63%.

Another predictor of CVD risk (alongside BP and CRF) is endothelial dysfunction (Bonetti et al. 2002). Reduced endothelial function may precede overt vascular disease by a number of years and can be estimated by brachial artery ultrasound scanning to detect flow mediated dilatation (FMD) in response to shear stress applied to the artery wall (Pyke and Tschakovsky 2005; Charakida et al. 2010; Faulx et al. 2003). This measure of endothelial plasticity is commonly used as a surrogate for cardiovascular health and has been validated as a means of assessing vascular responsiveness (Charakida et al. 2010). IHG has been shown to improve FMD in medicated hypertensive (McGowan et al. 2007b), but not normotensive subjects (McGowan et al. 2007a), over an 8-week period. Similarly, RIPC has shown promise with improvements in FMD demonstrated after 2-weeks intervention (Jones et al. 2014b) in a young normotensive healthy population. However, as with the aforementioned studies assessing the effects of these

'static' interventions on CRF and resting BP, almost all of the study subjects were male.

The current paucity of any substantial studies investigating these interventions in premenopausal females means no firm conclusions can yet be drawn as to whether they have any role to play in CVD risk modulation in this population. This is of increasing importance with many traditional CVD risk factors now increasing in this population (Garcia et al. 2016).

This study was therefore designed as a pilot study to investigate the efficacy of 4-weeks supervised RIPC or IHG on CRF, BP and endothelial function in inactive but otherwise healthy, premenopausal females. A 4-week intervention was chosen in order to try to minimise natural variation in physiological parameters within subjects due to phases of their menstrual cycle (Mihm et al. 2011). This pilot study aimed to develop the methods and protocols needed to perform a larger study in older adults and to perform exploratory analyses on the effects of RIPC and IHG on these outcomes in middle aged females.

## 3.3 Method

### 3.3.1 Subject characteristics

Institutional research ethics approval (University of Nottingham Medical School Ethics Committee) was obtained (J14112013 SoM MS GEM) before healthy (non-hypertensive) inactive female subjects aged 25-50 years (37.9 (7.9); BMI >18 or <30kg/$m^2$: (23.8 (3.1)) were recruited by local advertising. Activity status was defined as no participation in any formal exercise regime. After obtaining written informed consent, 30 subjects were recruited to this study. Twenty were randomised to either IHG or RIPC, with 10 subjects allocated separately to a non-intervention control group. Figure 17 shows a CONSORT diagram demonstrating the study conduct. There were no significant differences in any baseline demographic characteristics between the groups (Table 3). All methods were performed in accordance with the relevant guidelines and regulations. The study was registered with clinicaltrials.gov (NCT03473990) and complied with the 1964 Declaration of Helsinki.

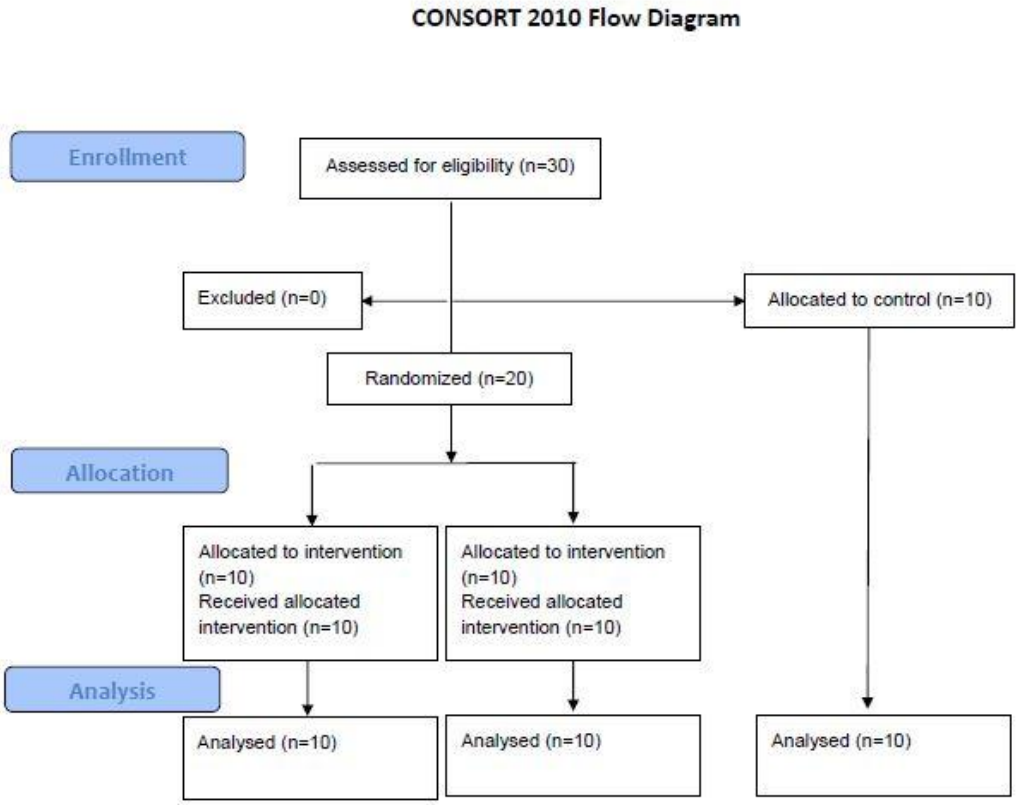


*Figure 17 CONSORT diagram*

*Table 3 Subject characteristics*

| Group | Age (y) | Weight (kg) | Height (m) | BMI ($kg/m^2$) |
|---|---|---|---|---|
| **RIPC** | 38(7) | 66.1(9.3) | 1.68(0.07) | 23.4(2.9) |
| **IHG** | 33(9) | 63.0(10.3) | 1.65(0.07) | 23.2(3.6) |
| **Control** | 38(7) | 66.0(7.0) | 1.62(0.05) | 25.0(2.0) |
| **p-value** | 0.34 | 0.69 | 0.20 | 0.33 |

Abbreviations: IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning;

Before baseline testing, all subjects underwent cardiovascular examination by a qualified medical doctor with exclusion criteria for further participation as per the ATS/ACCP guidelines for Cardiopulmonary Exercise Testing (CPET) (Weisman et al. 2003). At baseline testing participants underwent measurements of resting BP and FMD and completed a CPET. After the 4-week intervention (or control) period the same tests were repeated.

### 3.3.2 Pre- and Post-Intervention Testing

For both testing sessions subjects attended the laboratory fasted from midnight with supine BP measured in triplicate after a 5-minute supine resting period using oscillometry (Datascope trio patient monitor, Datascope, New Jersey, USA) and a blood pressure cuff (Welch Allyn, New York, USA) of appropriate size (British and Irish Hypertension society 2017) using the subject's right arm.

After 30-minutes supine in a 24°C temperature-controlled room FMD was assessed according to the International Brachial Artery Reactivity Task Force guidelines (Corretti et al.

2002b) using the subject's right arm. In brief, after a baseline measurement of brachial artery diameter for 1-minute, arterial occlusion distal to the brachial artery was induced using a BP cuff (Hokanson, Washington, USA) inflated to 200 mmHg for 5-minutes. The cuff was then deflated, and dilatation of the brachial artery assessed for a further 5-minutes. A linear array ultrasound transducer (17-5 MHz with Philips iU22 ultrasound machine; Philips Healthcare, Amsterdam, Netherlands) was used for the FMD imaging, with automated real-time arterial diameter measurements (Quipu Cardiovascular Suite FMD Studio version 3.2.0; Quipu, Pisa, Tuscany, March 2017) (Gemignani et al. 2008).

CPET was performed according to ATS/ACCP guidelines (Weisman et al. 2003) using a Lode Corival cycle ergometer (Lode Corival, Lode, Groningen) and inline gas analysis system (ZAN 680, nSpire Health, Colorado, USA). After 2-minutes of unloaded cycling, participants were instructed to maintain a cadence of 50-60 revolutions per minute while being encouraged to exercise to volitional exhaustion. A Bruce ramp protocol (Will and Walter 1999; Kaminsky and Whaley 1998) was selected (10-25 Watts per minute) based on the participant's body weight and self-reported level of habitual

physical activity to ensure the CPET was between 8 to 12-minutes in duration (Weisman et al. 2003; Buchfuhrer et al. 1983). Anaerobic threshold (AT) was determined using a combination of the V-slope and VE methods (Wasserman et al. 1973; Beaver et al. 1986) by two blinded independent assessors, with disagreement resolved by consensus.

The pre-intervention tests were carried out within 7 days of the first RIPC or IHG training session and the post-intervention tests carried out within 2-5 days of the final training session. This time interval duration was chosen so as to avoid the potential acute effect of the interventions on blood pressure from affecting the results (Farah et al. 2017).

### 3.3.3 Interventions

Subjects assigned to RIPC or IHG attended the laboratory three times each week for 4-weeks, with each session lasting approximately 15-minutes. All sessions were fully supervised by a member of the research team. Control subjects attended only for pre- and post-intervention testing sessions. All subjects were asked to maintain their habitual level of physical activity for the duration of the study.

RIPC subjects were conditioned with a manual non-invasive blood pressure cuff (Welch Allyn, NY, USA). At each session, this cuff was placed on their right arm and inflated to 200 mmHg for 3-minutes before deflating. This was repeated three times in each session with three minutes rest between each inflation (Figure 18). The decision to use a single, upper-limb protocol was taken based on this being a protocol employed by a number of studies in the most recent systematic review (Epps et al. 2016). Limb occlusion time in this study was less than in many previously studied protocols, in order to reduce the total time commitment for the training protocol, making it comparable with the IHG intervention.

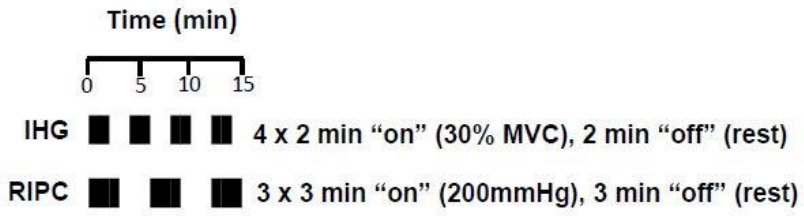


*Figure 18 Schematic representation of isometric handgrip training (IHG) and remote ischaemic preconditioning (RIPC) intervention protocols, each performed 3x/week for 4-weeks.*

At each session, subjects assigned to IHG completed four 2-minute repetitions of isometric handgrip at 30% of maximum

voluntary contraction (MVC) on an electronic hand dynamometer (Camry EH101, Zhongshan Camry Electronic Co. Ltd, Guangdong, China) using their dominant arm, with two minutes rest between each contraction. Handgrip MVC was assessed at the first IHG session using the same dynamometer as used for IHG, whilst subjects were seated with elbow flexion of 90 degrees (per the American Society of Hand Therapist recommendations (Fess 1992)). The maximum from three repetitions of maximum effort (allowing 1-minute rest between attempts) was recorded as MVC.

### 3.3.4 Statistics

All calculations were performed using Graphpad Prism Version 7.02 (California, USA). Data are presented as mean (SD). Participant demographics at baseline were compared using one-way ANOVA, whilst outcome data were compared using two-way ANOVA, as hypothesis-generating exploratory analyses. Significance was taken as an alpha of $p<0.05$.

## 3.4 Results

### 3.4.1 Feasibility

All participants completed the 4-week study with none lost to follow-up. All training was directly observed, and both interventions were well tolerated, with compliance 100%, including baseline testing and reassessment. There were no adverse events throughout the study (as defined by Good Clinical Practice)(ICH 2016) and verbal feedback from all participants indicated a high acceptability of the interventions.

### 3.4.2 Resting blood pressure

There was no statistically significant difference in either systolic blood pressure (SBP) or diastolic blood pressure (DBP) between any of the groups at baseline. There was a statistically significant reduction in SBP in the IHG group after training (Pre: 119.1 (10.8) mmHg vs. Post: 111.9 (7.9) mmHg, p=0.03, mean reduction 7.2(10.6) mmHg), but no significant change in either the RIPC group (Pre: 116.4 (14.0) mmHg vs. Post: 112.8 (15.5) mmHg, p=0.46) or the control group (Pre: 113.1(11.9) mmHg vs. 110.7(10.0) mmHg, p=0.75). There was a main effect of time on SBP (p=0.01) across all groups.

There was a statistically significant reduction in DBP in the IHG group after training (Pre: 77.1 (10.2) mmHg vs. Post: 71.1 (7.4) mmHg, p=0.03, mean reduction 6.0(9.4) mmHg), but no significant change in either the RIPC group (Pre: 71.1 (4.0) mmHg vs. 70.4 (6.6) mmHg, p=0.98) or the control group (Pre: 69.8 (8.6) mmHg vs. 72.0 (6.6) mmHg, p=0.69) (Figure 19). There was no main effect of time on DBP p=0.3 across all groups.

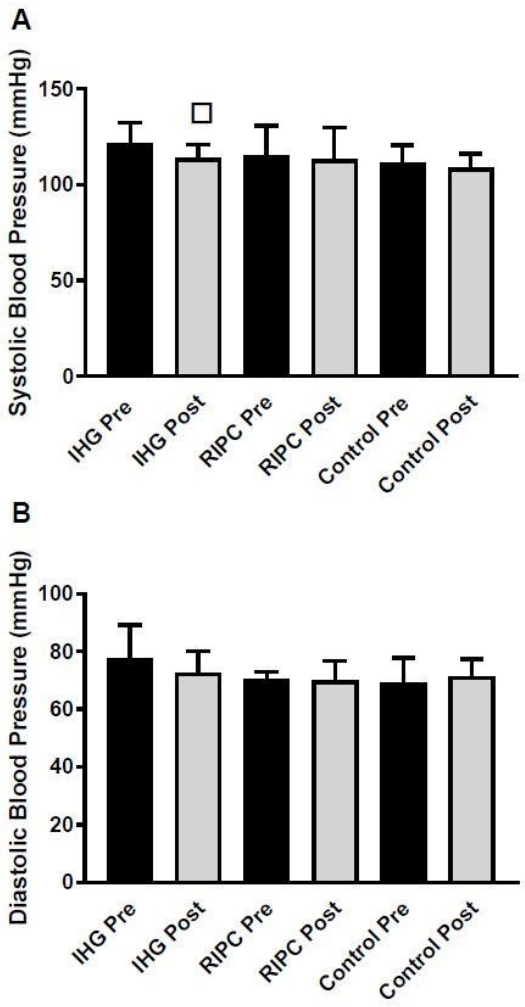


*Figure 19 Systolic (A) and diastolic (B) Blood Pressure before and after 4-weeks remote ischaemic preconditioning (RIPC), isometric handgrip training (IHG) or a control period.*

*Data is presented as Mean +/- SD. N=10 subjects per group*

### 3.4.3 Cardiopulmonary exercise testing

As was the case for blood pressure values, there were no statistically significant differences in CRF between the groups at baseline. There was no significant change in the CPET derived parameters of AT, $VO_2$ Peak or peak workload in any group (Table 4Table 4 Cardiorespiratory fitness before and

after 4-weeks remote ischaemic preconditioning (RIPC), isometric handgrip training (IHG) or a control period). There was no main effect of time across groups for any CPET variable (all p=0.3).

*Table 4 Cardiorespiratory fitness before and after 4-weeks remote ischaemic preconditioning (RIPC), isometric handgrip training (IHG) or a control period*

| | **AT (ml/kg /min) Pre** | **AT (ml/kg /min) Post** | **Change in AT** | **p-value** | **$VO_2$Peak (ml/kg /min) Pre** | **$VO_2$Peak (ml/kg /min) Post** | **Change in $VO_2$Peak** | **p-value** | **Peak Power (W) Pre** | **Peak Power (W) Post** | **Change in Peak Power** | **p-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **RIPC** | 15.8(3.4) | 16.8(4.4) | 1.1(1.4) | 0.43 | 32.8(7.3) | 33.7(5.7) | 0.8(2.1) | 0.74 | 184(35) | 188(33) | 4(8) | 0.90 |
| **IHG** | 19.0(5.3) | 18.7(5.0) | -0.3(3.2) | 0.98 | 38.7(7.2) | 36.5(6.9) | -2.2(3.3) | 0.06 | 203(30) | 215(35) | 13(33) | 0.16 |
| **Control** | 15.9(2.5) | 16.4(3.8) | 0.4(2.3) | 0.91 | 35.2(7.9) | 34.9(8.6) | -0.3(3.0) | 0.97 | 192(36) | 187(40) | -5(10) | 0.83 |

Abbreviations AT: Anaerobic threshold, $VO_{2peak}$: peak volume of oxygen consumption

### 3.4.4 Endothelial function

As with the other outcomes, there were no statistically significant differences in FMD at baseline between the groups. There was no significant change in FMD, our marker of endothelial function, after intervention in any group (IHG: 6.9(3.9) vs. 8.0(2.6)%, +1.1 (4.5)%, p=0.81; RIPC: 5.9(3.9) vs. 6.9(6.1)%, +1.0(4.7)%, p=0.84; CON: 5.8(2.5) vs. 6.1(3.4)%, +0.3 (2.9)%, p=0.99), with no main effect of time (p=0.3) across groups.

## 3.5 Discussion

This study is the first to compare the effects of IHG and RIPC on markers of cardiovascular health in inactive otherwise healthy premenopausal females. It demonstrates that IHG and RIPC are both well-tolerated in this group, with 100% compliance and no adverse events. Our hypothesis-generating exploratory analyses showed that IHG, conducted over 4 weeks can lead to statistically significant reductions in both SBP and DBP but was unable to affect any changes in CRF. We did not detect any significant changes in measures of cardiovascular health or fitness from our RIPC protocol, questioning the application of RIPC to reduce CVD risk in this specific population.

The significant reduction in SBP in our IHG group mirrors the findings of Gill *et al.* who observed a 6.9 mmHg reduction in SBP in normotensive females after 3-weeks of isometric exercise training (IET) (Gill et al. 2015). However, Gill *et al*. employed bilateral leg extensions as their mode of IET, thus recruiting a much larger muscle. We have demonstrated a similar reduction in BP using only a handgrip protocol, which may have greater potential for translation to real world use. Of note, a previous meta-analysis has demonstrated that IET reduces blood pressure most effectively in hypertensive males (Inder et al. 2016), as the subjects in this study were neither hypertensive nor male the potential for greater improvements in other cohorts remains.

The absence of a significant change in SBP and DBP in our RIPC group is in keeping with a previous study of RIPC in young adults which also showed no change in BP or exercise capacity in normotensive participants (Banks et al. 2016).

We did not demonstrate significant improvements in any CPET parameter in any group. This mirrors the findings of the only previous study assessing CPET variables before and after a

prolonged period of IHG (Blackwell et al. 2017). Similarly, this finding also agrees with two previous studies that have performed a CPET before and after repeated RIPC (Banks et al. 2016; Jones et al. 2014b). It is noteworthy that despite a lack of improvement in CPET parameters with repeated RIPC, a single-bout of RIPC performed immediately prior to exercise has been shown to improve athletic performance (Cruz et al. 2015; Jean-St-Michel et al. 2011). This suggests that the mechanisms by which a single-bout of RIPC improves athletic performance (postulated to be either through improved metabolic efficiency or increased limb blood flow (Incognito et al. 2016)) are not retained over the long-term and cannot be improved and/or maintained by repeated sessions.

This study also failed to demonstrate any significant improvement in FMD in any group. This is in keeping with previous studies that have shown IHG to be effective at increasing FMD in hypertensive subjects only (McGowan et al. 2007b, 2007a). However, the only previous study investigating the effect of RIPC on FMD (Jones et al. 2014b) did demonstrate a significant improvement in healthy normotensive young males, this is contrary to the findings reported herein. The lack of effect of RIPC observed in this study may indicate sexual

dimorphism in the effects of RIPC on FMD, a postulation that has also been suggested for other cardiovascular parameters in response to static interventions (Gill et al. 2015).

The main limitation of this study is its small sample size and that it was conducted as a pilot study without any formal calculation of a required sample size. Thus, it will have reduced power to detect changes in the measured parameters and increased the risk of error. Therefore, the results reported are hypothesis generating exploratory analyses rather than firm conclusions. One further limitation of this study was its inability to control for the length of the menstrual cycle of the subjects or their stage of menstrual cycle whilst participating in the study. Stage of menstrual cycle is known to effect physiological parameters and this may have confounded our results(Dunne et al. 1991). However, recruiting a group of subjects with identical menstrual cycles and running the pre- and post-intervention testing on set days in this cycle would not have been feasible in our institution. It must also be acknowledged that our single-arm, 3x 3 minutes of ischaemia protocol for our RIPC intervention involved less ischaemic time that other previous studies of RIPC (Banks et al. 2016; Epps et al. 2016). We devised this protocol in order to limit the time commitment

of training sessions, making it comparable to the IHG intervention, however in doing so we may have not delivered sufficient an ischaemic stimulus. Future studies of RIPC should consider using a longer ischaemic time or render a larger mass of muscle ischaemic.

Based on the findings of the exploratory analyses of this pilot study and the previous literature in this area, IHG, deliverable through a single arm handgrip protocol may lead to significant reductions in resting blood pressure in. Repeated RIPC using our single arm, 3-minute ischaemia protocol appears to be ineffective in this population. Further work is required to determine the effects of RIPC and IHG on physiological parameters associated with CVD risk in female subgroups in which this is elevated (e.g. hypertensives, older females). Given the tolerability of these simple, inexpensive interventions as shown in this study, if proven effective, these interventions may offer large public-health benefit as independent or adjuvant (i.e., alongside established exercise and dietary changes (Warburton et al. 2006; Cook et al. 2007)) lifestyle recommendations to improve cardiovascular health in specific groups.

# 4 Experimental methods

## 4.1 Chapter focus

This chapter details the methods followed in the experimental chapters within this thesis. The specific details of any additional methods used and the details of recruitment and randomisation (when applicable) are detailed separately in chapters 5, 6, 7 and 8 respectively.

## 4.2 Ethical approval

Ethical approval was sought and granted from the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (A12092016) before the first participant was recruited. The submission to the ethics committee, including the ethics form and participant information sheet are included in Appendices 3-5.

Subsequent ethical approval was sought to recruit the additional participants for the study described in chapter 8 via a minor amendment (A12092016, amendment 4, 25.08.2017, Appendix 6).

## 4.3 Recruitment

Participants were recruited for the studies in this thesis through a variety of methods. These included distributing the study recruitment poster to volunteers at the Royal Derby Hospital and to previous research participants who had been involved in other studies at the University of Nottingham and who had consented to have their contact details stored by the university for the purpose of informing them about future studies. Finally, the study recruitment poster was advertised in local community magazines with a distribution close to the Royal Derby Hospital site.

## 4.4 Screening

All potential participants who displayed an interest in taking part in one of the studies attended the University of Nottingham Medical School at the Royal Derby Hospital for a screening visit. Prior to this visit they were provided with a copy of the participant information sheet for the study they were interested in participating in.

On arrival, the volunteer was given another thorough explanation of the study before they gave written informed consent to be screened.

Screening involved a full and detailed medical history, physical examination, measurement of height and weight and subsequent calculation of BMI.

Resting BP was then measured after the participant had sat quietly for 5 minutes, with their dominant arm supported at the level of their heart, using an automated oscilometric device (Datascope trio, New Jersey, USA) and a cuff of appropriate size, according to the 2017 British and Irish Hypertension Society guidelines (British and Irish Hypertension society 2017). Blood pressure was measured 3 times in this way and the mean values of systolic and diastolic BP were recorded (Lackland et al. 2018).

After measurement of BP, a resting 12-lead ECG was recorded to assess for any occult cardiac disease before finally the patient was bled for measurement of full blood count, urea and

electrolytes, liver function tests, thyroid function tests, lipids, and fasting glucose.

## 4.5 Inclusion criteria

The inclusion criteria for the study were:

- Age 65-85 at date of randomisation
- Male or female
- Any baseline hypertensive status (if medicated, must have been on a stable dosage for >3months)
- Able to travel to the University of Nottingham Medical School at the Royal Derby Hospital regularly during the study
- Has capacity to consent to participate in the study

## 4.6 Exclusion criteria

The study exclusion criteria were broadly taken from the ATS/ACCP guidelines for CPET which primarily exclude for any cardiac contraindication to maximal exercise testing (Weisman et al. 2003). Additional exclusion criteria were defined as follows:

- Current participation in a formal exercise regime (defined as participation in any regularly organised exercise)
- A BMI < 18 or > 32 $kg \cdot m^2$
- Active cardiovascular disease:
    - uncontrolled hypertension (BP > 160/100),
    - angina,
    - heart failure (class III/IV),
    - significant arrhythmia,
    - right to left cardiac shunt,
    - recent cardiac event
- Taking beta-adrenergic blocking agents,
- Cerebrovascular disease:
    - previous stroke,
    - aneurysm (large vessel or intracranial)
- Epilepsy
- Respiratory disease including:
    - pulmonary hypertension,
    - significant COPD,
    - uncontrolled asthma
- Metabolic disease:
    - hyper and hypo parathyroidism,
    - untreated hyper and hypothyroidism,
    - Cushing's disease,

    - type 1 or 2 diabetes
- Active inflammatory bowel or renal disease
- Malignancy
- Clotting dysfunction
- Significant Musculoskeletal or neurological disorders
- Family history of early (<55y) death from cardiovascular disease
- Known sensitivity to Sonovue®

## 4.7 Measurement of Blood pressure

### 4.7.1 Resting blood pressure

Resting BP was measured as in the screening visit (section 1.4) Blood pressure was measured 3 times in this way and the mean values of systolic and diastolic BP were recorded. Blood pressure was recorded at the same time of day both before and after study intervention periods and in the same clinical laboratory. Time of day of recording is important due to well recognised diurnal variations in BP (Kawano 2011), as are external conditions e.g. ambient temperature (Wang et al. 2017), hence these were controlled to the best of our ability. BP measurements were also made after the participant had completed an overnight fast (from 2100 the previous day, taking only water) in order to eliminate any impact of

postprandial BP changes (Puisieux et al. 2000), which have the potential to be altered by different meal compositions (Potter et al. 1989).

### 4.7.2 Ambulatory blood pressure

Ambulatory blood pressure was measured using an A&D TM 2430 ambulatory blood pressure monitor (A&D Company Ltd., Tokyo, Japan) with an appropriate sized cuff applied to the non-dominant arm, with measurement intervals set at every 30 minutes during the day (0700-2200) and every 60 minutes overnight (2200-0700) (British and Irish Hypertension Society 2017). Participants were requested to maintain their habitual pattern of daily activity when wearing the ambulatory blood pressure monitor and to sleep at approximately the same time for each set of measurements.

Data were extracted and analysed from the ambulatory monitor using the manufacturer's recommended Doctor Pro 3 software (Version 3.03.00, A&D Company Ltd., Tokyo, Japan). Data were analysed according to the European Society of Hypertension 2013 guidelines (O'Brien et al. 2013), with an adequate number of measurements required for analysis; defined as ≥20 valid daytime measurements and ≥7 valid

night time measurements (O'Brien et al. 2013). No editing of raw values were performed, except for the deletion of physiologically impossible values (O'Brien et al. 2013).

If the participant was unable to wear the ambulatory blood pressure monitor for an entire 24 hour period (either through logistical reasons in the study protocol, or through personal circumstances), the same time period of measurement before and after the study was analysed in order to remove the possibility of bias (for example if night time readings were to comprise a larger proportion of the average measurement period, this could artificially lower the average). Average 24-hour, average daytime and average night-time systolic and diastolic blood pressures were calculated.

## 4.8 Cardiopulmonary exercise testing

### 4.8.1 CPET equipment

All CPET tests were carried out in accordance with both the ATS/ACCP and POETS guidelines for CPET (Weisman et al. 2003; Levett et al. 2018).

CPETs were performed using a metabolic cart (Zan 680 and ZAN GPI 3.00 software, nSpire Health, Longmont, Colorado, USA) and a cycle ergometer (Lode Corival, Lode BV, Groningen, NL) in the exercise laboratory of the Clinical, Metabolic & Molecular Physiology research group at the University of Nottingham Medical School, based at the Royal Derby Hospital. Both the metabolic cart and the cycle ergometer were regularly serviced in accordance with the manufacturers' instructions.

Routine calibration was performed for the metabolic cart prior to every CPET. Dual calibration was carried out for the gas analyser against a custom gas mixture of 5% carbon dioxide, 15% oxygen and 80% nitrogen (BOC, Guildford, UK) and against ambient air, controlling for ambient conditions recorded at the time of calibration (temperature, atmospheric pressure and humidity). Flow sensors were calibrated using the manufacturer's recommended 3L syringe.

After calibration, and in preparation for each CPET, flow sensors were fitted to a silicone face mask (7450 Series Silicone V2, Hans Rudolph Inc., Shawnee, Kansas, USA) sized appropriately for the participant, and the mask tightened on

each participant to ensure an adequate seal against a maximal forced expiration with the aperture occluded.

Continuous 12-lead ECG monitoring was performed throughout the CPET via exercise electrodes (Ambu® Blusensor R, Ambu A/S, Copenhagen, Denmark) and an ECG machine-USB interface (CardioDirect 12, SpaceLabs Healthcare, Snoqualmie, Washington, USA), with a trace from all 12 leads visible on the CPET monitor throughout the test.

Blood pressure during CPET was measured at 2-minute intervals using an acoustic exercise blood pressure cuff (Orbit-K™, SunTech Medical®, Morrisville, North Carolina, USA) around the participant's right arm, connected to the cycle ergometer.

Waveform pulse oximetry was recorded throughout the test using a pulse oximeter (Nellcor™, Medtronic, Watford, UK) placed on the index finger of the participants left hand, connected to a free-standing patient monitor (iMEC 8, Mindray medical, Shenzhen, China).

### 4.8.2 CPET protocol

Before beginning the CPET, participants were given a thorough safety briefing with the opportunity for them to ask any questions before the monitoring equipment was attached. All tests were conducted by two trained, experienced investigators with at least one of whom held a valid Advanced Life Support Provider certificate (Resuscitation Council UK, London, UK).

After attachment of all monitoring equipment, proper functioning of this was verified before the participant was guided to the cycle ergometer (Figure 20).



*Figure 20. Participant seated on the cycle ergometer with monitoring equipment attached before a CPET test commenced.*

The height of the seat of the cycle ergometer was adjusted to ensure participant comfort, with this measurement recorded and used in any subsequent tests.

On starting the test, participants were instructed not to talk unless in the event of a medical emergency so as not to influence respiratory gas data.

Each test began with a 3-minute period of observed rest on the ergometer, prior to a 2-minute warm up of unloaded cycling to ensure patient acclimatisation to the equipment.

Participants then began a ramp-incremental test period, whilst being instructed to maintain a cadence of 50-60rpm. A ramp of between 5-25 W/min was chosen for each participant, estimated based on their weight and self-reported level of activity, aiming to ensure a to ensure a duration of work of between 8 and 12-minutes (Weisman et al. 2003). Participants were given enthusiastic verbal encouragement by two investigators to exercise to volitional exhaustion, unless any of the laboratory's pre-defined termination criteria were met (Table 5) (Weisman et al. 2003) or if the participant was

unable to maintain a cadence of ≥50 rpm despite verbal encouragement.

*Table 5 ATS/ACCP Indications for Exercise Termination, Taken from Weisman et al. 2003*

| |
|---|
| Chest pain suggestive of myocardial ischaemia |
| Ischaemic ECG changes |
| Complex ectopy |
| Second- or third-degree heart block |
| Fall in SBP >20 mmHg |
| Significant hypertension (>250 mmHg systolic; >120 mmHg diastolic) |
| Severe desaturation ($SpO_2$ <80%) |
| Sudden pallor |
| Loss of coordination |
| Mental confusion |
| Dizziness or faintness |
| Signs of respiratory failure |

On termination of the ramp protocol, participants continued to cycle against a resistance reduced to 10 W for 5 minutes of observed recovery cycling. After this, they were allowed to dismount the cycle ergometer, remove the facemask and rest

in a comfortable chair with ongoing continuous ECG and pulse oximetry monitoring and intermittent BP measurements. Monitoring was discontinued when that participant indicated that they felt they had recovered, and their heart rate had reduced to <100 bpm.

### 4.8.3 CPET analysis

Breath-by-breath data was displayed graphically as an average of 5 breaths, to minimise the influence of biological "noise" prior to interpretation (Levett et al. 2018). Gas exchange variables were calculated relative to participant bodyweight, measured on the day of each test and reported in ml/kg/min.

#### *4.8.3.1 Defining $VO_{2peak}$*

$VO_{2peak}$ was defined for each test as the highest average $VO_2$ determined by the metabolic cart over the final 30s of the ramp-incremental and early recovery parts of the test (Levett et al. 2018) and was calculated automatically by the integrated analysis package.

*4.8.3.2 Defining AT*

AT was defined for each test by two experienced CPET investigators using the methods advocated in the POETS and ATS/ACCP guidelines (Weisman et al. 2003; Levett et al. 2018). This involved identifying excess $VCO_2$ relative to $VO_2$ (indicated by the breakpoint in the $VCO_2/VO_2$ graph by the V-slope method), identifying hyperventilation relative to oxygen (identifying an increase in the $VE/VO_2$ $-VO_2$ graph and an increase in the $P_{ET}O_2$ – $VO_2$ graph) and excluding hyperventilation relative to $CO_2$ (ensuring the $VE/VCO_2$ graph does not rise with the $VE/VO_2$ graph and ensuring no reciprocal fall in $P_{ET}CO_2$ with the increase in $P_{ET}O_2$) (Levett et al. 2018) (Figure 21).

Both assessors determined the AT in conjunction with each other, but were blinded to participant ID, their intervention allocation and whether the test had been carried out before or after an intervention. Any disagreement was resolved by consensus and involvement of a third investigator if required.

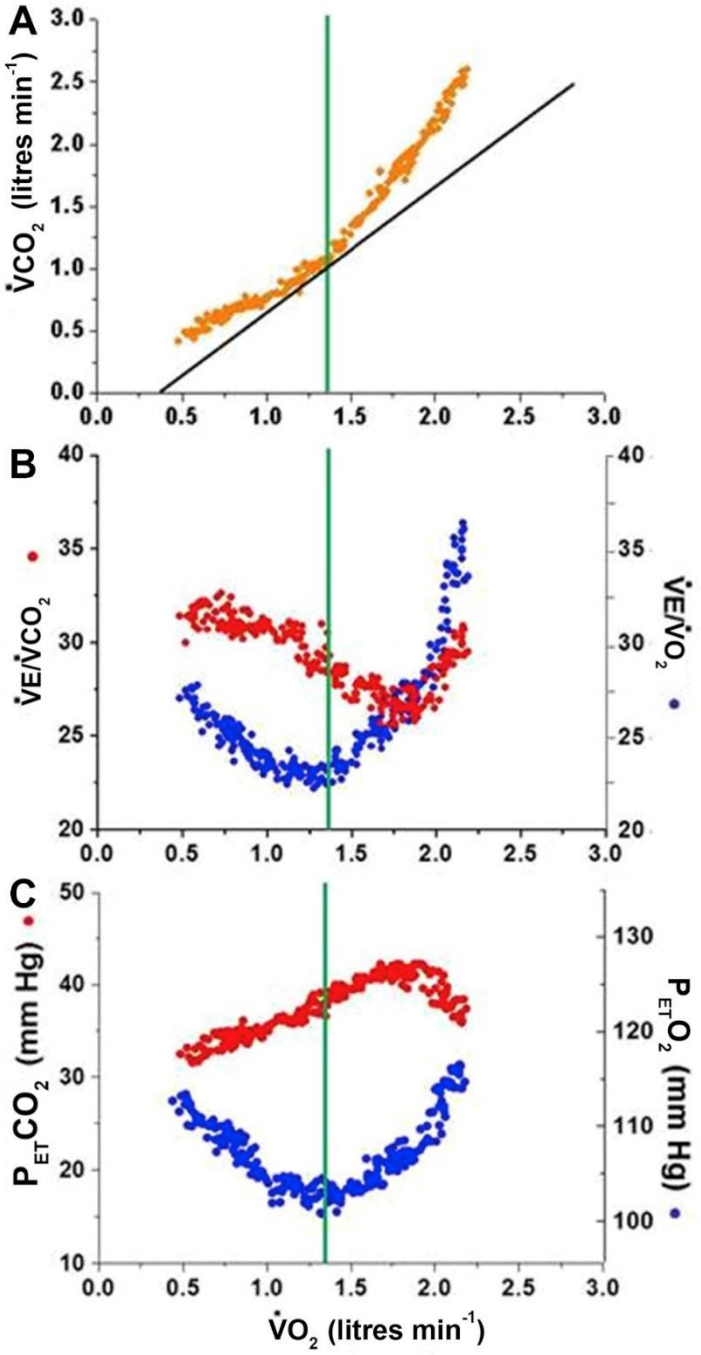


*Figure 21. Determination of anaerobic threshold (AT)*

*Green line represents AT. Abbreviations: $VCO_2$, Volume of $CO_2$; VE, Minute Ventilation; $P_{ET}CO_2$, End-tidal partial pressure of $CO_2$; $VO_2$, Volume of $O_2$. (Taken from Levett et al., 2018. Br J Anaest)*

#### *4.8.3.3 Defining peak exercise tolerance ($W_{peak}$)*

Peak exercise tolerance was defined as the power output of the cycle ergometer at the termination of the ramp-incremental part of the CPET. This was directly related to the duration of the test (and hence exercise tolerance). As the same ramp protocol was used for CPETs before and after intervention periods, $W_{peak}$ could be directly compared.

## 4.9 Oral glucose tolerance testing (OGTT)

A modified version of the standard World Health Organisation OGTT (World Health Organisation 2006) was carried out for the studies contained within this thesis.

### 4.9.1 Conduct of the OGTT

For each OGTT, subjects presented to the laboratory at 0900h fasted from 2100h the preceding evening. On arrival, a 22G intravenous cannula (BD Venflon™ Pro, Beckton Dickson, Franklin Lakes, New Jersey, USA) was inserted retrogradely into a vein in the dorsum of a hand of the participant's choice to facilitate repeated blood sampling. The hand was inserted into a hand warming box (manufactured in-house by the Medical Engineering department of the University of

Nottingham) (Figure 22) and heated to 55°C to arterialise the venous blood in order to minimise any contribution of uptake of either glucose or insulin by the forearm or hand (Liu et al. 1992; Jackson et al. 1973). The cannula was connected to a continuous slow infusion of normal (0.9%) saline (Baxter International Inc., Deerfield, Illinois, USA) to allow flushing of the cannulae and ensure line patency.

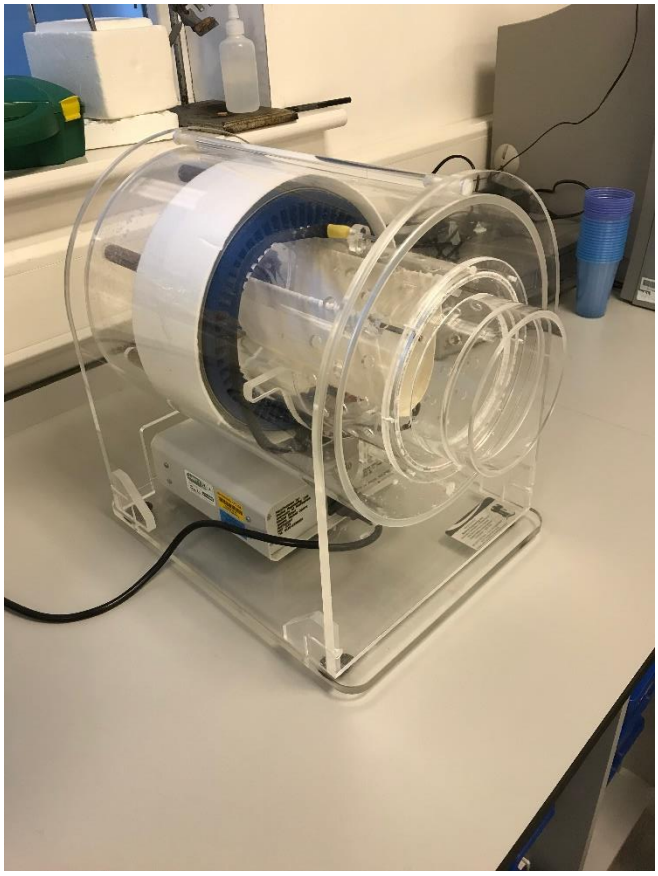

*Figure 22. Hand-warming box used to arterialise blood* (Liu et al. 1992) *during oral glucose tolerance tests (OGTT).*

Three millilitres of blood were drawn at each time-point during the OGTT. This was used for real-time quantification of blood glucose concentrations using a near-patient glucose analyser (YSI Life sciences, Ohio, USA), whilst the remaining blood was centrifuged and plasma aliquoted and frozen at -80$^{o}$C for later quantification of insulin concentrations.

After baseline sampling, participants drank 75g oral dextrose (Myprotein, Northwich, UK) dissolved in 200mL of water.

Blood samples were then drawn 15, 30, 45, 60, 80, 100, 120, 140, 160 and 180 minutes after the dextrose drink. A test duration of 180 minutes was chosen in line with an increasing body of literature suggesting that extending the standard WHO 2-hr OGTT (World Health Organisation 2006) may provide additional information on insulin sensitivity as some individuals may have delayed peak glucose and insulin concentrations (Wang et al. 2018; Pannacciulli et al. 2007; Robinson et al. 2004; Wopereis et al. 2009).

### 4.9.2 Insulin quantification

Plasma insulin concentrations were determined using an ultrasensitive enzyme linked immunosorbent assay (ELISA) (Mercodia Ultrasensitive Insulin ELISA, Mercodia AB, Uppsala, Sweden).

Plasma samples were thawed, vortexed and centrifuged before any further preparation. Fasted samples and samples taken at 180 minutes were analysed without dilution, whilst samples from all other time points were diluted 1:12 to ensure that the insulin concentration fell within the working reference range of the kit.

Samples and calibrator solutions were incubated on an automated plate shaker at room temperature for 1 hour with the enzyme conjugate solution to ensure complete mixing and binding of insulin to the pre-coated plate wells, before manual washing with the recommended wash buffer as per the manufacturer's instructions.

Plates were then incubated with substrate TMB for 30 minutes to ensure the colorimetric substrate was completely bound

before the stop solution was added. Plates were then read (Figure 23) at 450nm on an optical plate reader (Thermo Scientific Multiskan Ascent, ThermoFisher Scientific, Waltham, Massachusetts, USA) and calculation of insulin concentration performed automatically by the plate reader software in reference to the calibrator solutions included on each plate (calibrator concentrations: 0.15mU/L, 1mU/L, 3mU/L, 10mU/L and 20mU/L).

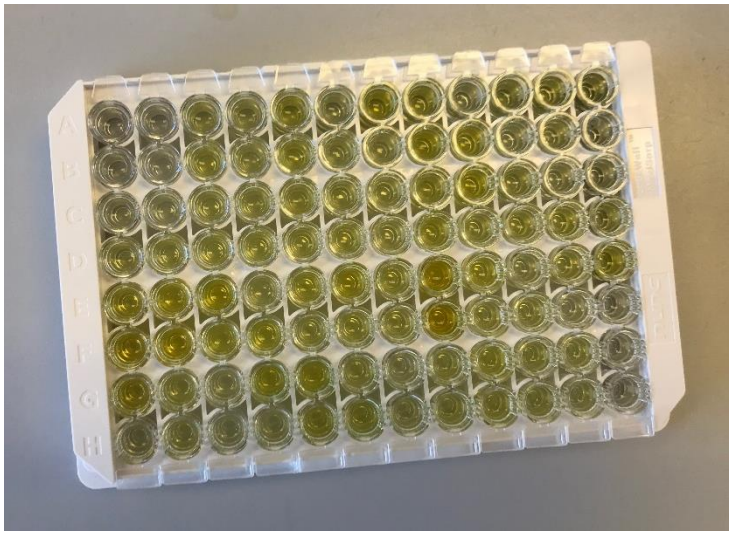

*Figure 23. Ultrasensitive enzyme linked immunosorbent assay (ELISA) plate for insulin immediately prior to analysis on an optical plate reader.*

### 4.9.3 Data interpretation

Glucose and insulin levels taken from the OGTTs were plotted against time and areas under the curves for glucose and insulin concentrations we calculated. Glucose and insulin data were

also used to calculate three established indices of insulin sensitivity:

i) the homeostatic model assessment of insulin resistance (HOMA-IR) (Matthews et al. 1985),

ii) the Matsuda index of insulin sensitivity (Matsuda and DeFronzo 1999), and

iii) the Cederholm index of insulin sensitivity (Cederholm and Wibell 1990).

HOMA-IR was calculated using the formula:

$$\frac{[Fasting\ insulin(mUL^{-1})] \times [Fasting\ glucose(mmolL^{-1})]}{22.5}$$

As described in section 1.4.5.3, this is a static measure of insulin resistance, relying solely on fasted values. Higher values represent increasing degrees of insulin resistance.

The Matsuda index was calculated using the formula:

$$\frac{10{,}000}{\sqrt{([Fasting\ Glucose] \times [Fasting\ Insulin] \times [Mean\ Glucose] \times [Mean\ Insulin]}}$$

Data for time points at 0, 60 and 120 minutes were used for the calculation of the Matsuda index as described by the Matsuda group in a subsequent publication, as samples were not taken at 90 minutes (a 90 minute sample is required for calculation using the original formula) (DeFronzo and Matsuda 2010). Higher values of the Matsuda index indicate increasing degrees of insulin sensitivity.

The Cederholm index was calculated using the formula (which includes conversion factors to allow glucose to be inputted in mmol/L (Patarrão et al. 2014):

$$\frac{75000 + ([Fasting\ Glucose] - [Glucose_{120min}]) \times 1.15 \times 180 \times 0.19 \times Body\ mass}{120 \times [Mean\ Glucose] \times \log[Mean\ Insulin]}$$

Data for time points at 0, 30, 60 and 120 minutes were used for the calculation of the Cederholm index as described in the

original paper by Cederholm (Cederholm and Wibell 1990). Higher values of the Cederholm index indicate increasing levels of insulin sensitivity. As described in section x, both HOMA-IR and the Matsuda index, by having either a total reliance or a strong emphasis on fasting values of insulin and glucose, provide a greater emphasis on the hepatic component of insulin sensitivity, whilst the Cederholm index is more weighted to reflect peripheral insulin sensitivity (Cockcroft et al. 2017; Gutch et al. 2015; Patarrão et al. 2014).

## 4.10 Handgrip strength

Handgrip strength was measured for both the dominant and non-dominant hand of each participant. Grip strength was measured according to the American Society of Hand Therapists clinical assessment recommendations, with the participant seated, the shoulder adducted and neutrally rotated and the elbow flexed to 90 degrees (Fess 1992). Each participant was given three attempts to measure a maximal voluntary contraction (MVC) for each hand using a handgrip dynamometer (Camry EH101, Zhongshan Camry Electronic Co. Ltd, Guangdong, China), with 1-minute rest between each attempt at MVC. The MVC was recorded as the highest reading obtained for each hand.

## 4.11 Measurement of body composition (DXA)

Body composition was measured using a whole-body dual-energy X-ray absorptiometry (DXA) scan. The integrated software associated with the scanner (Lunar Prodigy II, GE Medical Systems, Buckinghamshire, UK) provided automated data for whole-body body lean mass (LM), body fat percentage (FM%) and lean leg mass (LLM). Participants were scanned by an Ionising Radiation (Medical Exposure) Regulations (IR(ME)R)) trained operator, ensuring they had voided their bladder immediately prior to scanning to avoid any impact of a full bladder. Scans were performed at the same time of day before and after intervention periods.

## 4.12 Measurement of leg strength

Measurement of 1-repetition maximum (1-RM) for unilateral leg extension was performed on a leg extension machine (ISO Leg extension, Leisure Lines (GB) Ltd., Hinckley, UK) as an assessment of strength. This leg extension machine which was also used for the assessment of macro and microvascular responsiveness in sections 4.13 and 4.14 below (Figure 24).



*Figure 24 Leg extension machine used in the studies in this thesis.*

The range of motion of the leg extension machine was set at 0 with an aim 90° at extension. Participants were familiarised with the equipment by performing 6-8 repetitions with each leg with a light weight (~50% estimated 1-RM) and asked to rate their level of exertion on a modified Borg scale of 1-10 (Borg 1982). Based on this, after 1 minute of rest the weight was increased and the participant asked to lift the load again, this time only once. After each successful attempt, the participant rested for one minute and then the load was increased for a further attempt. Load increases between the

repetitions were based on the investigator's discretion based on previous experience. To minimise the potential effect of fatigue on 1-RM assessments a maximum of 3 attempts was allowed to achieve 1-RM (Levinger et al. 2009).

## 4.13 Measurement of leg blood flow

Measurements of (macrovascular) leg blood flow were made in the morning after the participant had completed a fast from 9pm the preceding evening (allowed water to drink *ad libitum*). Leg blood flow was measured using a Doppler ultrasound (iU22, Phillips Healthcare, Guildford, UK) and a linear 9-3mHz ultrasound probe. The common femoral artery of the non-dominant leg was insonated, approximately 2-3 cm proximal to its bifurcation in order to minimize any effect of turbulence (Phillips et al. 2012), with an insonation angle $<60^{o}$ in order to minimize any error in velocity measurement (Logason et al. 2001), after the participant had rested in the supine position for 5 minutes. Blood flow was calculated as the product of the artery cross sectional area and the mean blood velocity over 3 cardiac cycles (Phillips et al. 2012). Three measurements of leg blood flow were made (Figure 25).

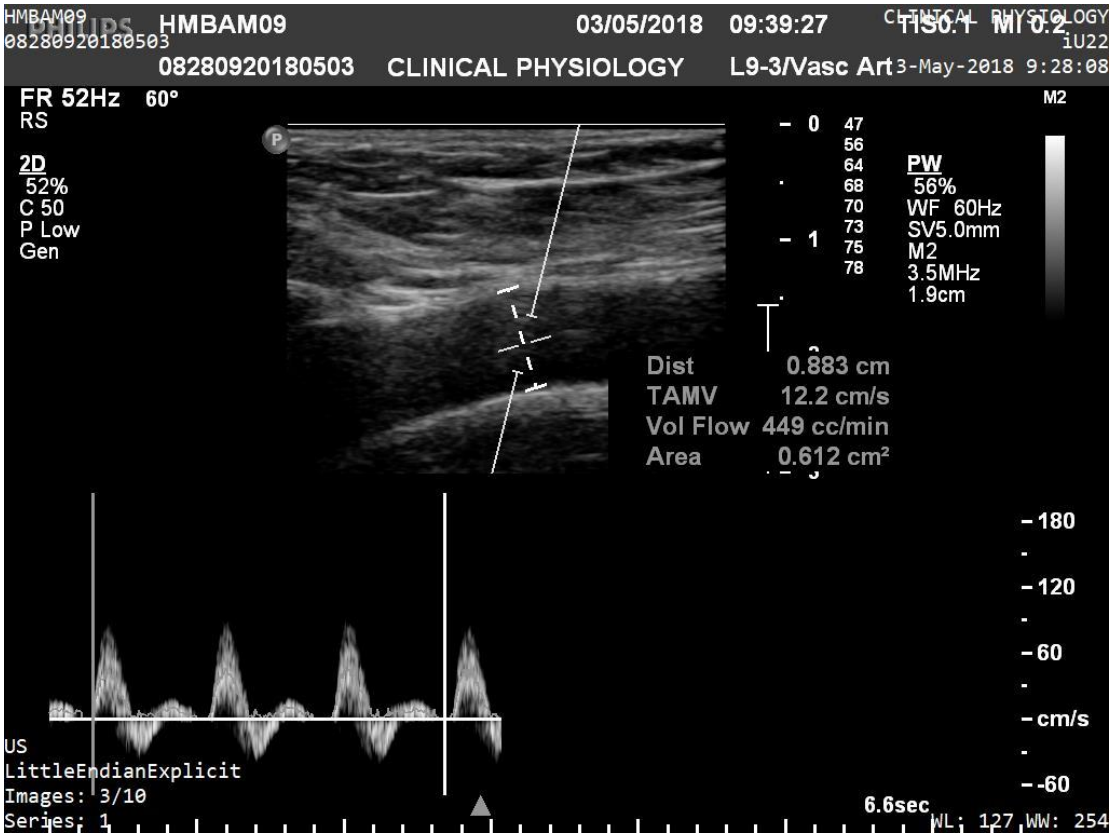


*Figure 25. A Doppler ultrasound image of the method used to assess leg blood flow.*

*Abbreviations: Dist, distance between the two calipers; TAMV, time-averaged mean velocity; Vol Flow, calculated blood volume flow, PW; pulsed wave ultrasound, WF, wall filter; SV, ample volume; M2, medium 2 packet size (preset).*

After a measurement of resting blood flow, the participant moved to the leg extension machine and were instructed to perform 6 leg extensions at 50% 1-RM with the non-dominant leg. They were then instructed to immediately return to the scanner couch (located <1m away) for a measurement of post-exercise leg blood flow. This was measured in the same fashion as resting leg blood flow immediately after the patient lay down, with 3 recordings of leg blood flow made and the largest recorded as the peak blood flow response.

## 4.14 Measurement of microvascular blood flow

Muscle microvascular blood flow was measured using contrast enhanced ultrasound (CEUS).

### 4.14.1 CEUS equipment

Measurements of muscle microvascular blood flow were made in the (vastus lateralis) of the participant's dominant leg, according to the method described by our laboratory (Mitchell et al. 2013). All measurements were made on the same morning as those of leg blood flow (section 4.13) with the participant maintaining their fast from overnight.

The same ultrasound machine (iU22, Phillips Healthcare, Guildford, UK) and the same linear 9-3mHz probe as used for the measurement of leg blood flow were used for the measurement of muscle microvascular blood flow. The probe was secured to the participant's thigh using a custom-made plastic probe-holder (manufactured in-house by the Medical Engineering department of the University of Nottingham) and Velcro straps (Figure 26).

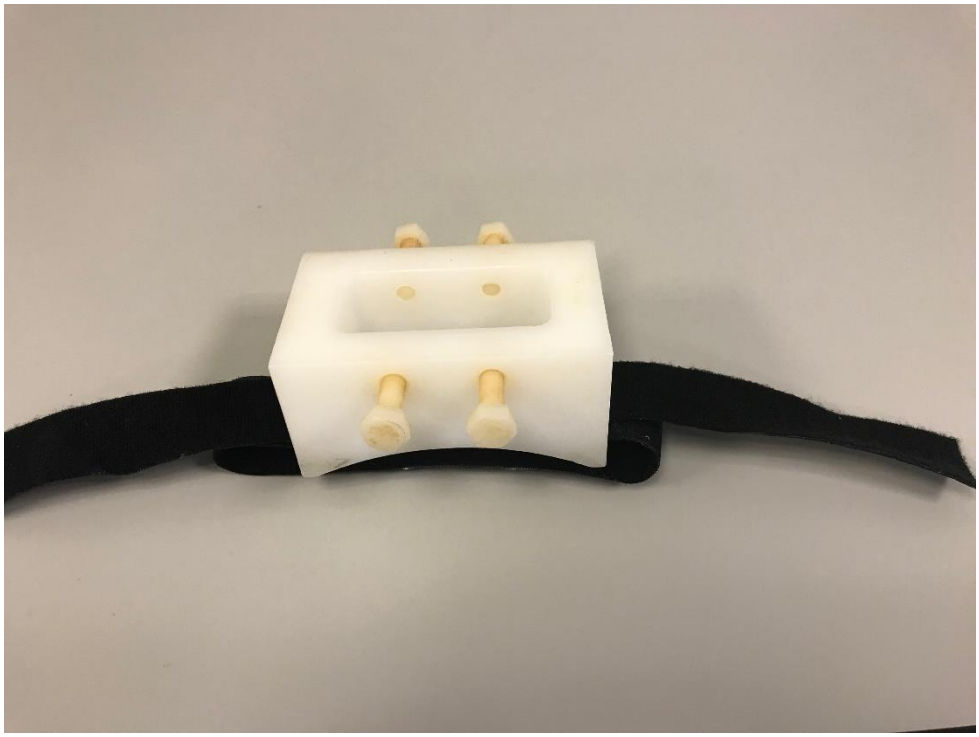

*Figure 26 Plastic probe holder with Velcro strap, used to secure the probe to the patient's thigh during contrast enhanced ultrasound studies.*

The probe, probe-holder and straps were further secured to the patient's thigh by Micropore™ tape (3M™, Maplewood, Minnesota, USA) to prevent movement of the probe during the experiment. The probe was secured approximately at the mid-thigh level, with the distance between the most inferior aspect of the probe-holder and the superior border of the participant's patella noted to facilitate repeated measures in the same place after the intervention period. The probe was attached to the participant's thigh for a minimum of 20 minutes before CEUS measurements to allow the probe temperature to equilibrate with that of the participant's skin (Sjøberg et al. 2011).

Sonovue™ (Bracco, Milan, Italy) was used as the microbubble ultrasound contrast agent to obtain the CEUS images in the studies in this thesis. This was reconstituted according to the manufacturer's instructions with 5ml normal saline (contained within the packaging) shaken vigorously and then drawn up into the manufacturer's recommended 20ml syringe (ACIST® Medical Systems, Eden Prairie, Minnesota, USA). An additional 1ml normal saline (Baxter International Inc., Deerfield, Illinois, USA) was then instilled into the Sonovue ampoule, shaken vigorously and then also drawn up into the 20ml syringe in order to increase the yield of contrast agent. This was then connected to the recommended syringe-patient connection line (ACIST® Medical Systems, Eden Prairie, Minnesota, USA) and the line then primed with the Sonovue™. The 20ml syringe was then inserted into a Vueject® ultrasound contrast infusion pump (Bracco, Milan, Italy), which continually oscillated to ensure the contrast agent remained in suspension. Finally, the syringe-patient connection line was connected to a 20G intravenous cannula (BD Venflon™ Pro, Beckton Dickson, Franklin Lakes, New Jersey, USA) inserted into a vein in the antecubital fossa of the participants left arm. Continuous 3-lead ECG monitoring (Datascope Trio™, Mindray North America, Mahwah, New Jersey, USA) of the patient was conducted according to the manufacturer's safety precautions,

given a reported incidence of hypersensitivity reactions of between 1/1000 and 1/10,000. During the studies in this thesis, no adverse events in relation to Sonovue™ administration were encountered.

### 4.14.2 CEUS protocol

The infusion protocol described by Mitchell and colleagues (Mitchell et al. 2013) was used to obtain CEUS images before and after unilateral leg extensions. The patient was seated on the same leg extension machine utilised in sections 4.12 and 4.13. After the equipment was set-up, the participant was given a thorough briefing of the test protocol and then instructed to keep their leg as still as possible until instructed to perform leg extensions. Sonovue™ was infused at a rate of 2ml/min for 1 minute before the rate of infusion was reduced to 1ml/min until the syringe was empty. Each vial of Sonovue™ provided approximately 3.5-4 minutes of infusion time. Systemic steady state was achieved after approximately 90 seconds of infusion (Mitchell et al. 2013), thus after this measurements of muscle microvascular blood flow were started. Microbubbles under the probe which had accumulated under systemic steady state were destroyed by providing a brief "flash" impulse of high mechanical index ultrasound from

the probe (Mitchell et al. 2013). The flash and the replenishment of the contrast agent to the muscle underlying the probe was then recorded on 30 second cine clips recorded through the probe. Two recordings of a "flash" and replenishment were made at rest, after which the participant was instructed to perform 6 unilateral leg extensions at 50% 1-RM over approximately 30 seconds. The participant was then asked to once again keep their leg as still as possible and another "flash" was then delivered as soon as the leg had come to rest. A recording of this flash and the subsequent replenishment was made in order to measure post-exercise muscle microvascular blood flow.

### 4.14.3 Analysis of CEUS image files

DICOM video files of CEUS studies were exported from the ultrasound machine and analysed using QLAB image quantification software (Version 10, Phillips Healthcare, Amsterdam, Netherlands) using the Region of Interest (ROI) plug-in, using the method previously described by our research group (Mitchell et al. 2013). ROIs were drawn freehand on each cine clip including as much muscle as possible, whilst excluding connective tissue or any large rapid filling vessels

(distinct large vessels visibly containing contrast within the first 0.05 seconds of recording) (Figure 27).

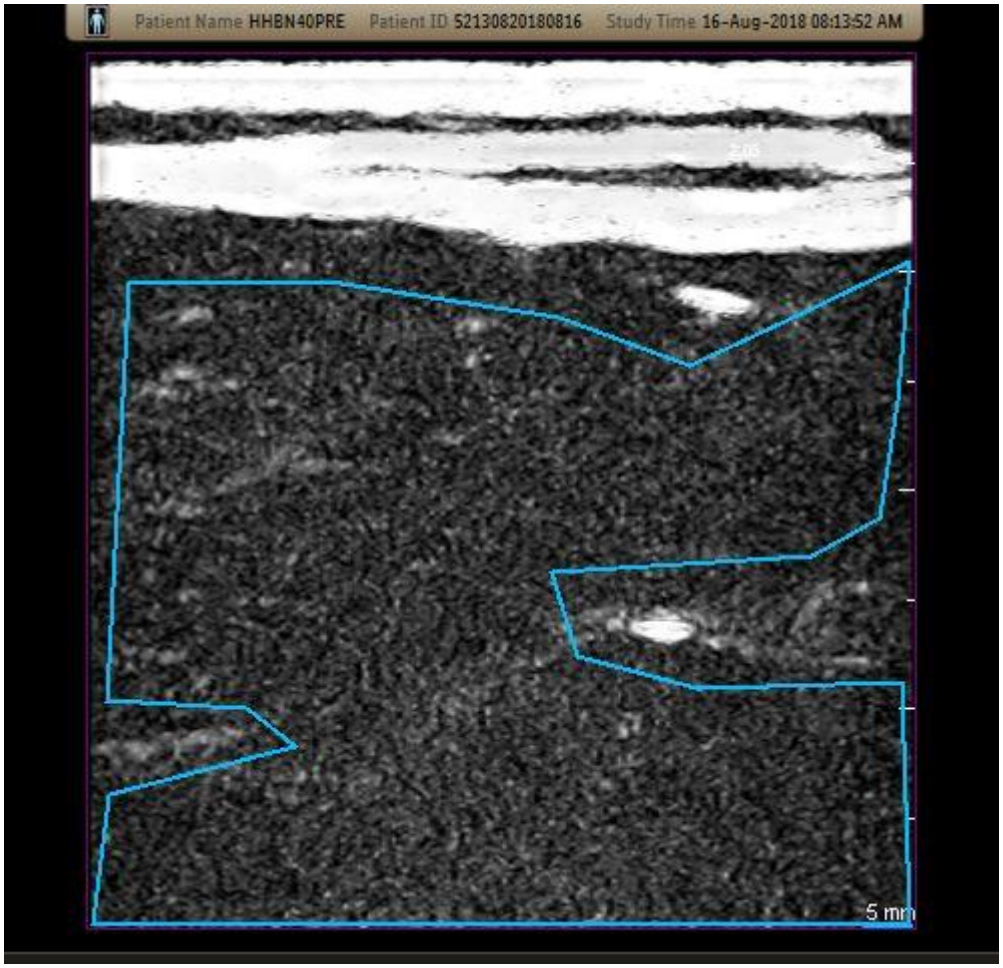


*Figure 27. Region of interest (ROI) of* vastus lateralis muscle *drawn on a CEUS DICOM file, excluding connective tissue and rapid filling vessels.*

*Bright areas at the top of the image represent skin and connective tissue.*

The parameters of the freehand ROI shape used for each participant were copied across all of the cine clips for that participant on a study day to ensure consistency of

measurements. QLAB then provided a value for the mean acoustic index (AI)(measure of the echogenicity of the tissue, which increases proportionately to the concentration of the Sonovue™ (Mitchell et al. 2013)), throughout the ROI for each frame of the cine clip. The mean AI for the first 6 frames immediately post "flash" was designated the background AI of the tissue within the ROI and subsequently the subtracted from all subsequent AI values.

The mean AI data from two pre-exercise cine clips were averaged to give one measure of pre-exercise microvascular blood flow which was subsequently compared to the post-exercise AI data.

AI values from both before and after exercise were then used to plot curves of AI against time, and the exponential function of one-phase association using Prism 7 (Graphpad software, San Diego, California, USA):

$$y = y_0 + A(1 - e^{-\beta t})$$

Using this equation in this setting, A denotes the level of the plateau of the graph and is analogous to microvascular blood volume. β in this equation denotes the rate of increase in the gradient of the graph and is analogous to microvascular flow velocity. The product of A x β is therefore arguably representative of microvascular blood flow (Mitchell et al. 2013).

## 4.15 Measurement of endothelial function

Endothelial function was measured using flow mediated dilation (FMD).

### 4.15.1 FMD equipment

Flow mediated dilation was measured in the brachial artery of the non-dominant arm according to the guidelines set out by the International Brachial Artery Reactivity Task Force (Corretti et al. 2002b). Participants presented to the laboratory at 9am, fasted from 9pm the preceding evening (having been allowed to drink only water *ad libitum*). Measurements of FMD were made in a quiet room, with the ambient temperature controlled to 24$^{o}$C. The participant was then instructed to lie

supine on the bed on which the measurements were to be taken (Harris et al. 2010).

Images were obtained using an iU22 ultrasound machine (Phillips healthcare, Guildford, UK) and linear 17-5MHz probe. This probe was secured in a position whereby it was providing longitudinal views of the distal portion of the participant's brachial artery, using a designated probe holder for FMD studies that was adjustable in 3 axes (Quipu, Pisa, Italy) (Figure 28).

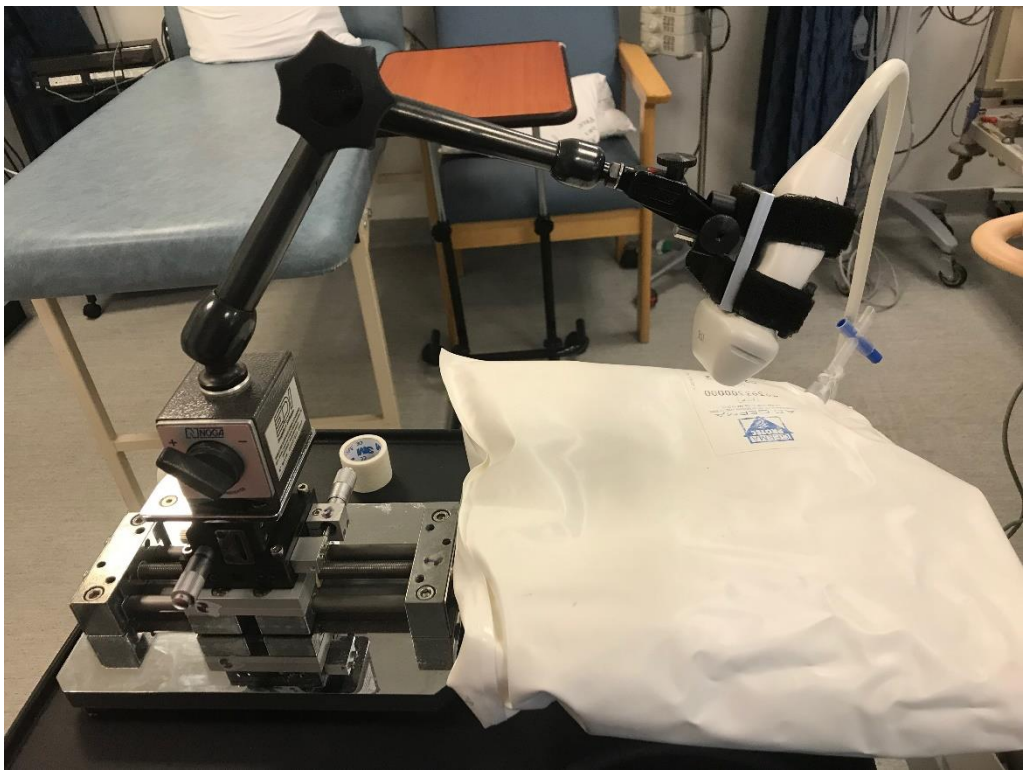

*Figure 28. Mechanical probe holder for flow mediated dilation (FMD) measures of endothelial function with support bean bag to try and minimise participant movement.*

The participants arm was further stabilised by supporting it on a support bean bag with the air extracted (Vacuum Pillow White, AB Germa, Kristianstad, Sweden). A vascular tourniquet cuff (Hokanson® SC10™, D.E. Hokanson Inc., Bellevue, Washington, USA) was applied immediately distal to the participant's elbow to provide the ischaemic stimulus.

Images from the ultrasound machine were transferred in real-time to a computer running Cardiovascular Suite, FMD Studio (Version 3.3.0, Quipu, Pisa, Italy) to facilitate real-time analysis. This software uses an edge detection algorithm to measure the diameter of the artery in real time, making 25 measurements per second across a defined region of interest and has been previously validated for the assessment of endothelial function (Gemignani et al. 2008, 2007).

### 4.15.2 FMD protocol

Once the participant had completed the required 30 minutes of quiet, supervised supine rest and the equipment was set up as above, they were instructed to keep their arm as still as possible for the duration of the measurements.

The diameter of the brachial artery was measured at rest for 1 minute, after which the cuff was inflated to and maintained at 250 mmHg for 5 minutes to occlude both the radial and ulnar arteries. Immediately before cuff deflation, the participant was again reminded of the importance of keeping their arm still, despite any paraesthesia they might experience in the arm on cuff deflation. The cuff was then deflated and measurement of the brachial artery diameter made for a further 4 minutes. The %FMD was calculated automatically by the cardiovascular suite software as:

$$\%\boldsymbol{FMD} = \frac{\boldsymbol{d_{max} - d_{baseline}}}{\boldsymbol{d_{baseline}}}$$




*Figure 29 Flow mediated dilatation (FMD) studio, contained within Cardiovascular suite software.*

*The top-left panel shows the graph plotting real-time diameter of the brachial artery, top-right panel shows the input from the ultrasound machine, the bottom-left panel plots arterial shear rate (in order to demonstrate that occlusion has led to a rapid increase in flow, the bottom-right paned displays the calculated FMD*

## 4.16 Measurement of exercise tolerance

### 4.16.1 Exercise tolerance test equipment

Measurements of exercise tolerance (determined as a test of cycling endurance) were made using an identical Lode Corival cycle ergometer to that which was used for CPET (4.8.1). The ergometer was set to "hyperbolic" mode, allowing the work to remain constant, independent of the participant's cadence. The load of the ergometer was set to 50% of their power output at the termination of the ramp-incremental stage of their most recent CPET. Participants were seated on the ergometer at a comfortable height. Routine monitoring was conducted throughout the test, including continuous 3-lead ECG, pulse oximetry (Datascope Trio™, Mindray North America, Mahwah, New Jersey, USA) and intermittent measurements of blood pressure by auscultation (A&D UM101 Mercury-free Sphygmomanometer, A&D Company Ltd., Tokyo, Japan). All exercise tolerance tests were conducted by 2 trained, experienced investigators with at least one of which holding a valid Advanced Life Support Provider certificate (Resuscitation Council UK, London, UK). As no analysis of respiratory gases was performed during the endurance test, participants were free to drink (only) water throughout the test.

### 4.16.2 Exercise tolerance test protocol

After a thorough briefing, participants were instructed to begin cycling, maintaining a cadence of between 50-100 rpm at a pace they found comfortable. They were asked to continue to cycle until they were unable to continue either through breathlessness, muscular fatigue or discomfort. They were also notified that if their cadence dropped below 50 rpm, they would be given encouragement to increase to above 50 rpm and if they were unable to do this the test would be concluded. Indications for terminating the exercise tolerance test for safety reasons were the same as those used for CPET (Section 4.8.2, Table 5). The maximum allowed cycling duration was set at 60 minutes by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee.

The total time spent cycling until either medical termination, 60 minutes had been reached or volitional exhaustion was recorded.

## 4.17 Clinical Biochemistry

Ten millilitres of venous blood were taken from all participants at screening (in 3 x BD Vacutainer® tubes (Beckton Dickson, Franklin Lakes, New Jersey, USA) (one Purple Ethylenediaminetetraacetic acid (EDTA) tube, one Yellow serum separating (SST™) tube and one Grey Fluoride tube) and sent to the department of Biochemistry and Haematology at the Royal Derby Hospital for measurement of a routine full blood count, urea and electrolytes, liver function tests, thyroid function tests, lipids, and fasting glucose to confirm eligibility for the studies.

## 4.18 Statistical analysis

All data in this thesis is reported as mean (SD), median (IQR) or number (n) (%) as appropriate. Groups were tested at baseline for significant differences using one-way ANOVA, whilst between group changes were tested by 2-way ANOVA (group allocation and pre/post intervention), with significant differences interrogated further by Sidak's multiple comparison testing. Significance was set at $P<0.05$. All calculations on empirical data were performed using Prism Graphpad Version 7.04 (Graphpad Software Inc., San Diego, California, USA).

## 4.19 Interventions

The three interventions used in the studies in this thesis were all delivered at the Medical School at the Royal Derby Hospital. All intervention sessions were fully supervised by an appropriately trained member of the research team and an attendance and compliance log was kept for all interventions. All interventions were delivered 3 times per week at the participant's convenience.

### 4.19.1 High intensity interval training

The high-intensity interval training (HIIT) protocol used in the studies in this thesis was the same 5x1 minute protocol described in chapter 1 (section1.6.1.1) (Phillips et al. 2017a; Boereboom et al. 2016b). All training sessions were fully supervised by an investigator holding a valid Advanced Life Support Provider certificate (Resuscitation Council UK, London, UK) and conducted with full ECG, BP and pulse oximetry monitoring using the same cycle ergometer and monitoring equipment as used for CPETs (outlined in detail in section 4.8.1).

#### *4.19.1.1 HIIT assessment session*

The first HIIT session for each participant consisted of an assessment session to determine the training load for each subsequent HIIT session. This session consisted of 5 x 1-minute working intervals, each of increasing intensity, interspersed with 90s recovery periods of unloaded cycling. The work intensity of each interval was: 90%, 95%, 100%, 105% and finally 110% of the peak power output at the termination of the pre intervention CPET ($W_{peak}$). Participants were given verbal encouragement to continue cycling throughout the working interval at a cadence of approximately 90rpm. If a participant was unable to complete an interval at the designated intensity, their training intensity for the remaining intervals in the assessment session and their subsequent training sessions was set at the intensity of the last interval they were able to complete.

#### *4.19.1.2 HIIT sessions*

After the assessment session, participants completed HIIT sessions with 5 x 60s working intervals set at their designated training intensity interspersed with 90s recovery periods of unloaded cycling (Figure 30). Participants were given verbal encouragement to complete all working intervals at a cadence

of approximately 90rpm, and to reach a minimum of 85% predicted maximum heart rate. If a participant's cadence was falling and they were unable to increase this back to 90rpm, despite verbal encouragement, then the work intensity was reduced in 5W increments in order to allow them to complete the 60s of work.

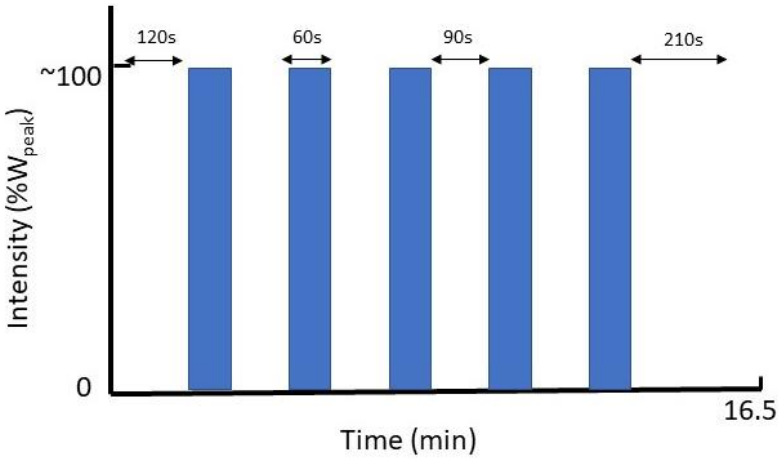


*Figure 30 High intensity interval training (HIIT) protocol used in the studies in this thesis.*

*Abbreviations $W_{peak}$; peak power output at termination of ramp incremental cardiopulmonary exercise test*

After 3 weeks of training (at the end of the 9$^{th}$ training session), participants were shown a modified Borg scale of 1-10 and asked to rate their perceived exertion. A rating of ≤8 led to an increase in the intensity of the working intervals of

the next 3 week's training sessions by 10% of their current working intensity (Phillips et al. 2017a).

### 4.19.2 Isometric handgrip training

The isometric handgrip (IHG) protocol used was as previously described by our research group (Blackwell et al. 2017). The dynamometer used for IHG training was the same dynamometer as was used for measuring the handgrip MVC of the participants (Camry EH101, Zhongshan Camry Electronic Co. Ltd, Guangdong, China) (section 4.10). This model of dynamometer has a digital display of the force applied to the handle, with the measurement updated in real time as the force applied varies, allowing the participant to sustain the correct force for the duration of each contraction. The member of the research team supervising the IHG training sessions directly observed the device display to verify the protocol was being adhered to.

In each training session, participants completed four x 2-minute repetitions of isometric handgrip at 30% of MVC using their dominant arm, with two minutes rest between each contraction.

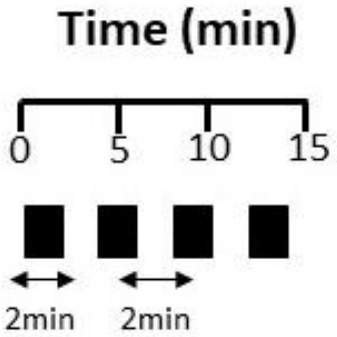


*Figure 31 Isometric handgrip (IHG) training protocol used in the studies in this thesis*

### 4.19.3 Remote ischaemic preconditioning

The remote ischaemic preconditioning (RIPC) protocol used in the studies in this thesis was adapted from study protocols used in the most recent systematic review of RIPC (Epps et al. 2016). The decision to shorten limb occlusion time in this protocol to less than in many previously studied protocols (Epps et al. 2016) was made in order to reduce the total time commitment for the training protocol, making it comparable with the IHG and HIIT protocols.

RIPC was performed using a manual non-invasive blood pressure cuff (Welch Allyn, NY, USA). At each session, this cuff was placed on the participant's dominant arm and inflated to 200 mmHg for 3-minutes. The supervising member of the research team ensured that the cuff pressure remained above 200 mmHg for the duration of the 3-minute period of ischaemia and topped up the cuff bladder as required to compensate for any gradual air leak. After each 3-minute period of ischaemia, the cuff was rapidly deflated to 0 mmHg to allow a period of 3 minutes of reperfusion. Each training session consisted of 3 x 3-minute periods of ischaemia, interspersed with 3 minutes of reperfusion.

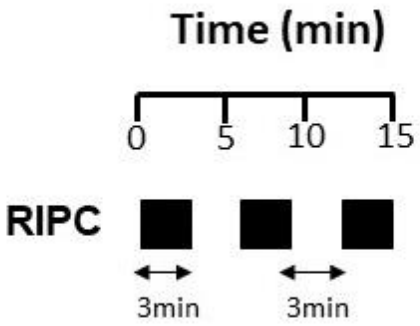


*Figure 32. Remote ischaemic preconditioning (RIPC) protocol used in the studies in this thesis.*

# 5 The effects of 6 weeks supervised high-intensity interval training, isometric handgrip training or remote ischaemic preconditioning versus control on resting and ambulatory blood pressure and cardiorespiratory fitness; a randomised controlled trial

## 5.1 Chapter focus

This chapter describes a randomised controlled trial comparing the effects of 6 weeks HIIT, IHG, RIPC or non-intervention control on both resting and ambulatory blood pressure and measures of cardiorespiratory fitness.

## 5.2 Introduction

National guidelines state that the control of hypertension is key to the management of cardiovascular risk (Eckel et al. 2014). The importance of hypertension control is emphasized by a large meta-analysis of antihypertensive therapy, comprising 464,000 patients demonstrating that a 10 mmHg reduction in systolic BP is associated with a vast reduction in cardiovascular disease risk (Law et al. 2009).

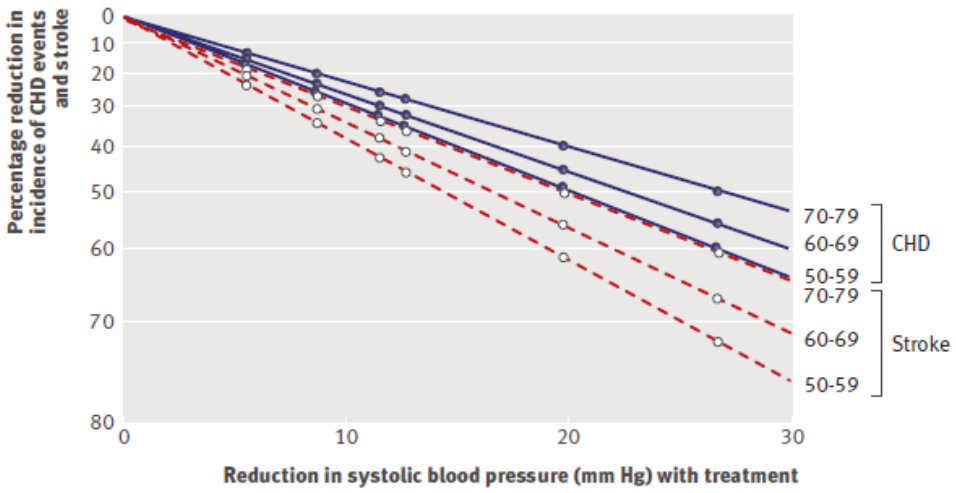


*Figure 33 Percentage reduction in incidence of CHD (coronary heart disease) events and stroke risk, by reduction in systolic blood pressure, by patient age.*

*(Figure taken from Law et al. 2009).*

Many national guidelines make recommendations on lifestyle modifications to reduce BP (National Institute for Health and Care Excellence 2011; Mancia et al. 2013), however their applicability to older adults remains untested, with the majority of evidence coming from studies in young adults (Eckel et al. 2014). This is despite the well described association between advancing age and hypertension, with prevalence increasing from 8% of men and 2% of women in the 16-24 age range to 66% of men and 78% of women aged 75 and over (Knott and Mindell 2012).

The systematic review that forms chapter 2 of this thesis demonstrated the benefits of aerobic exercise, dynamic resistance exercise, combined training and isometric handgrip training when carried out by older adults, however the majority of interventions lasted 3 months or more, with sessions often exceeding 60 minutes in duration. Furthermore, the majority of interventions took place in a fully supervised fashion, requiring participants to travel to be trained and thus increasing the time required. As has already been discussed in detail in chapter 1, lack of time remains a significant barrier to exercise. As such, there remains a need for an effective and time-efficient lifestyle intervention to reduce BP in older adults.

In addition to BP control, improving cardiorespiratory fitness (CRF) in older adults may also bring clinical benefit, with increased physical activity/ exercise required to achieve this (Dunn et al. 1999). High levels of CRF have been consistently associated with improved life expectancy (Mandsager et al. 2018; Clausen et al. 2018) and low levels are associated with poorer survival in a range of patient cohorts including diabetics (Wei et al. 2000) and patients with ischaemic heart disease (Nichols et al. 2018).

HIIT (Costa et al. 2018), IHG (Jin et al. 2017) and RIPC (Epps et al. 2016), may all be of use in favourably affecting cardiovascular parameters such as BP and CRF, however all have yet to be assessed in a RCT of older adults over a short timeframe. This study aimed to compare the effects of 6 weeks of HIIT, IHG and RIPC *vs.* non-intervention control on BP and CRF in healthy older adults.

## 5.3 Methods

Ethical approval was obtained to recruit adults aged 65-85 years to participate in a trial of 6 weeks fully supervised HIIT, RIPC, IHG or non-intervention control (Ethical approval code from the University of Nottingham Medical School Research Ethics Committee: A12092016, see Appendix 5 by local advertising. The study was prospectively registered with clinicaltrials.gov (NCT03019146). All participants gave written informed consent. Participants already prescribed antihypertensives were included, providing no alterations were made between 3 months before enrollment and study close. The screening process and exclusion criteria for the study are listed in chapter 4.6.

### 5.3.1 Measurements

#### *5.3.1.1 Resting BP*

The primary outcome of the study was resting SBP. Resting BP was measured using an automated oscilometric device (Datascope trio, New Jersey, USA) and a cuff of appropriate size after the participant had sat quietly for 5 minutes, with their dominant arm supported at the level of their heart, in line with the 2017 British and Irish Hypertension Society guidelines (British and Irish Hypertension society 2017). Blood pressure was measured 3 times in this way and the mean values of systolic and diastolic BP were recorded. Blood pressure was recorded at the same time of day both before and after study intervention periods and in the same clinical laboratory.

#### *5.3.1.2 Ambulatory BP*

Measurement of ambulatory BP was carried out as described in detail in chapter 4.7.2 both before randomisation and after the intervention period.

#### *5.3.1.3 CPET*

Ramp-incremental CPETs were conducted to volitional exhaustion using a cycle ergometer and are described in detail

in chapter 4 (Section 4.8). Baseline CPET was performed before randomisation and between 2-5 days after each participant's final training session in the intervention period.

### 5.3.2 Randomisation

After their baseline measurements, participants were randomised to either HIIT, RIPC, IHG or control using a computerised randomisation system (sealedenvelope.com), using random permuted blocks of size 4 and 8 to ensure allocation concealment. Randomisation was stratified to baseline resting SBP (<140 mmHg or ≥140 mmHg/taking prescribed antihypertensive medication).

### 5.3.3 Interventions

Subjects randomised to either HIIT, RIPC or IHG trained 3 times per week, under full supervision in the Medical School at the Royal Derby Hospital. The interventions are described in detail in chapter 4 (sections 4.19.1, 4.19.2 and 4.19.3). All study training sessions were designed to last roughly 15 minutes. In brief, HIIT sessions consisted of 5, 1-minute high intensity cycling intervals interspersed with 90-seconds recovery; RIPC involved 3, 3-minute inflations of a blood

pressure cuff to 200 mmHg around the upper arm with 3 minutes of recovery; and IHG consisted of 4, 2-minute contractions interspersed with 2 minutes of recovery.

In addition, participants in the HIIT group were shown a modified Borg rating of perceived exertion (RPE) scale (Borg 1982) immediately after finishing their 5$^{th}$ working interval of session 9 (the half way point of the study) and asked to rate their perceived exertion. Any participant scoring ≤8 was deemed to be suitable for an increase in training load of 10% for subsequent sessions. No interim alterations were made to the intervention for those in the RIPC or IHG groups. Participants allocated to the control group were asked to continue their normal activities of daily living throughout the study period but were not given any intervention.

### 5.3.4 Re-assessment

All participants repeated the study measurements after the intervention period, within 2 – 5 days of their last intervention session.

### 5.3.5 Statistical analysis

#### *5.3.5.1 Power calculation*

This study was designed with 90% power to detect a clinically relevant 10 mmHg reduction in SBP (Law et al. 2009) vs. control with an α of 0.05 and β of 0.1. As no previous study has compared our chosen interventions for this end point, the study was powered from data for the standard deviation of change of SBP of 9 mmHg from a previous study conducted in our laboratory, albeit in middle aged participants, which investigated the same IHG and HIIT protocols (Blackwell et al. 2017). This power calculation determined a sample size of 48 participants (12 per group), thus allowing for an anticipated drop-out rate of 25%, permission was sought to recruit 60 participants. Recruitment was planned to be terminated after complete data for the primary outcome for 48 participants was obtained.

#### *5.3.5.2 Analysis*

All data is presented as n (%) or mean (SD) as appropriate. Data were tested for between group differences at baseline by one-way ANOVA or a chi square test as appropriate. Differences in change from baseline between groups was analysed using 2-way ANOVA with Sidak's multiple

comparisons post hoc testing. The correlation between resting values and change values was analysed using simple linear regression, whilst exploratory secondary analyses based on baseline hypertensive status were analysed using unpaired t-tests. All data was analysed on an intention to treat basis.

## 5.4 Results

### 5.4.1 Participant characteristics

Forty-eight participants of mean (SD) age 71(4) years (46% male) were recruited to the study. Participant demographics are displayed in Table 6.

*Table 6 Participant demographics*

| Group | Age (Years) | Male/Female | BMI ($kg/m^2$) (SD) | Hypertensive (n) |
|---|---|---|---|---|
| HIIT (n=13) | 70(3) | 5/7 | 27.7 (2.4) | 7 |
| IHG (n=11) | 72(4) | 4/7 | 24.4 (3.0) | 7 |
| RIPC (n=12) | 69(4) | 6/6 | 26.5 (3.5) | 7 |
| CON (n=12) | 72(6) | 7/5 | 26.3 (2.9) | 5 |
| *P* | 0.24 | 0.73 | 0.09 | 0.74 |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### 5.4.2 Study adherence

All participants remained in the study and none were lost to follow up for the primary endpoint of the study. The study consort diagram is displayed in Figure 34. Two participants declined to perform a post intervention CPET (one allocated to IHG, one to control), however they allowed measurement of all other variables. Mean training compliance was 100% in the HIIT group, 98% in the IHG group and 100% in the RIPC group.

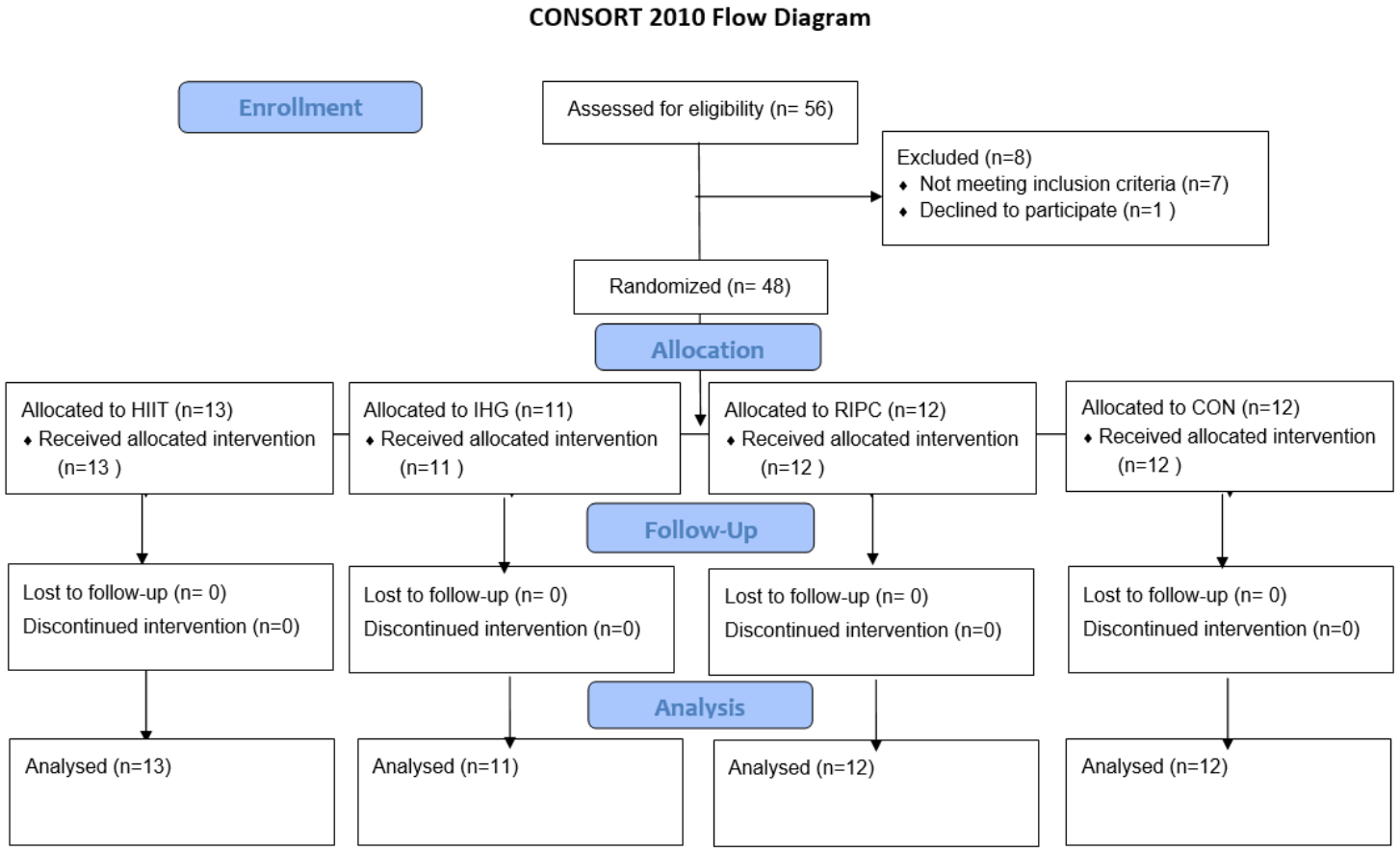


*Figure 34 Consort diagram*

### 5.4.3 Resting blood pressure

#### *5.4.3.1 Primary analysis of resting blood pressure*

All 48 participants had resting BP measured both before and after intervention. There was no significant difference between the groups at baseline for either systolic BP (*P*=0.15) or diastolic BP (*P*=0.30).

Data for both resting systolic BP and diastolic BP at baseline and after intervention are displayed in Table 7, Figure 35 and Figure *36*. There was a reduction in resting systolic BP in both the HIIT and IHG groups, but no change in the RIPC or CON groups, with a main effect of time (*P*<0.001) and a significant group x time interaction (*P*=0.04) on 2-way ANOVA. There was no change in resting diastolic BP in any group, although there was a main effect of time (*P*=0.01) but no significant group x time interaction (*P*=0.83).

*Table 7. Resting systolic blood pressure data before (pre) and after (post) 6-week intervention periods in older adults.*

| | | Systolic blood pressure (mmHg) | | | |
|---|---|---|---|---|---|
| | Pre | Post | Change | 95%CI | *P* |
| HIIT n=13 | 142(15) | 133(11) | -9(9) | -4,-15 | <0.001 |
| IHG n=11 | 139(15) | 131(12) | -9(9) | -3,-15 | 0.002 |
| RIPC n=12 | 138(15) | 134(14) | -4(5) | +1,-10 | 0.165 |
| CON n=12 | 130(10) | 128(10) | -1(6) | +5,-7 | 0.959 |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

*Table 8 Resting diastolic blood pressure data before (pre) and after (post) 6-week intervention periods in older adults*

| | Diastolic blood pressure (mmHg) | | |
|---|---|---|---|
| | Pre | Post | Change |
| HIIT n=13 | 85(13) | 81(8) | -4(10) |
| IHG n=11 | 81(6) | 76(10) | -5(9) |
| RIPC n=12 | 78(5) | 77(7) | -2(6) |
| CON n=12 | 80(10) | 77(7) | -3(11) |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

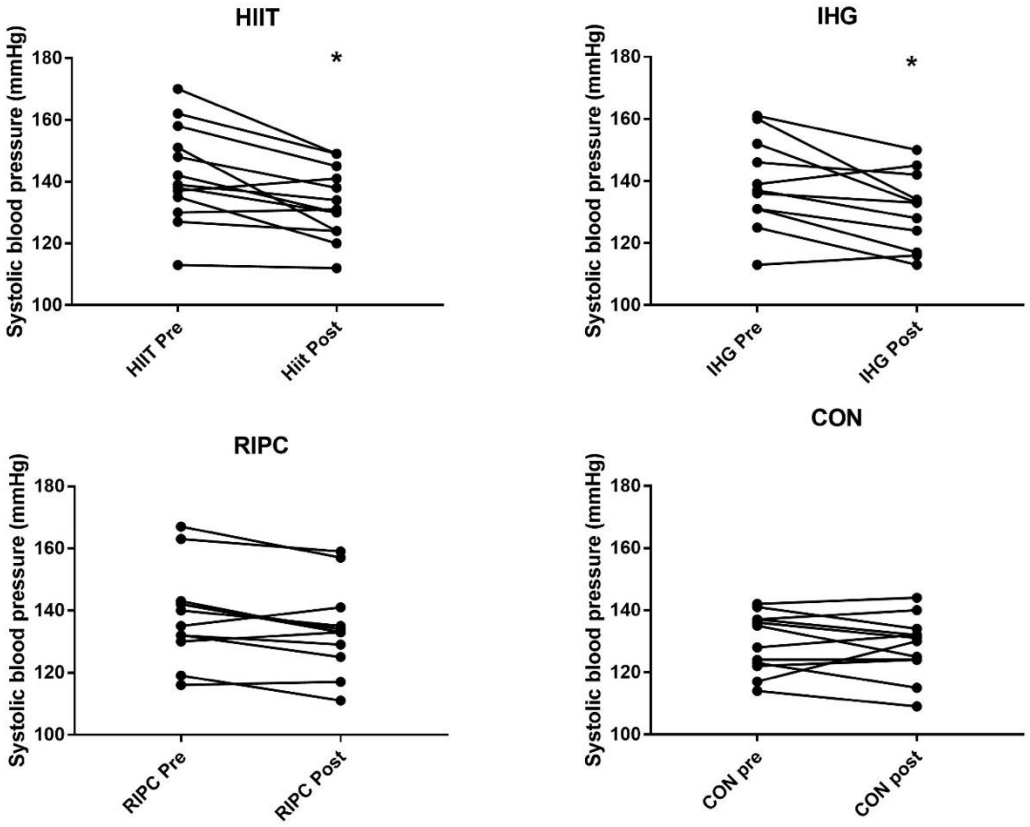


*Figure 35. Resting systolic blood pressure before (pre) and after (post) 6-week intervention periods in older adults.*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=11); RIPC: remote ischaemic pre-conditioning (n=12), CON: control (no intervention) (n=12). *denotes P<0.05 vs. pre-intervention.*

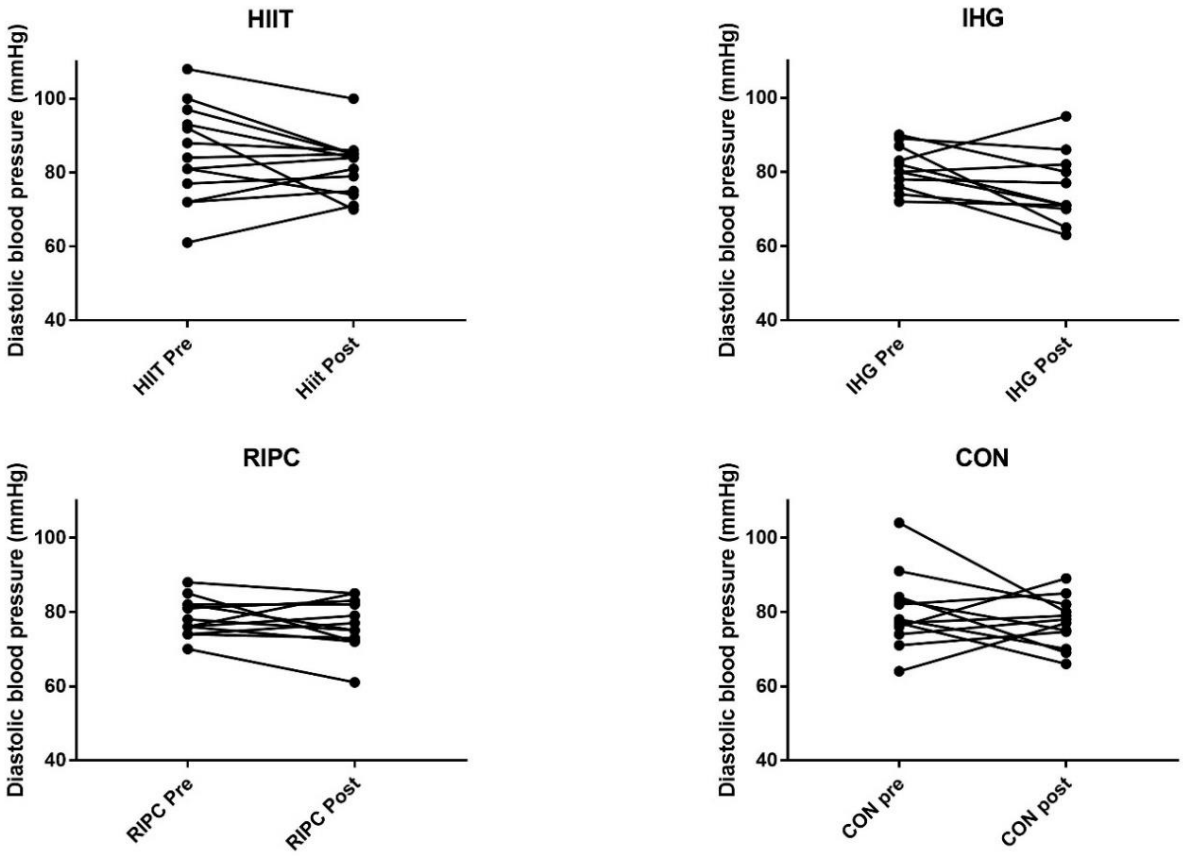


*Figure* 36**.** *Resting diastolic blood pressure before (pre) and after (post) 6-week intervention periods in older adults.*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=11); RIPC: remote ischaemic pre-conditioning (n=12), CON: control (no intervention) (n=12).*

#### *5.4.3.2 Secondary exploratory analyses of significant changes in resting systolic blood pressure*

Both the contribution of pre-intervention resting systolic BP and baseline hypertensive status on the change in resting systolic BP in the HIIT and IHG groups (which demonstrated significant reductions in group means above) are now explored in this section.

##### 5.4.3.2.1 Influence of pre-intervention resting systolic blood pressure on change in resting systolic blood pressure

There was a significant correlation between pre-intervention resting systolic BP and the change in systolic BP in the HIIT group ($P$=0.01, $R^2$= 0.47) (Figure 37 A) and a trend towards a correlation in between pre-intervention resting systolic BP and the change in systolic BP in the IHG group ($P$ =0.08, $R^2$= 0.29) (Figure 37 B).

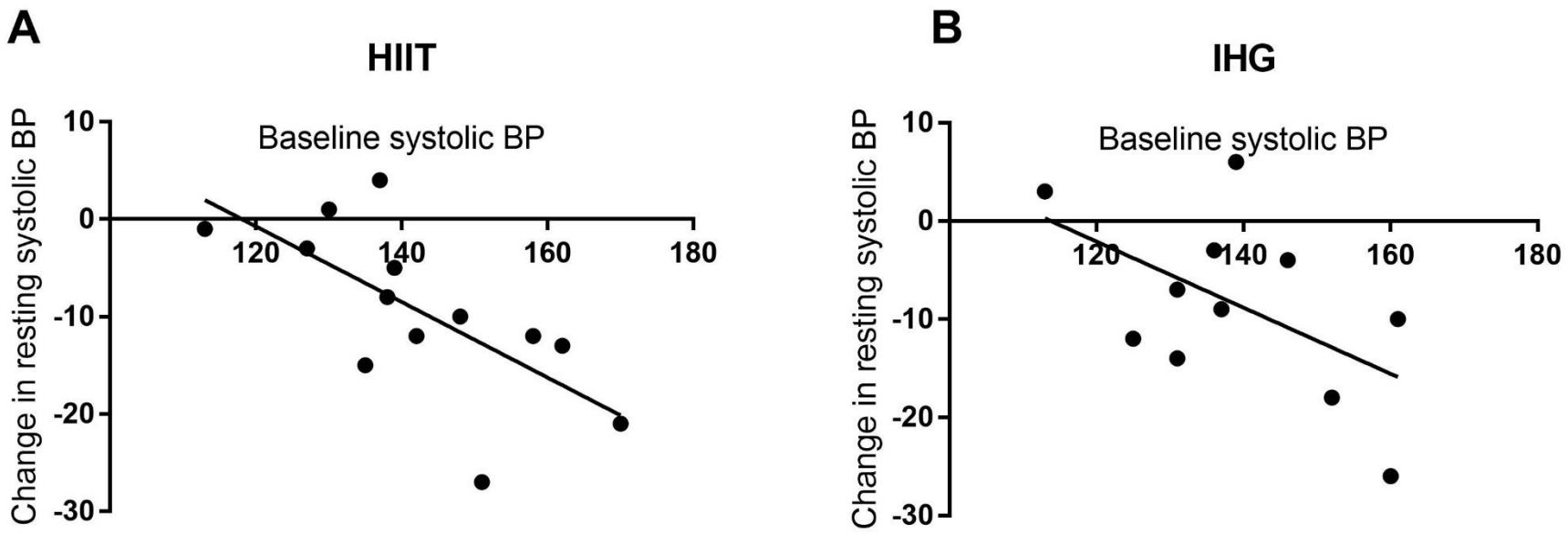


*Figure 37 Correlations between baseline resting systolic blood pressure and the change in resting systolic blood pressure after intervention*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=11), BP: Blood pressure*

#### 5.4.3.2.2 Influence of baseline hypertensive status on change in resting systolic blood pressure

There was a trend towards a difference in the magnitude of reduction in resting systolic BP between hypertensives or normotensives in the HIIT group (-13(10) mmHg *vs.* -5(6) mmHg, *P* =0.10) but no difference in the magnitude of reduction in resting systolic BP between hypertensives or normotensives in the IHG group (-10(10) mmHg *vs.* -7(8) mmHg, *P* = 0.58).

### 5.4.4 Ambulatory blood pressure

For ambulatory BP measurements to be deemed valid, as described in chapter 4, we required a minimum of 20 valid daytime measurements and 7 valid night time measurements in each measurement period (O'Brien et al. 2013). Only 34 (71%) participants provided sufficient measurements of ambulatory blood pressure to be considered valid (7 (58%) CON, 10 (77%) HIIT, 8 (73%) IHG and 9 (75%) RIPC). Of those excluded, twelve participants were non-compliant with night-time measurements (fewer than the required 7 measurements both before and after intervention) whilst 2

further participants were non-compliant with daytime measurements.

#### *5.4.4.1 24-hour ambulatory systolic blood pressure*

There was no significant difference in baseline 24Hr ambulatory diastolic BP between groups (*P*=0.21). There were no significant changes in 24Hr ambulatory systolic BP in any of the groups, with no main effect of time (*P*=0.74) and no group x time interaction (*P*=0.96) (Table 9).

*Table 9 24Hr ambulatory systolic blood pressure data before (pre) and after (post) 6-week intervention periods in older adults*

| | Pre (mmHg) | Post (mmHg) | Change (mmHg) |
|---|---|---|---|
| HIIT n=10 | 138(10) | 138(15) | 0(10) |
| IHG n=8 | 126(10) | 128(7) | +2(8) |
| RIPC n=9 | 132(10) | 131(10) | -1(9) |
| CON n=7 | 134(18) | 136(19) | 2(26) |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

#### *5.4.4.2 24Hr Ambulatory diastolic blood pressure*

As with systolic BP, there was no significant difference in baseline 24Hr ambulatory diastolic BP between groups (*P*=0.12). There were no significant changes in 24Hr ambulatory diastolic BP in any of the groups, with no main effect of time (*P*=0.27) and no group x time interaction (*P*=0.43) (Table 10).

*Table 10 24Hr ambulatory diastolic blood pressure data before (pre) and after (post) 6-week intervention periods in older adults.*

| | Pre | Post | Change |
|---|---|---|---|
| HIIT n=10 | 81(9) | 79(9) | -2(3) |
| IHG n=8 | 75(4) | 75(5) | +1(7) |
| RIPC n=9 | 73(3) | 73(3) | 0(4) |
| CON n=7 | 79(11) | 75(3) | -4(11) |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

## 5.4.5 Cardiorespiratory fitness

All 48 participants performed a CPET to volitional exhaustion at baseline, however 2 participants did not perform a repeat CPET after the intervention period (1 IHG and 1 CON).

### *5.4.5.1 Anaerobic threshold*

There was no significant difference in baseline AT between groups (*P*=0.70). Participants in the HIIT group significantly increased their AT after intervention, however there was no change in AT in any other group (Table 11, Figure 38). There was a main effect of time (*P*=0.009) and a significant group x time interaction (*P*=0.03)

*Table 11 Anaerobic threshold before and after intervention (ml/kg/min)*

| | Pre | Post | Change | *P* |
|---|---|---|---|---|
| HIIT n=13 | 13.2(3.4) | 15.3(3.8) | +2.0(1.7) | <0.001 |
| IHG n=10 | 14.8(3.3) | 15.1(4.2) | +0.4(2.0) | 0.94 |
| RIPC n=12 | 14.0(2.8) | 14.4(2.6) | +0.4(1.8) | 0.92 |
| CON n=11 | 15.1(6.0) | 15.2(6.4) | +0.1(1.1) | >0.99 |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

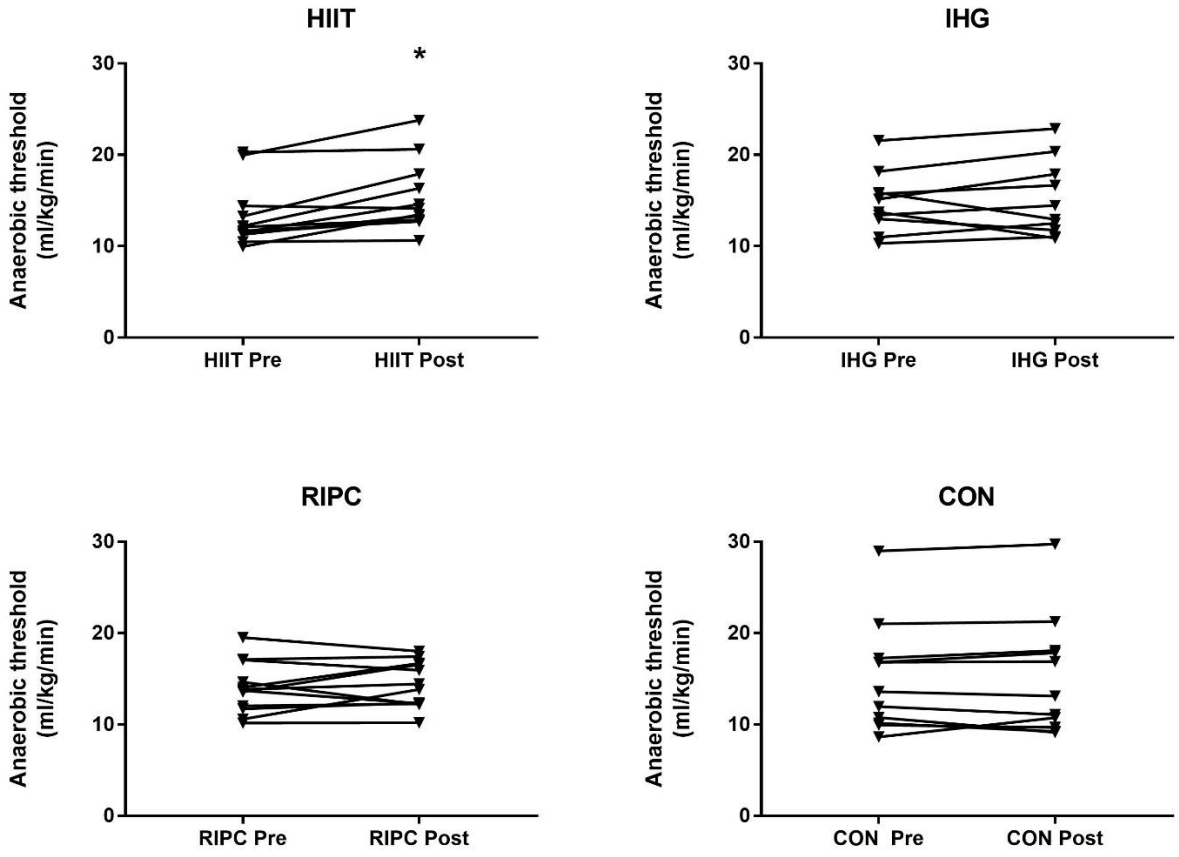


*Figure 38 Anaerobic threshold before and after intervention.*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=10); RIPC: remote ischaemic pre-conditioning (n=12), CON: control (no intervention) (n=11). *denotes P<0.05 vs. pre-intervention.*

#### *5.4.5.2 $VO_{2peak}$*

There was no significant difference in baseline $VO_{2peak}$ between groups (*P*=0.65). Participants in the HIIT group significantly increased their $VO_{2peak}$ after intervention, however there was no change in $VO_{2peak}$ in any other group (Table 12,Figure 39). There was no main effect of time (*P*=0.15) but there was a trend towards a significant group x time interaction (*P*=0.06).

*Table 12 $VO_{2peak}$ before and after intervention (ml/kg/min)*

| | Pre | Post | Change |
|---|---|---|---|
| HIIT n=13 | 22.5(7.1) | 25.4(5.5) | +2.8(4.8) |
| IHG n=10 | 26.2(7.3) | 25.5(7.1) | -0.8(2.4) |
| RIPC n=12 | 24.1(4.9) | 24.2(4.4) | 0.0(2.4) |
| CON n=11 | 25.3(9.7) | 26.1(9.4) | +0.8(2.5) |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

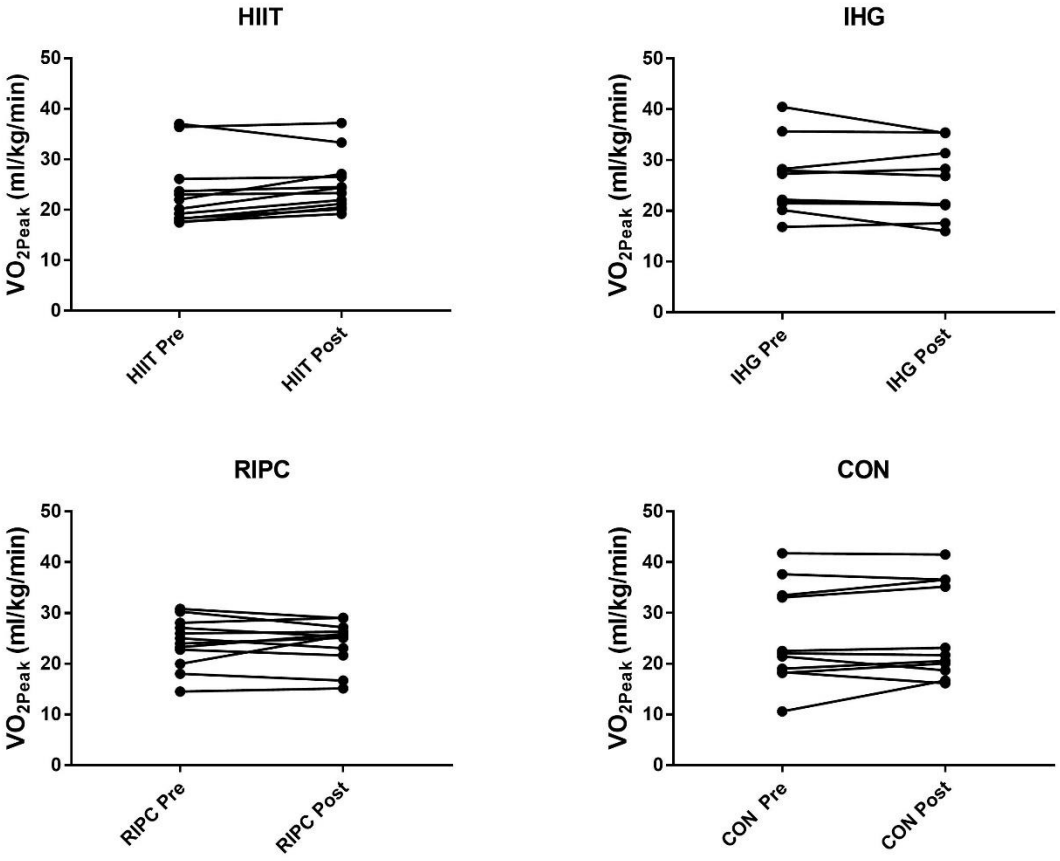


*Figure 39 $VO_{2peak}$ before and after intervention.*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=10); RIPC: remote ischaemic pre-conditioning (n=12), CON: control (no intervention) (n=11). *denotes P<0.05 vs. pre-intervention*

## 5.4.6 Exercise tolerance

### *5.4.6.1 Power output at termination of CPET ($W_{peak}$)*

There was no significant difference in baseline $W_{peak}$ between groups (*P*=0.97). Participants in both the HIIT and RIPC groups significantly increased their $W_{peak}$ after intervention, however there was no change in $W_{peak}$ in either the IHG or CON groups (Table 13, Figure 40). There was a main effect of time (*P*<0.001) and a significant group x time interaction (*P*<0.001)

*Table 13 $W_{peak}$ before and after intervention (W)*

| | Pre | Post | Change | *P* |
|---|---|---|---|---|
| HIIT n=13 | 145(60) | 159(59) | +14(11) | <0.001 |
| IHG n=10 | 137(54) | 136(56) | -1(4) | >0.99 |
| RIPC n=12 | 135(48) | 143(52) | +7(8) | 0.01 |
| CON n=11 | 144(63) | 145(62) | +1(6) | >0.99 |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

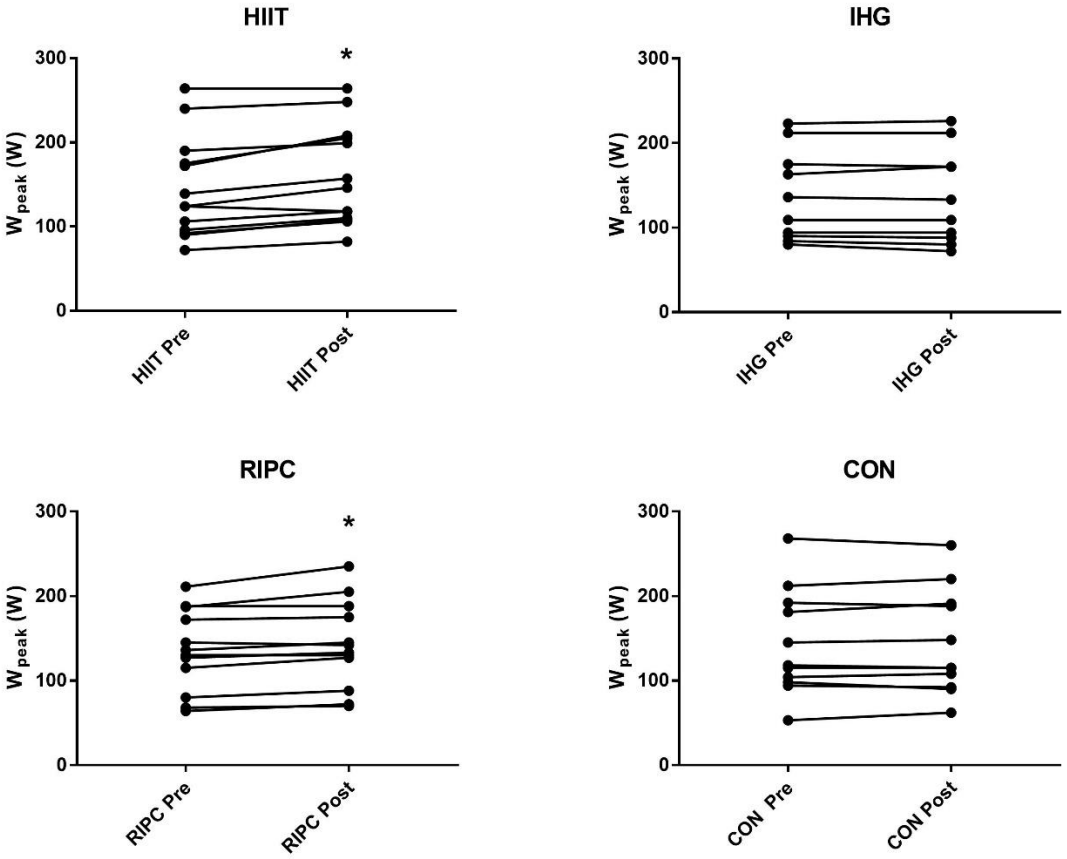


*Figure 40 $W_{peak}$ before and after intervention.*

*HIIT: high-intensity interval training (n=13), IHG: isometric handgrip training (n=10); RIPC: remote ischaemic pre-conditioning (n=12), CON: control (no intervention) (n=11). *denotes P<0.05 vs. pre-intervention*

*5.4.6.2 Cycling endurance trial*

Before and after intervention data for the cycling endurance trial was only available for 40 participants. Eights participants declined to participate in this test after the intervention period (3 HIIT, 3 IHG, 1 RIPC, 1 CON). There was no significant difference in baseline cycling endurance trial performance between groups (*P*=0.69). There was no significant change in cycling endurance trial performance in any group (Table 14). There was a trend towards an effect of time (*P*=0.06) and a trend towards a significant group x time interaction (*P*=0.09)

*Table 14 Cycling endurance trial duration before and after intervention (minutes)*

| | Pre | Post | Change |
|---|---|---|---|
| HIIT n=13 | 39.1(17.7) | 40.6(14.5) | +1.5(10.1) |
| IHG n=10 | 30.0(16.6) | 37.0(21.2) | +7.0(11.4) |
| RIPC n=12 | 33.2(14.8) | 38.2(14.8) | +5.0(7.2) |
| CON n=11 | 36.1(17.7) | 33.3(18.0) | -2.8(6.7) |

HIIT: high-intensity interval training, IHG: isometric handgrip training; RIPC: remote ischaemic pre-conditioning, CON: control (no intervention). Data is presented as mean (SD).

## 5.5 Discussion

This study has demonstrated that 6-weeks of supervised IHG or HIIT can reduce the resting systolic BP of healthy older adults by approximately 9 mmHg. This reduction is of a clinically relevant magnitude likely to substantially reduce a patient's risk of myocardial infarction by as much as 21% and of stroke by 41% (Law et al. 2009). Even with the small sample size of this study, the lower bound of the 95% CI for these two interventions was -4 mmHg for HIIT and -3mmHg for IHG, reductions of such a magnitude would also be expected to lead to significant reductions in risk, if not as dramatic as the reductions stated above. The fact that these interventions are time efficient, with each session lasting only 15 minutes, and effective in as short a time frame as 6 weeks, could lead to their use as either a brief lifestyle intervention that could be prescribed by general practitioners to patients either for their general cardiovascular risk status, or as specific lifestyle advice treatment of hypertension (NICE 2011). The short time frame over which they work could also allow them to be prescribed as prehabilitation prior to major surgery (Boereboom et al. 2016b). However, this utility may be limited to benign disease as currently 6 weeks of training would exceed cancer waiting time targets enforced in the UK NHS such as the 31-day decision to treat target (Cancer Research UK 2015).

Previous studies using HIIT in both young healthy subjects (Ciolac et al. 2010) and middle-aged hypertensive subjects (Olea et al. 2017) have shown that HIIT can reduce blood pressure, and it appears from our findings that this is transferrable to older adults. Of note, the only other study reporting blood pressure results from a short-term HIIT protocol in older adults (men only), showed a similar magnitude of change (Grace et al. 2018). The training cohort study by Grace et al., with a mean age of 62 years asked their volunteers to perform 9 sessions of HIIT over a 6-week intervention period, with each session comprising 6, 30-second sprints on a cycle ergometer, at 40% of peak power output determined using a 6-second test (with a reported training mean training intensity at 141% of the Wpeak at VO2peak (Knowles et al. 2015)), interspersed with 3-minute resting intervals. Thus, it would appear that our reduced intensity 5x1 HIIT protocol can achieve similar reductions in systolic BP, with a shorter session duration in a mixed sex cohort. The magnitude of change in systolic BP in our study exceeds the mean change found in the systematic review and meta-analysis reported in chapter 2 of this thesis, from both moderate continuous aerobic exercise, dynamic resistance exercise or a combination of the two modalities (Herrod et al.

2018), over a greatly reduced duration of intervention. This could imply that HIIT is superior to these interventions in this age group.

The effect of the IHG protocol on SBP in this study was approximately equal to the mean change found in the meta-analysis of the two previous trials of IHG in older adults, which were conducted over 8 weeks (Millar et al. 2008) and 10-weeks (Taylor et al. 2003) respectively. It may therefore be that maximal improvements in systolic BP are achieved by 6 weeks, with prolonged training unable to produce further benefit.

It appears that there is a correlation between higher baseline values of systolic BP and greater reductions in BP from our HIIT protocol whilst there is a similar trend to a significant association from our IHG protocol, despite there seemingly being either only a trend to larger reductions in the hypertensives in our HIIT group and no significant difference between the reduction in systolic BP in hypertensives and normotensives in our IHG group. One reason for this apparent discrepancy may be that participants classified in both the HIIT and IHG groups as hypertensive by virtue of them taking prescribed antihypertensive medication, had relatively lower

systolic blood pressures at baseline than some in the normotensive groups. Or it may be simply that the study was not adequately powered to make these comparisons (especially with only 4 normotensives in the IHG group). Nevertheless, it would appear that both interventions may be more effective at reducing resting systolic BP in individuals with higher initial blood pressures.

That the 5x1 minute HIIT protocol used in this study also led to gains in one of the measures of CRF (AT) and an improvement in exercise performance with excellent adherence and no adverse events this suggests that HIIT has additional benefits over our IHG protocol. It must however be considered that IHG requires significantly less exertion on behalf of the participant and has the potential as a home-based intervention with minimal equipment, which may allow it to have greater uptake in the general population. That our HIIT protocol led to a 2ml/kg/minute increase in AT, greater than the accepted minimum clinically important difference of 1.5ml/kg/minute before cancer surgery (Dunne et al. 2016), supports its superiority to IHG in the preoperative setting. HIIT protocols have recently been shown in a large systematic review and meta-analysis to be superior to either control

interventions or moderate continuous aerobic exercise interventions at improving measures of CRF in older adults in short time-frames (Blackwell et al. 2018) and the results of this study add further weight to this evidence. That our IHG protocol led to no change in any measure of CRF agrees with the only other study which has investigated this (in younger adults) (Blackwell et al. 2017) and the study reported in chapter 3 of this thesis (Herrod et al. 2019a).

However, despite the significant effects of both IHG and HIIT on resting systolic BP, they had no effect on either resting diastolic BP or ambulatory BP. A lack of statistical power to detect changes in resting diastolic BP likely contributed to the former, as is demonstrated by the standard deviation of the change in resting diastolic BP for all groups being approximately double that of the mean change. Future studies looking to investigate the effect on resting diastolic BP will need to be adequately powered for this.

The ambulatory BP results were also affected by a lack of power to detect a change, again with standard deviations of the changes far exceeding the group mean changes. This lack of power was compounded by only 70% of participants

providing sufficient measurements for a valid recording of ambulatory BP. It is important that the discrepancy found between the resting BP and ambulatory BP results is evaluated further by larger studies, powered for ambulatory BP as the primary endpoint as ambulatory BP is now considered the gold standard for the diagnosis of hypertension in the UK (NICE 2011).

It would appear that RIPC conducted using a unilateral arm 3x3 minute occlusion time protocol does not have any effect on either BP or CRF. This was the first RCT to examine the effects of unilateral upper limb RIPC with only 3 minute ischaemic periods in older adults and did not reproduce the significant reduction in resting blood pressure found by Jones and colleagues (Jones et al. 2014a). This may be that reducing the ischaemic time to 3 minutes (from 5 minutes) in this study is responsible for this discrepancy or that the effect of RIPC in the younger subjects in Jones' study is not transferrable to older age.

Interestingly however RIPC did lead to a small, but statistically significant improvement in exercise performance as demonstrated by an increase in the participants' $W_{peak}$. The

reason for this may be an increase in the participants' tolerance of painful stimuli from having being subjected to intermittent bouts of limb ischaemia over the preceding 6 weeks (Sharma et al. 2015). Previous studies of ischaemic preconditioning, both remote and local, carried out in younger athletes have suggested that it may have an effect on exercise performance. *Jean-St-Michael et al.* previously demonstrated that subjecting one arm to an IPC protocol immediately before a series of swimming exercises led to a significant improvement in swimming performance (Jean-St-Michel et al. 2011). Likewise, De Groot *et al.* used bilateral lower limb ischaemia applied immediately before a CPET to demonstrate both an increase in $VO_{2max}$ and $W_{peak}$ (De Groot et al. 2010). However these results have been contentious as other studies in sprinters have failed to replicate the results (Gibson et al. 2013). Of note these athletic studies all examine the acute effects of IPC applied immediately prior to exercise and to our knowledge this is the first study in older adults to assess the effect of repeated RIPC on exercise performance.

One interesting facet of both IHG and HIIT are that they have the potential for future use as home-based unsupervised interventions. HIIT could also be delivered in the community

as a group intervention in community gyms or other fitness facilities. Future studies will be required to confirm that the same magnitude of benefit to both resting systolic BP and CRF in the case of HIIT as previous studies using aerobic exercise have suggested unsupervised training may lead to inferior outcomes (Haykowsky et al. 2005; Hageman et al. 2018).

### 5.5.1 Limitations

This randomised controlled trial was adequately powered to draw valid conclusions on its primary outcome, resting systolic BP, however it's lack of power to detect changes in both resting diastolic BP and ambulatory BP limit its ability to draw firm conclusions on the effectiveness of the interventions on these outcomes. Future studies investigating these should be adequately powered in this regard.

That this study sought to find and was powered to detect a difference of 10 mmHg in systolic BP, a vast difference with profound clinical implications (Law et al. 2009), was potentially too harsh a smallest worthwhile difference to detect, in view of the beneficial effects on population disease risk that could have been brought by differences only half this (Law et al.

2009). Future studies could consider using a less ambitious target, but should be powered accordingly.

The 4-arm design of this study has also limited its ability to determine whether each intervention was effective in its own right, rather, comparing each intervention with each other intervention. Future studies should consider limiting the number of trial arms, in order to be able to make a more valid finding on the effectiveness of each.

One further limitation of this study is that it provides no evidence as to the longevity of any adaptations these interventions may bring as no follow up after the reassessment testing session was attempted. Future studies will be required to assess both the time course of maladaptation following cessation of these interventions, and the longevity of them if training is continued.

## 5.6 Chapter conclusions

This chapter has demonstrated that 6 weeks of either HIIT or IHG can lead to statistically significant and clinically relevant reductions in resting systolic BP, whilst HIIT can also significantly improve AT. Our specific unilateral upper limb RIPC protocol appears to be ineffective at improving either of these cardiorespiratory facets.

6 The effects of 6 weeks supervised high-intensity interval training, isometric handgrip training or remote ischaemic preconditioning on glucose handling, insulin resistance and body composition

## 6.1 Chapter focus

This chapter describes secondary endpoints from the RCT of HIIT, IHG, or RIPC versus a non-intervention control in healthy older adults as fully described in Chapter 5. This chapter reports the effect of these interventions on both glucose and insulin kinetics, body composition and handgrip strength.

## 6.2 Introduction

Insulin resistance is defined as the resistance of cells to insulin mediated glucose uptake (Reaven 1988). Insulin resistance is both an important precursor to the development of type 2 diabetes mellitus and central to its pathogenesis (Taylor 2012) with an increasing prevalence with advancing patient age (Shimokata et al. 1991). Older age may also be an independent risk factor for insulin resistance in addition to other factors such as body composition. For example, in a large American cohort study, insulin resistance was increased in those aged over 60 compared to those below 60, even after adjusting for confounding variables such as cardiorespiratory fitness and body composition (Shimokata et al. 1991). Insulin resistance is also linked to the development of other non-communicable diseases including hypertension, hyperlipidaemia and atherosclerosis (Reaven 1988). Insulin

resistance and hypertension may be linked in their pathogenesis; higher circulating levels of insulin may lead to increased renal sodium reabsorption, and sympathetic nervous system activation, whilst at the same hypertension may impair the peripheral microcirculation and cause insulin resistance (Salvetti et al. 1993). Insulin resistance may also lead to hyperlipidaemia through a reduction in the insulin mediated inhibition of lipolysis in adipocytes, leading to increased circulating free fatty acids and very low density lipoprotein (VLDL) cholesterol (Ormazabal et al. 2018). Obesity and in particular abdominal obesity appear to be key to the pathogenesis of insulin resistance, with various potential molecular mechanisms, including chronic low-grade inflammation from visceral adipocytes (Frasca et al. 2017).

In addition to changes in adipose distribution, ageing is associated with the loss of lean muscle mass; a condition known as sarcopenia (Dodds et al. 2017). Although a true consensus on the clinical cut-off's for sarcopenia is still to be reached, with much debate in this area (Beaudart et al. 2015; Baumgartner et al. 1998; Janssen et al. 2002; Studenski et al. 2014; Cruz-Jentoft et al. 2010), sarcopenia is widely accepted to be an age-related decline in both skeletal muscle mass and

function. As such, the most recent guidelines for its assessment and diagnosis recommend measurements of both muscle mass and function (Cruz-Jentoft et al. 2019) (Chapter 1). Although, aspects of body composition including muscle mass can be relatively easily estimated through anthropometric measures such as skinfold thicknesses (Durnin and Womersley 1974), imaging techniques such as dual energy X-ray absorptiometry (DXA) can quantify these to a greater degree of accuracy (Clasey et al. 1999; Duren et al. 2008). As discussed in Chapter 1, DXA, although not the gold standard imaging modality for measuring body composition (an honor held by MRI (Ross et al. 2006)), can give rapid, reliable, and reproducible measurements of body composition (Cheung et al. 2016). DXA, whilst being far cheaper and less technically demanding to utilize than MRI and hence widely used both for both research purposes and clinically (Bruyère et al. 2016), has several limitations intrinsic to its method of measurement. DXA, as suggested by its name, uses X-rays of two different energies and measures the attenuation of these two X-rays as they pass through tissue, which are dependent on both the thickness of the tissue and the attenuation coefficient of the tissue it penetrates (Borga et al. 2018). The X-ray source in a DXA machine remains fixed below the subject, and thus data is produced in 2 dimensions, and

several assumptions are made in order to give measurements of a 3 dimensional subject (Lee and Gallagher 2008). Initially, the DXA machine will divide the subject's body into a 2-component model (bone and soft tissue) and calculates the proportions of each over pixels in which it has detected bone (relative to its calibrator phantom) (Laskey 1996). In pixels in which it detects no bone, and only soft tissue, the algorithm can use the relative attenuation of the two X-rays to divide the tissue into lean mass and fat mass, then using these proportions to extrapolate what the composition of the lean tissue in pixels containing bone should be (Laskey 1996).

As with muscle mass, different techniques can be used for the assessment of muscle function. Maximal handgrip strength is one validated measure of muscle function that is easily measurable with minimal equipment and as such is recommended in the European consensus statement on sarcopenia (Cruz-Jentoft et al. 2019). Previous work has shown that low handgrip strength is strongly associated with mortality in older adults (Bae et al. 2019; Al Snih et al. 2002; Rantanen et al. 2003) and reductions in grip strength over a 2 year period in a large observational study also associated with an increased risk of subsequent mortality (Prasitsiriphon and

Pothisiri 2018). Low handgrip strength has also been associated with both morbidity and mortality after major surgery (Sato et al. 2016; Sultan et al. 2012; Sato et al. 2018).

In addition to both being age-associated, sarcopenia and insulin resistance may also have some mechanistic links in common, both potentially mediated through the sub-clinical chronic inflammatory state caused through obesity, whereby macrophage infiltration may lead to both muscle atrophy and simultaneously lead to insulin resistance (Cleasby et al. 2016). Current management strategies for intervening in patients with insulin resistance are based on lifestyle modification, through both dietary and exercise interventions and both may be effective in older adults with good adherence (Ryan 2000). For example, a small RCT which recruited obese older adults randomised subjects to 6 months of a calorie controlled diet or 6 months of the same diet with the addition of a combined aerobic and resistance exercise training programme and led to both groups making significant improvements in insulin sensitivity (Shah et al. 2009). Similarly, another RCT demonstrated that 6 months of either aerobic or combined aerobic and resistance exercise training can lead to improvements in insulin sensitivity, without a dietary

intervention (Davidson et al. 2009). However if these are not strictly adhered to they may be ineffective as shown by the work of McAuley, in which only an exercise group randomised to a strict intensive programme made improvements in insulin sensitivity, whilst those on modest programmes made no improvements (McAuley et al. 2002). Reasons for non-adherence to exercise interventions were discussed in detail in section 1.3, with reasons for non-compliance to dietary interventions reported as including a lack of subject understanding of the importance of the intervention (Mcnaughton and Shucksmith 2014) and a lack of intrinsic motivation to make a dietary alteration (Ebrahim et al. 2014). It has however been shown that short-bout, less time consuming interventions may bring greater adherence (Hemmingsson et al. 2001).

As discussed in section 1.6.1, one fairly well researched exercise training modality that offers time efficiency is HIIT. In relation to the endpoints assessed in this experimental chapter, two previous studies have examined the effect of HIIT training protocols in older adults on both insulin resistance and body composition with both reporting positive outcomes for both parameters (Robinson et al. 2017; Søgaard et al. 2018).

However the protocol used by Robinson *et al.,* was fairly onerous with participants training 5 times each week for 12 weeks (Robinson et al. 2017). The Søgaard protcol was very similar to that used in the studies in this thesis, comprising 6 weeks of training, with participants performing 5 x 1 minute high intensity intervals interspersed with 90s rest, 3 days a week. However the Søgaard study only recruited participants with a BMI >27, with the final cohort mean BMI of >30 (Søgaard et al. 2018). In addition, neither the Robinson study or the Søgaard study used a non-exercising control; Robinson *et al.* randomised participants to either HIIT, RET or a combination of the two modalities, whilst Søgaard *et al*. reported a cohort study without any control (Søgaard et al. 2018; Robinson et al. 2017). Therefore, an evidence gap as to the beneficial effects of a time efficient, feasible HIIT protocol in normal weight older adults still exists.

As discussed in section 1.6.2, IHG offers a potential intervention for lowering BP (Kelley and Kelley 2010), and is an intervention which may be easily deliverable to an older population, however as yet its effect on any aspect of body composition or insulin resistance has not been quantified in a clinical study. Given the postulated links between the aetiology

of hypertension and insulin resistance (Zhou et al. 2014) potentially via chronic inflammation, obesity and sedentary behaviour, it may be that an intervention able to reduce BP is also able to improve aspects of insulin sensitivity. Further, although several studies of IHG (in mainly younger adults) have been conducted with differing secondary endpoints beyond BP (e.g., arterial elasticity (Pagonas et al. 2017), heart rate variability (Farah et al. 2018) or cardiorespiratory fitness (Blackwell et al. 2017)), none report reassessment of handgrip strength after the intervention.

Finally, as introduced in section 1.6.3, an emerging body of work is investigating the effect of repeated RIPC on both athletic performance (Caru et al. 2019) and markers of cardiorespiratory health (Epps et al. 2016). However, to date no study has evaluated any possible effect of RIPC on body composition or insulin resistance. There is limited evidence from small-scale studies that RIPC may improve endothelial function and a possibly reduce blood pressure (Epps et al. 2016), both of which may be implicated in the pathogenesis of insulin resistance (Muniyappa and Sowers 2013; Zhou et al. 2014) given the clear relationship between insulin and vasodilation (Muniyappa et al. 2007; Baron et al. 1995) and

the aforementioned potential link between blood pressure and insulin resistance.

Therefore, the work presented in this chapter aimed to assess the effect of 3 different time-efficient 6-week health interventions on insulin resistance, body composition and handgrip strength in healthy older adults.

## 6.3 Methods

As described in detail in Chapter 4 (section 4.2) ethical approval was obtained to recruit adults aged 65-85 years to participate in a trial of 6-weeks fully supervised HIIT, RIPC, IHG or non-intervention control (ethical approval number A12092016) by local advertising. The screening process and exclusion criteria for the study are listed in Chapter 4 (sections 4.4 and 4.6).

### 6.3.1 Measurements

At baseline, participants had a DXA scan (Lunar Prodigy II, GE Medical Systems, Buckinghamshire, UK) followed by a frequently sampled oral glucose tolerance test (OGTT) with 75g dextrose (World Health Organisation 2006), after

completing a 12-hour overnight fast. Blood samples from the OGTT were analyzed at the point of collection for blood glucose concentration using a near-patient glucose analyzer (YSI Life Sciences, Ohio, USA), and plasma then frozen and stored for later measurement of insulin concentration by enzyme linked immunosorbent assay (ELISA). Glucose and insulin data were used to calculate area under the concentration curves, HOMA-IR (Matthews et al. 1985), the Matsuda index of insulin sensitivity (Matsuda and DeFronzo 1999) and the Cederholm index of insulin sensitivity (Cederholm and Wibell 1990).

DXA images were automatically analysed by the integrated software package to provide automated data for whole-body body lean mass (LM), body fat percentage (FM%) and lean leg mass (LLM).

Handgrip strength was measured according to the American Society of Hand Therapists clinical assessment recommendations, with the participant seated, the shoulder adducted and neutrally rotated and the elbow flexed to 90 degrees (Fess 1992). Each participant was given three attempts to measure a maximal voluntary contraction (MVC) for each hand using a dynamometer (Camry EH101,

Zhongshan Camry Electronic Co. Ltd, Guangdong, China), with 1-minute rest between attempt at MVC. The MVC was recorded as the highest reading obtained for each hand (Fess 1992).

The protocols for the OGTT, DXA scans, insulin ELISAs and subsequent derivation of indices of insulin sensitivity are described in detail in Chapter 4 (sections 4.9 and 4.11).

### 6.3.2 Randomisation

After their baseline measurements, participants were randomised to either HIIT, RIPC, IHG or a non-intervention control group using a computerised randomisation system (sealedenvelope.com), using random permuted blocks of size 4 and 8 to ensure allocation concealment.

### 6.3.3 Interventions

Subjects randomised to either HIIT, RIPC or IHG trained 3 times per week at the University of Nottingham, Royal Derby Hospital Centre. Each intervention is described in detail in Chapter 4 (sections 4.19.1, 4.19.2 and 4.19.3). All participants, irrespective of group assignment were asked to

maintain their normal activities of daily living and dietary habits throughout the study period.

### 6.3.4 Re-assessment

Participants repeated the pre-intervention study measurements 2 to 5 days after their last intervention session.

### 6.3.5 Statistical analysis

All data is presented as n (%) or mean (SD) as appropriate. Data were tested for between group differences at baseline by one-way ANOVA. Differences in change from baseline between groups was analysed using 2-way ANOVA with Sidak's multiple comparisons post hoc testing. All calculations on empirical data were performed using Prism Graphpad Version 7.04 (Graphpad Software Inc., San Diego, California, USA).

## 6.4 Results

### 6.4.1 Participant characteristics and study adherence

Forty-eight participants with a mean age of 71(4) years (46% male) were recruited. Participant characteristics and adherence to the interventions are described in detail in Chapter 5 (section 5.4.1 and 5.4.2). Three participants did not perform a post-intervention OGTT (one participant in the IHG group due to ill-health, one in the control group who withdrew consent and one in the HIIT group due to repeated failure of intravenous cannulae, leading to OGTT termination). DXA scans were not performed on 4 participants (one participant in the control group, one participant in the RIPC group and two participants in the IHG group) due to them having received a dose of ionising radiation in the preceding year (for diagnostic medical imaging in all cases) greater than that allowed in our ethical approval. One participant in the control group did not have a post intervention DXA scan after withdrawing consent (the same participant who withdrew consent for OGTT).

### 6.4.2 Glucose and insulin data

#### *6.4.2.1 Glucose concentration curves*

The mean glucose concentration curves for each group, before and after their respective intervention are displayed in Figure 41.

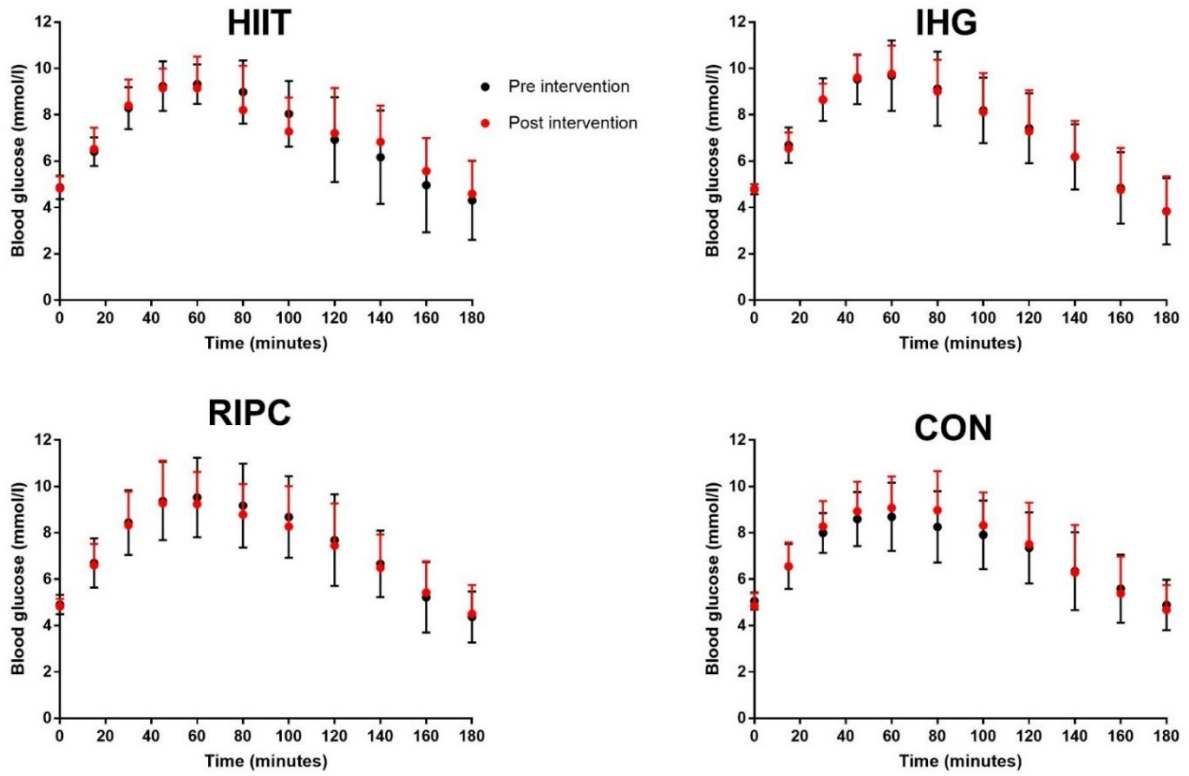


*Figure 41: Blood glucose concentration curves before (in black) and after (in red) a 6-week intervention or control period.*

*(HIIT, high intensity interval training n=12; IHG, isometric handgrip training n=10; RIPC, remote ischaemic preconditioning n= 12 or CON, control n= 11) Data are presented as mean (SD).*

There was no difference in the glucose AUC between groups at baseline (*P*=0.84), nor were there any changes in glucose AUC in any of the groups after the intervention period (Table 15Table 9). There was no significant main effect of time (*P*=0.95) and no group x time interaction (*P*=0.96).

*Table 15: Glucose AUC before (pre) and after (post) a 6-week intervention period (mmol/l/180min)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 1305(218) | 1316(209) | +11(100) |
| **IHG** | 1332(142) | 1324(150) | -8(118) |
| **RIPC** | 1364(193) | 1333(112) | -31(145) |
| **CON** | 1306(140) | 1325(204) | +19(104) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *6.4.2.2 Insulin concentration curves*

The mean insulin concentration curves for each group, before and after their respective intervention are displayed in Figure 42.

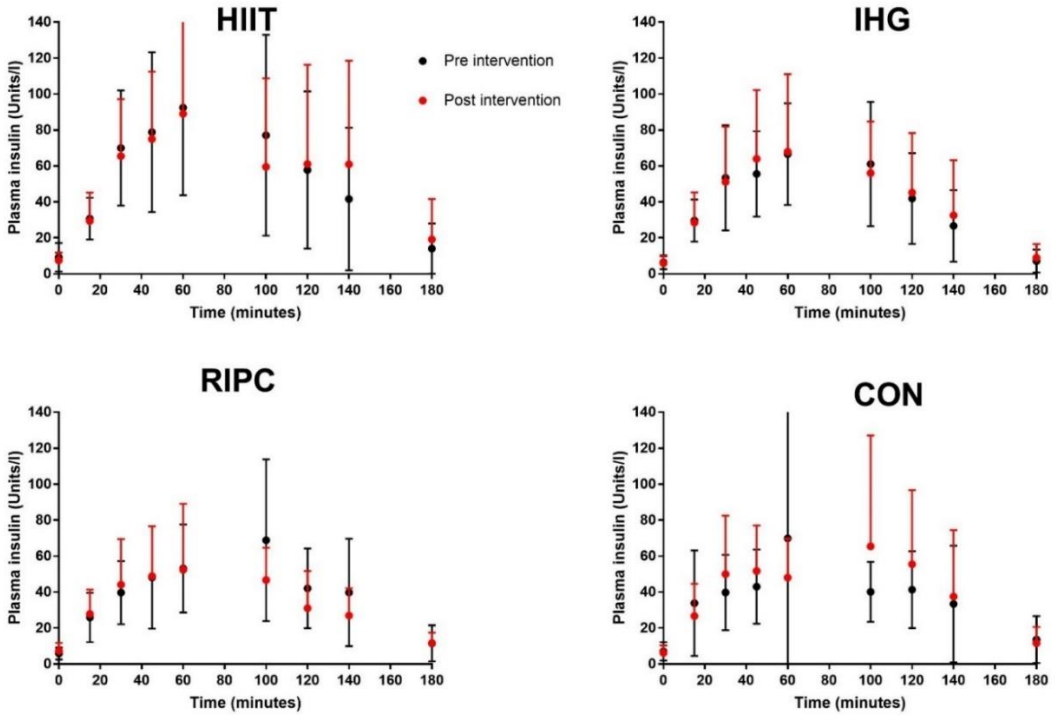


*Figure 42 Plasma insulin concentration curves before and after a 6-week intervention or control period*

*(HIIT, high intensity interval training n=12; IHG, isometric handgrip training n=10; RIPC, remote ischaemic preconditioning n= 12 or CON, control n= 11) Data are presented as mean (SD).*

As was seen for glucose AUC values, there was no difference in the insulin AUC between groups at baseline (*P*=0.22), nor were there any significant changes in insulin AUC in any of the groups after the intervention period (Table 16). There was no significant main effect of time (*P*=0.95) and no group x time interaction (*P*=0.92).

*Table 16 Insulin AUC before and after intervention (mUnits/ml/180min)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 10294 (5572) | 10265 (6694) | -29.08 (4869) |
| **IHG** | 7513 (3124) | 7739 (3277) | +225.9 (1284) |
| **RIPC** | 7448 (3417) | 6363 (2321) | -1085 (2555) |
| **CON** | 6981 (3915) | 7644 (4314) | +662.6 (3519) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

## 6.4.3 Indices of insulin sensitivity

### *6.4.3.1 HOMA-IR*

There was no significant difference in baseline HOMA-IR between groups (*P*=0.40), nor were there significant changes in HOMA-IR in any of the groups after the intervention period

(Table 17). There was also no significant main effect of time (*P*=0.32) and no group x time interaction (*P*=0.19).

*Table 17 HOMA-IR before and after intervention*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 2.0(1.7) | 1.6(0.9) | -0.4(1.0) |
| **IHG** | 1.4(0.80) | 1.2(0.8) | -0.1(0.6) |
| **RIPC** | 1.3(0.8) | 1.6(1.0) | +0.3(0.8) |
| **CON** | 1.6(1.3) | 1.4(1.0) | -0.2(0.7) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *6.4.3.2 Matsuda index*

There was no significant difference in baseline Matsuda index between groups (*P*=0.59), nor were there any significant changes in the Matsuda index in any of the groups after the intervention period (Table 18). There was no significant main effect of time (*P*=0.58) and no group x time interaction (*P*=0.70).

*Table 18 Matsuda index before and after intervention*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 6.8(5.7) | 7.1(5.0) | +0.2(2.9) |
| **IHG** | 8.0(4.2) | 8.0(3.6) | 0.0(1.9) |
| **RIPC** | 11.0(11.1) | 9.9(10.6) | -1.1(5.2) |
| **CON** | 8.7(5.6) | 11.1(9.7) | +2.4(6.2) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *6.4.3.3 Cederholm index*

As with the Matsuda index, there was no significant difference in baseline Cederholm index between groups (*P*=0.72), nor were there any significant changes in the Cederholm index in any of the groups after the intervention period (Table 19Table 20). There was no significant main effect of time (*P*=0.35) and no group x time interaction (*P*=0.63).

*Table 19 Cederholm index before and after intervention*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 48.2(13.0) | 48.3(14.2) | +0.2(5.8) |
| **IHG** | 48.8(12.0) | 49.1(8.6) | +0.2(9.4) |
| **RIPC** | 60.4(51.3) | 65.0(53.1) | +4.6(9.0) |

| | | | |
|---|---|---|---|
| **CON** | 53.0(13.1) | 53.5(18.9) | +0.5(13.2) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

## 6.4.4 Body composition

### *6.4.4.1 Body Fat Percentage*

Although there was no significant difference in the body fat percentage between the groups at baseline, there was a statistical trend for the HIIT group to have a higher body fat percentage than the other three groups (*P*=0.054). There were no significant changes in body fat percentage in any of the groups after the intervention period (Table 20). There was no significant main effect of time (*P*=0.09) and no group x time interaction (*P*=0.45).

*Table 20 Body fat percentage before and after intervention (%)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 38.3(5.6) | 37.7(5.4) | -0.6(0.9) |
| **IHG** | 34.5(6.0) | 34.5(4.9) | -0.1(1.4) |
| **RIPC** | 35.8(7.3) | 35.4(7.1) | -0.4(0.7) |
| **CON** | 30.4(7.4) | 30.4(7.1) | 0.0(0.9) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *6.4.4.2 Total lean mass*

As with the body fat percentage, there was a statistical trend for the control and HIIT groups to have higher total lean mass compared to the IHG and RIPC groups (*P*=0.10). There were no significant changes in total lean mass in any of the groups after the intervention (Table 21). There was no significant main effect of time (*P*=0.56) and no group x time interaction (*P*=0.99).

*Table 21 Total lean mass before and after intervention (Kg)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 47.6(11.5) | 47.8(11.4) | +0.1(0.8) |
| **IHG** | 44.9(9.5) | 45.0(9.4) | +0.1(1.0) |
| **RIPC** | 46.2(11.2) | 46.2(11.3) | +0.1(0.8) |
| **CON** | 49.9(5.1) | 49.9(4.7) | 0.0(1.1) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *6.4.4.3 Total fat mass*

The HIIT group had significantly higher total fat mass than the other groups at baseline (*P*=0.0495). There was a significant main effect of time (*P*=0.04) but no group x time interaction (*P*=0.35).

*Table 22 Total fat mass before and after intervention (Kg)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 29.0(5.1) | 28.4(5.1) | -0.6(0.9) |
| **IHG** | 23.6(5.3) | 23.6(4.7) | 0.0(0.9) |
| **RIPC** | 25.5(6.5) | 25.1(6.7) | -0.3(0.6) |
| **CON** | 22.2(6.7) | 22.2(6.3) | -0.1(0.8) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### 6.4.5 Handgrip strength

#### *6.4.5.1 Dominant hand*

There was no significant difference in baseline handgrip strength in either the dominant (*P*=0.55) or the non-dominant (*P*=0.43) hand between groups. There were no significant changes in dominant hand grip strength in any of the groups

(Table 23) after the intervention, with no main effect of time (*P*=0.46) and no group x time interaction (*P*=0.40).

*Table 23 Dominant hand grip strength before and after intervention (Kg)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 31.5(7.5) | 31.4(7.8) | 0.0(3.0) |
| **IHG** | 29.6(5.6) | 28.8(5.8) | -0.8(3.5) |
| **RIPC** | 32.3(9.5) | 33.4(9.8) | +1.1(1.9) |
| **CON** | 34.5(8.8) | 35.6(9.9) | +1.1(3.7) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### *6.4.5.2 Non-dominant hand*

As with the dominant hand, there were no significant changes in non-dominant hand grip strength in any of the groups after the intervention period (Table 24). There was also no main effect of time (*P*>0.99) and no group x time interaction (*P*=0.80).

*Table 24 Non-dominant hand grip strength before and after intervention (Kg)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 30.7(8.6) | 31.0(9.2) | +0.3(3.3) |
| **IHG** | 27.8(4.8) | 28.3(8.0) | +0.4(3.5) |
| **RIPC** | 32.8(9.9) | 32.1(10.6) | -0.8(3.0) |
| **CON** | 33.1(9.3) | 33.1(9.0) | 0.0(3.0) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

## 6.5 Discussion

This study has demonstrated that 6-weeks of either HIIT, IHG or RIPC, using the protocols investigated in this study, have no impact on any of our used markers of either insulin resistance or body composition. In addition, these interventions had no effect on dominant or non-dominant handgrip strength.

The lack of effect of our HIIT protocol on markers of insulin sensitivity is in contrast to several other studies which have demonstrated positive effects of HIIT on insulin sensitivity both young (Richards et al. 2010), middle aged (Whyte et al. 2010) and older adults (Søgaard et al. 2018; Robinson et al. 2017). Postulated reasons for HIIT induced improvements in insulin sensitivity include HIIT rapidly depleting muscle glycogen

stores (Gibala et al. 2009) which may lead to greater GLUT4 mediated cellular glucose uptake and hence improved insulin sensitivity (Metcalfe et al. 2012). Alternatively, others have suggested that increased skeletal muscle mitochondrial content and function may have a role (Little et al. 2011). However, in our study an improvement in insulin sensitivity was not observed. The reason for this discrepancy may be due to differences in participant demographics, differences in HIIT protocols and/ or study design, or due to factors not controlled in this study such as dietary intake. For example, the participants in the HIIT group of our study were on average 8 years older than that in the study by Søgaard and colleagues with a mean BMI nearly 5kg/$m^2$ lower. Given the known links between adiposity and insulin resistance, it may have been easier for Søgaard and colleagues to demonstrate a significant improvement in insulin sensitivity in their obese subjects. Whilst the baseline demographics of the participants in the study by Robinson and colleagues were much closer to those of our participants their HIIT regime employed a much greater training frequency (training 5 days a week) on a combination of both cycle ergometer and treadmills, with much longer (4-minute) working intervals). Although previous research, albeit in younger individuals, has demonstrated that a higher volume of HIIT, both in terms of session frequency and

working intervals may not be more favourable for improving insulin sensitivity (Jelleyman et al. 2015), however the vast disparity in protocol between our protocol and that employed by Robinson et al., may explain the conflicting results. It must however be considered, that a high training volume is not necessarily a requirement to improve insulin sensitivity via HIIT as the work of Babraj *et al* has shown that in as few as 6 sessions over 2 weeks, insulin sensitivity can be improved (Babraj et al. 2009). This was again in younger individuals and was also at traditional "Wingate", all-out intensities, a feat that is likely not manageable for the majority of sedentary older adults (Hardcastle et al. 2014).

Whilst the protocol of Søgaard and colleagues may have been similar to ours (5 x 60 seconds training intervals 3 x per week on a cycle ergometer at a similar intensity to that employed in our study), the overweight profile of their participants may have made improvements in a metabolic facet easier to achieve. Being overweight or obese is linked to the development of insulin resistance and type 2 diabetes mellitus (Dandona et al. 2004), with the participants in the Søgaard study having baseline glucose AUCs during an OGTT ~ 25% greater than the participants in our study, despite them being

almost a decade younger than our cohort. As such it may have been that our cohort with "normal" insulin sensitivity could not be improved upon further with our HIIT intervention. The relationship between exercise induced improvements in insulin sensitivity and baseline insulin sensitivity has previously been demonstrated in the large meta-analysis by *Jelleyman et al.* where on meta-regression baseline values of insulin sensitivity, fasting insulin and fasting glucose was the only significant predictor of change in insulin sensitivity (Jelleyman et al. 2015).

In relation to study design, although our study and both the studies by Robinson and Søgaard made their post intervention assessments >48h from the last intervention session to prevent any acute carry-over effect of the last session (Lithgow and Leggate 2018), the Robinson and Søgaard studies both employed the gold-standard euglycaemic hyperinsulinaemic clamp to measure insulin sensitivity (DeFronzo et al. 1979). This technique, which we deemed too invasive and burdensome for our participants given that insulin sensitivity was only a secondary endpoint, may have had more sensitivity to detect change. For example, in a recent study comparing the Matsuda index to the euglycaemic hyperinsulinaemic

clamp, the area under the ROC curve for classifying insulin resistance was 0.897 (Lorenzo et al. 2015) and in its original validation paper, the correlation coefficient between the Matsuda index and the euglycaemic hyperinsulinaemic clamp was 0.73 (Matsuda and DeFronzo 1999). Although this suggests there is good agreement between the two methods, this is not perfect and thus may have been a source of error in the current study.

Finally, the study by Søgaard and colleagues was a cohort study with no control group and as such any change cannot be compared to a randomised controlled trial.

That our HIIT intervention also failed to elicit favourable changes in body composition is also in contrast to a number of other HIIT studies, including a systematic review of 13 studies conducted in younger overweight adults (Wewege et al. 2017) and the study by Søgaard discussed in the section above. (Søgaard et al. 2018). As described above, the Søgaard study included participants who were significantly younger than those in our study but with a significantly higher BMI. Søgaard *et al.,* demonstrated numerical reductions in body fat percentage and total fat mass that were similar to those we

report, however as their study lacked a control group and had 22 participants in the HIIT group, they likely had greater statistical power to detect a significant difference. Of note, another recent HIIT study in participants of a similar age range to ours but exclusively recruiting obese participants, with a 12-week training protocol, also failed to demonstrate improvements in body fat percentage, fat mass or lean mass (Buckinx et al. 2019). A previous study in our own institution which recruited normal weight older adults also failed to show any body composition benefit (Boereboom et al. 2016b). One potential explanation for this, especially given that many of the positive studies involve younger participants, is the concept of 'adaptive blunting' in older adults. Although this concept in relation to HIIT has not yet been explored, one recent study our research group explored adaptation to resistance exercise training (RET) as a function of age and reported that reductions in both whole-body fat and abdominal fat were negatively associated with advancing age (Phillips et al. 2017b).

It was also somewhat surprising to us, given the available literature, that our HIIT protocol did not elicit improvements in total body lean mass or our healthy older adults. Again, as with the adipose-based changes in body composition discussed

above, studies in younger adults (Wewege et al. 2017) and in those using more intense (Sculthorpe et al. 2017) or longer (Sperlich et al. 2017) HIIT protocols have shown muscle hypertrophy in response to HIIT. HIIT studies in older adults are however more contentious when measuring hypertrophy (Robinson et al. 2017; Søgaard et al. 2018) and previous work from our research group using the exact protocol used in this study, albeit for four and not six weeks, supports the view that our 'reduced-intensity' (vs. Wingate, for example) HIIT is not a particularly strong hypertrophic stimulus in older adults(Boereboom et al. 2016b).

The concept of adaptive blunting with advancing age may go some way to explain this, as in the Phillips *et al.,* RET study outlined above, blunted hypertrophic adaptations to RET with advancing age were observed, despite all individuals performing the same relative work in fully-supervised conditions (Phillips et al. 2017b). One underlying mechanism for this adaptive blunting in relation to muscle hypertrophy maybe the now widely accepted concept of anabolic resistance to both exercise (Kumar et al. 2009) and nutrition (Atherton and Smith 2012) that is associated with advancing age. Anabolic resistance describes the reduced magnitude of

muscle protein synthetic responses to key anabolic stimuli (nutrition and exercise) that presents with advancing age (Kumar et al. 2009), inactivity (Morton et al. 2018) and a variety of muscle wasting conditions (e.g., diabetes (Pereira et al. 2008) and cancer (Horstman and Sheffield-Moore 2015). Although, there is not yet a substantial enough body of work to determine if anabolic, or indeed overall adaptive blunting in response to HIIT is a feature of advancing age

Finally, the lack of body composition changes in the HIIT group of our study may be secondary to factors that were uncontrolled in this study such as dietary intake and/ or habitual physical activity. Although participants were instructed to maintain their pre-study habitual behaviours in relation to both of these factors, there is a possibility that HIIT may have led to an increase in appetite and subsequent energy intake which may have diminished body fat reductions (King et al. 2012). Similarly, after HIIT participants may have unconsciously reduced their habitual physical activity behaviours due to physical fatigue and or psychological aspects (Schutz et al. 2014; Drenowatz et al. 2015). Habitual physical activity (Swift et al. 2012) and energy intake (Blundell et al. 2015) are both known to have key roles in adiposes

losses, as are habitual physical activity and protein intake with regard to muscle mass (Tieland et al. 2012)

The single hand IHG protocol investigated in this study, although demonstrating clinically relevant effects on resting BP (reported in Chapter 5) appears to have no demonstrable effect on either insulin resistance or measures of body composition. To our knowledge this is the first study to investigate this. The pathogenesis of both essential hypertension and that of insulin resistance have been postulated to be linked (Salvetti et al. 1993; Zhou et al. 2014) via obesity and chronic inflammation and have a tendency to co-exist in individuals (Saad et al. 2004). In support of this, some anti-hypertensive medications, such as thiazolidinediones have been reported to act to both reduce BP and insulin resistance (Raji et al. 2003), however it would appear that the mechanisms by which IHG may work to reduce BP is different to this, and does not have a role to play in improving insulin sensitivity.

As with our IHG intervention, it would appear that our single, upper limb, 3x3 minute RIPC protocol has no effect on either

insulin resistance or body composition and to our knowledge this is the first study to investigate this.

None of the interventions in this study produced any change in either dominant or non-dominant hand grip strength. The reason why the IHG protocol failed to lead to any locally driven increases in MVC in the dominant training hand may be that the contraction intensity (30% MVC) of training was insufficient to elicit improvements in MVC. This intensity is much lower than that used in protocols designed explicitly to increase handgrip strength (often used in rehabilitation (Eng 2004) or hand surgery (Matsumura et al. 2008) settings for example), where typical protocols employ shorter (2-3 seconds), more intense (50-100% MVC) contractions (Kim and Yim 2017; Olafsdottir et al. 2008).

As all three protocols failed to cause an increase in lean mass, it is unsurprising that no change in handgrip strength resulted from them either, as there is a fairly large body of work showing a relationship between handgrip strength and whole body lean mass (Marin et al. 2010; Sánchez Torralvo et al. 2018). However this is not an absolute association, especially in relation to intervention induced changes in lean mass, with

previous work showing that exercise protocols which lead to gains in lower body muscle mass or function do not always lead to improvements in handgrip strength (Yaginuma et al. 2017).

### 6.5.1 Limitations

As already discussed above, the data presented in this chapter was limited by the lack of control of dietary intake and habitual physical activity by our participants throughout the intervention period, instead relying on them to maintain their usual habits as requested. It was also limited by our choice not to assess insulin sensitivity using the euglycaemic-hyperinsulinaemic clamp technique. That our loss of participants for follow-up approached nearly 10% for the OGTT and that a number of our participants were not able to have a DXA scan due to radiation exposure are also limitations to this work and may have limited the statistical power of certain data sets. This study was also limited by its 4-arm trial design which, will have reduced its power to demonstrate the effectiveness of each of the interventions in their own right.

## 6.6 Chapter conclusions

The work described in this chapter has demonstrated that 6-weeks of either HIIT, IHG or RIPC using the protocols described within this thesis, have no demonstrable effect on measures of either insulin sensitivity or body composition, or on a widely utilised measure of physical function.

# 7 The effects of 6 weeks of supervised high-intensity interval training, isometric handgrip training or remote ischaemic preconditioning on macro- and microvascular function

## 7.1 Chapter focus

This chapter describes the results of secondary endpoints from the RCT of HIIT, IHG or RIPC versus a non-intervention control in healthy older adults described in Chapter 5. This chapter reports the effect of these interventions on both macro and microvascular function.

## 7.2 Introduction

Impairment of the function of the peripheral vasculature becomes increasingly common with advancing age (Shammas 2007) with both macrovascular and microvascular dysfunction often co-existing (Scioli et al. 2014). Even in the absence of overt, symptomatic peripheral vascular disease, older adults exhibit reductions in both whole-limb and microvascular blood flow and in response to exercise when compared to younger adults (Donato et al. 2006; Durham et al. 2010; Dinenno et al. 2001). In terms of the clinical relevance of these deficits in vascular function there is a possibility that they may form part of the pathogenesis of insulin resistance (Julius et al. 1992) and may potentially also be implicated in the pathogenesis of hypertension (Dharmashankar and Widlansky 2010). The potential role of vascular dysfunction in relation to insulin resistance (which is discussed in more detail in Chapter 6) may

centre around the impaired delivery of insulin to peripheral tissues (Cersosimo and DeFronzo 2006; Lindner 2018). Similarly, the potential complex role of vascular dysfunction in the development of hypertension (discussed further in Chapter 1) is potentially related to endothelial function. However, there remains a point of contention as to whether endothelial dysfunction causes or is a result of hypertension. Laboratory studies have demonstrated that acutely causing pharmacological endothelial dysfunction, by inhibiting nitric oxide synthase, leads to increased blood pressure (Sander et al. 1999) and a large observational cohort study demonstrated that endothelial dysfunction demonstrated in normotensive postmenopausal women may predict the later onset of hypertension (Rossi et al. 2004). However this finding is not uniform, as another larger and more recent observational study found that endothelial dysfunction was not a significant predictor of subsequently developing hypertension after adjusting for covariates (Shimbo et al. 2010). Others have suggested that there is potentially a bidirectional relationship between hypertension and endothelial dysfunction (Dharmashankar and Widlansky 2010). One additional clinical implication of reduced vascular function that has been suggested is that it is a potential mechanism contributing to age-related anabolic blunting and subsequent sarcopenia

(discussed in more detail in chapter 6) (Zempo et al. 2017). However, despite this suggestion being supported by a number of studies showing both an association between reduced macro and microvascular blood flow and reduced protein synthesis in older adults in response to either an infusion of amino acids (Rasmussen et al. 2006) or acute exercise (Durham et al. 2010), other research, including that by our research group has suggested that neither macro or microvascular blood flow responsiveness is rate-limiting for muscle protein anabolism based on studies of exercise (Phillips et al. 2015), pharmacological (Phillips et al. 2014) and nutritional enhancement (Phillips et al. 2016) of vascular function. Thus, it may be that peripheral vascular dysfunction although implicated in the pathogenesis of sarcopenia, is just one component in its development.

Aside from age-associated reductions in vascular function, habitual exercise is known to have a beneficial effect on vascular function in older age; a systematic review and meta-analysis of 485 participants (210 habitual exercisers and 275 sedentary older adults) from 10 cross-sectional studies demonstrated that habitual exercisers had a mean difference

of +2.1% FMD compared to the sedentary older adults (Campbell et al. 2019).

Demonstrating the ability of exercise training to improve vascular function in only a short period of time, a systematic review of exercise studies with a mean duration of 8 weeks in adolescents by Dias *et al*., demonstrated a mean improvement in FMD of 1.5% (Dias et al. 2015). Similar results have been found in some intervention studies in older adults, with Alkatan *et al.* demonstrating a ~3% rise in FMD after a 12-week moderate continuous aerobic swimming intervention in a group with a mean age of 59 years (Alkatan et al. 2016), however exercise studies in older adults have not been uniformly positive, with a recent systematic review and meta-analysis finding no overall benefit to short term exercise on FMD in older adults (Campbell et al. 2019).

Despite the reported benefit of lifelong exercise and exercise uptake on vascular function, concerns regarding the low adherence to 'traditional', often time-consuming exercise interventions have been outlined a number of times throughout this thesis and are made in detail in chapter 1 (section 1.3). In Chapter 1 the potential benefit of HIIT as an

alternative training mode in terms of its time-efficiency and likely adherence were described. In addition to this, and of direct relevance to the endpoints reported in this chapter, HIIT has also been demonstrated to be more effective than moderate continuous aerobic exercise at improving macrovascular function in obese younger adults (Sawyer et al. 2016) and middle-aged diabetics (Hollekim-Strand et al. 2014), and has also been shown superior to resistance exercise training in obese middle-aged adults (Stensvold et al. 2010). However, studies examining the effect of HIIT on the vascular function of older adults are lacking.

As is the case for HIIT, studies which have attempted to measure the impact of IHG protocols on vascular function have demonstrated improvements have largely consisted of measurement of endothelial function via FMD, but primarily in younger participants (Bentley et al. 2018). There is however some evidence from cohort studies to suggest that IHG may have a positive effect on endothelial function via FMD in older adults (Dobrosielski et al. 2009).

Very few studies have evaluated the effect of RIPC on vascular function, however those which have, have demonstrated

significant improvements in FMD, although these studies have exclusively been carried out in young healthy men (Jones et al. 2014b, 2014a).

Therefore, the aim of this chapter is to assess the effect of 6 weeks of HIIT, IHG or RIPC versus a non-control intervention period on measures of macro- and microvascular function in healthy older adults.

## 7.3 Methods

As described in detail in chapter 4 ethical approval was obtained to recruit adults aged 65-85 years to participate in a trial of 6-weeks fully supervised HIIT, RIPC, IHG or non-intervention control (A12092016) by local advertising. The screening process and exclusion criteria for the study are listed in chapter 4 (sections 4.4 and 4.6).

### 7.3.1 Measurements

#### *7.3.1.1 Measurement of endothelial function*

Endothelial function was assessed using the technique of flow mediated dilation (FMD). Measurement of FMD was carried out in the participant's non-dominant arm (the arm not used in the training protocol of either IHG or RIPC). For a detailed

description of the method and equipment used to measure FMD, see chapter 4 (section 4.15). In brief, FMD was measured using the brachial artery after a 12-hour overnight fast, at the same time of day before and after the intervention period, with the overall aim of FMD to assessing vasodilatory responsiveness following a sudden increase in post-occlusion blood flow and as such shear stress in the artery (Corretti et al. 2002a). The diameter of the brachial artery was measured at rest for 1 minute, before the radial and ulnar arteries in the proximal forearm were occluded using a tourniquet cuff inflated to 250 mmHg for 5 minutes. The cuff was then rapidly deflated and the diameter of the brachial artery measured in real time for the subsequent 4 minutes using an automated software package (Cardiovascular Suite, FMD Studio Version 3.3.0, Quipu, Pisa, Italy) (Figure 43).

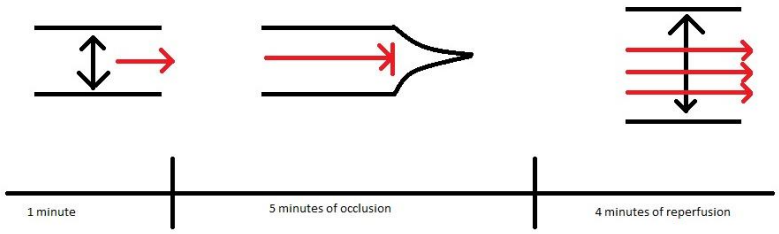


*Figure 43: Schematic of FMD measurement*

*7.3.1.2 Measurement of microvascular blood flow*

Measurements of microvascular blood flow were made using contrast enhanced ultrasound (CEUS) of the vastus lateralis muscle of the participant's dominant leg, after a 12hr overnight fast. For a detailed description of the methods and equipment used to perform CEUS see chapter 4 (section 4.14). In brief, after infusion of Sonovue® to achieve systemic steady state, microbubbles under the ultrasound probe were destroyed by a high mechanical index "flash" and the subsequent reperfusion of the muscle recorded. Two destruction-reperfusion cycles were measured at rest, following which the participant performed 6 leg extensions at 50% 1-repetition maximum. Immediately after the exercise, a further destruction-reperfusion cycle was measured. Cine video clips of each destruction-reperfusion cycle were analyzed as detailed in chapter 4 (section 4.14.3) to produce reperfusion curves for before and after unilateral leg extensions. These curves were then analyzed to produce values for the change in both microvascular blood volume and microvascular flow velocity in response to the exercise as a measure of microvascular responsiveness. Due to the nature of CEUS measurements, resting measures before and after the intervention period could not be directly compared as it is not possible to ensure that the ROI is identical on two separate

time-points even with relevance to anatomical landmarks (e.g., the mid-point of the patella) as changes in the composition of the tissue beds will impact this.

#### *7.3.1.3 Measurement of resting and post-exercise leg blood flow*

Leg blood flow in the common femoral artery of the participant's non-dominant leg was measured using Doppler ultrasound. The protocol for the measurement of leg blood flow is described in detail in chapter 4 (section 7.4.3). In brief, 3 measurements of blood flow in the femoral artery were made after the participant had rested supine for 5 minutes. Participants then performed 6 unilateral leg extensions before another 3 measurements were taken immediately.

### 7.3.2 Randomisation

After their baseline measurements, participants were randomised to either HIIT, RIPC, IHG or a non-intervention control group using a computerised randomisation system (sealedenvelope.com), using random permuted blocks of size 4 and 8 to ensure allocation concealment.

### 7.3.3 Interventions

Subjects randomised to either HIIT, RIPC or IHG trained 3 times per week, in the Medical School at the Royal Derby Hospital. The interventions are described in detail in chapter 4 (sections 4.19.1, 4.19.2 and 4.19.3). Participants allocated to the control group were not given any intervention. All participants were asked to continue their normal activities of daily living and dietary intake throughout the study period.

### 7.3.4 Re-assessment

Participants repeated the study measurements two to five after the intervention period to prevent any acute effects from the last intervention session.

### 7.3.5 Statistical analysis

All data is presented as n (%) or mean (SD) as appropriate. Data were tested for between group differences at baseline by one-way ANOVA. Differences in change from baseline between groups was analysed using 2-way ANOVA with Sidak's multiple comparisons post hoc testing. All calculations on empirical data were performed using Prism Graphpad Version 7.04 (Graphpad Software Inc., San Diego, California, USA).

## 7.4 Results

### 7.4.1 Participant characteristics and study adherence

Forty-eight participants of with a mean age of 71(4) years (46% male) were recruited to the study. Participant characteristics and adherence to the interventions are described in detail in chapter 5 (section 5.4.1).

### 7.4.2 Flow mediated dilation

FMD data was available for 41 (85%) participants (12 HIIT, 9 IHG, 11 RIPC and 9 CON). Reasons for FMD not being available in all participants was technical failure in 5 instances (software malfunction or license expiry) and participants withdrawing from the study in 2 instances. FMD data at baseline and after the intervention period are displayed in Table 25. There was no significant difference in FMD between the groups at baseline ($P$=0.82). There was no change in FMD in any group after the intervention period. There was a trend towards a main effect of time ($P$=0.06) across the groups, but no significant group x time interaction ($P$=0.64).

*Table 25 FMD before and after intervention (%)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 6.5 (2.3) | 8.6 (3.3) | +2.1 (3.8) |
| **IHG** | 6.3 (3.2) | 7.3 (4.5) | +1.0 (5.5) |
| **RIPC** | 5.7 (1.9) | 7.9 (3.4) | +2.0 (4.7) |
| **CON** | 5.7 (2.6) | 5.6 (2.3) | 0.0 (3.0) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control. Data is presented as mean (SD).

## 7.4.3 Leg blood flow

### *7.4.3.1 Resting blood flow*

There was no significant difference in resting leg blood flow between the groups at baseline ($P$=0.93). Resting leg blood flow data at baseline and after intervention are displayed in Table 26. There was no significant change in resting leg blood flow in any group with no main effect of time ($P$=0.32) and no group x time interaction ($P$=0.98).

*Table 26 Resting leg blood flow before and after intervention (ml/min)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 359(144) | 397(126) | +38(139) |
| **IHG** | 356(109) | 370(120) | +13(170) |
| **RIPC** | 387(162) | 403(145) | +15(128) |
| **CON** | 357(108) | 380(113) | +23(174) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### *7.4.3.2 Increase in blood flow in response to exercise*

There was no significant change in the increase leg blood flow in responsiveness to a single set of 6 unilateral leg extensions, in any group after the intervention period (Table 3). There was no main effect of time ($P$=0.96) and no group x time interaction ($P$=0.40).

*Table 27 Increase in leg blood flow in response to 6 unilateral leg extensions, before and after intervention (ml/min)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | +444(349) | +360(193) | -94(439) |
| **IHG** | +346(269) | +429(320) | +83(273) |
| **RIPC** | +393(260) | +477(271) | +84(202) |
| **CON** | +396(293) | +321(161) | -75(289) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### 7.4.4 Muscle microvascular blood flow

Forty-six participants had CEUS performed before and after the intervention period. CEUS assessment failed in one participant (HIIT group) due to cannula failure leading to contrast agent extravasation into the soft tissue of the arm and lack of delivery to the leg. One participant withdrew consent for reassessment (CON group). CEUS reperfusion curves for each group are displayed in *Figure 44*.

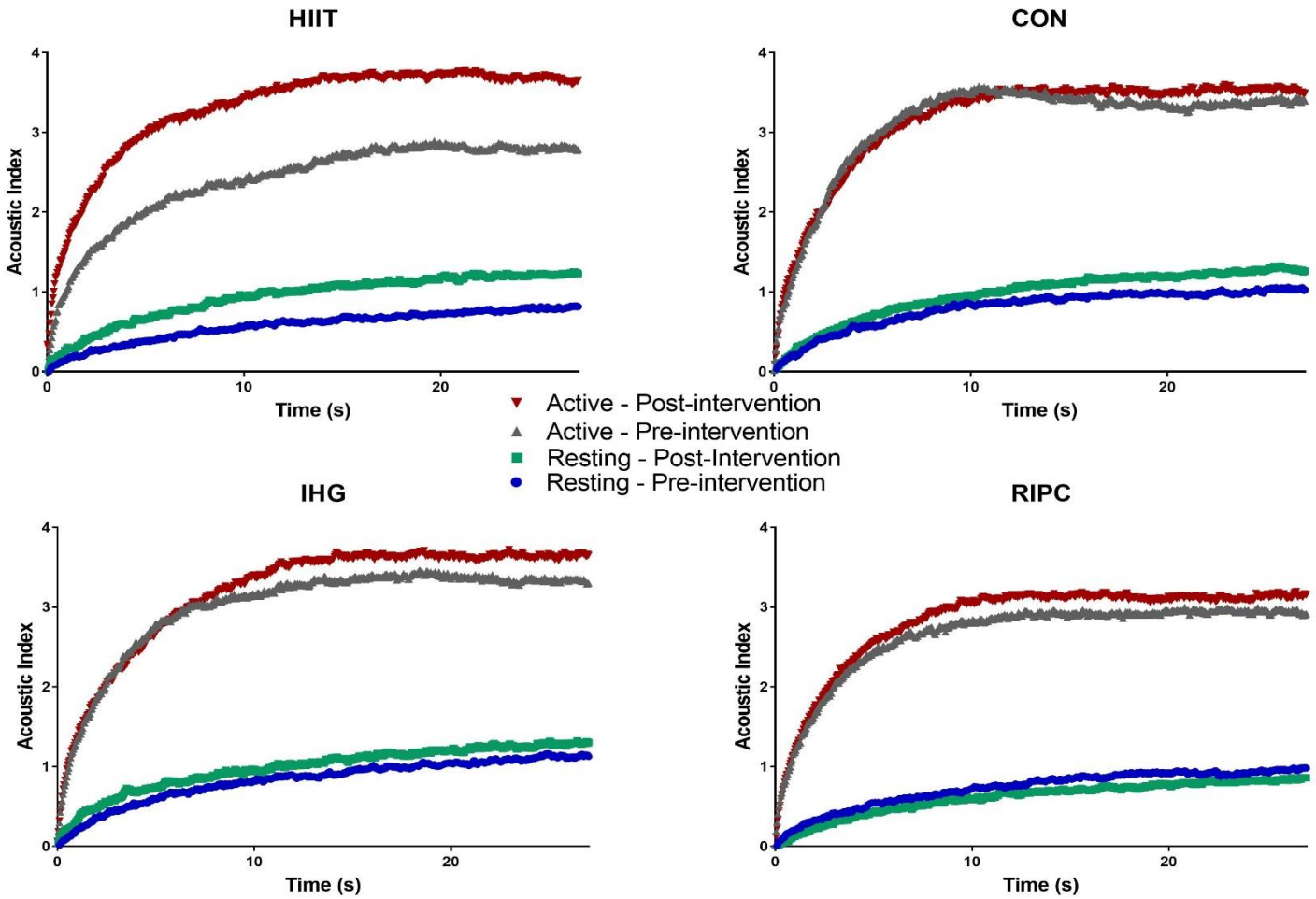


*Figure 44 CEUS reperfusion curves at rest and after 6 leg extensions, before and after a 6-week intervention or control period*

*(HIIT, high intensity interval training n=12; IHG, isometric handgrip training n=11; RIPC, remote ischaemic preconditioning n= 12 or CON, control n= 11).*

### *7.4.4.1 Microvascular blood volume (MBV)*

There was no significant difference in MBV responsiveness to a single set of 6 unilateral leg extensions between the groups at baseline (*P*=0.50). MBV data at baseline and after intervention are displayed in Table 28. There was a main effect of time (*P*=0.02) across the groups but no significant group x time interaction (*P*=0.20).

*Table 28 Change in microvascular blood volume in response to 6 unilateral leg extensions, before and after intervention (acoustic index)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 1.8(0.63) | 2.3(0.8) | +0.5 (0.3) |
| **IHG** | 2.2(0.73) | 2.3(0.8) | 0.1(1.0) |
| **RIPC** | 1.9(0.8) | 2.3(0.6) | 0.4(0.8) |
| **CON** | 2.2(1.2) | 2.2(1.0) | 0.0(0.6) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

### *7.4.4.2 Microvascular flow velocity (MFV)*

There was no significant difference the MFV responsiveness to a single set of 6 unilateral leg extensions between the groups

at baseline (*P*=0.23). MFV data at baseline and after the intervention period are displayed in Table 29. There was no main effect of time (*P*=0.71) or any group x time interaction (*P*=0.39).

*Table 29 Change in microvascular flow velocity in response to 6 unilateral leg extensions, before and after intervention*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **HIIT** | 0.2(0.2) | 0.3(0.2) | +0.1(0.2) |
| **IHG** | 0.20(0.1) | 0.2(0.1) | 0.0(0.1) |
| **RIPC** | 0.3(0.3) | 0.3(0.2) | 0.0(0.2) |
| **CON** | 0.2(0.2) | 0.3(0.4) | +0.1(0.5) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

#### *7.4.4.3 Microvascular blood flow (MBF)*

Reported to be a product of MBV multiplied by MFV (Mitchell et al. 2013) there was no significant difference in MBF responsiveness to a single set of 6 unilateral leg extensions between the groups at baseline (*P*=0.32). MBF data at baseline and after the intervention period are displayed in Table 30.

There was no main effect of time ($P$=0.20) and no group x time interaction ($P$=0.45).

*Table 30 Change in microvascular blood flow in response to 6 unilateral leg extensions, before and after intervention (acoustic index)*

| | Pre | Post | Change |
|---|---|---|---|
| **HIIT** | 0.7(0.4) | 1.2(0.9) | +0.5(0.7) |
| **IHG** | 0.9(0.3) | 0.9(0.4) | 0.0(0.4) |
| **RIPC** | 1.1(0.8) | 1.1(0.9) | -0.1(0.9) |
| **CON** | 1.0(0.8) | 1.4(1.5) | +0.3(1.8) |

Abbreviations: HIIT, high intensity interval training; IHG, isometric hand grip training; RIPC, remote ischaemic preconditioning; CON, control

## 7.5 Discussion

The work presented in this chapter has demonstrated that neither 6 weeks of HIIT, IHG or RIPC using the protocols presented within this thesis can significantly change either microvascular blood volume responsiveness or microvascular flow velocity responsiveness to acute exercise in the *vastus lateralis* muscle of older adults. To our knowledge, this is the first study to evaluate changes in microvascular function in response to HIIT training, in any age group. None of the three interventions presented in this thesis (HIIT, RIPC or IHG)

appear to have any effect on endothelial function in the brachial artery as measured by FMD, nor any effect on resting or post-acute exercise whole leg blood flow.

That this study did not detect any meaningful change in MFV responsiveness in the HIIT group, may be due to the inherent poor signal-to-noise ratio associated with the MFV measurements (Durham et al. 2010) and is highly susceptible to variation with subtle changes in probe angulation with respect to the direction of the muscle fibres and their associated capillaries (Mitchell et al. 2013). The majority of previous studies evaluating microvascular adaptions to exercise training, especially using CEUS, often only report changes in MBV and not MFV (Vincent et al. 2002), whilst some studies report MBV alone (Dawson et al. 2002; Sjøberg et al. 2011). The rationale for calculating and reporting MBF was based on studies investigating the microvascular response to feeding stimuli, in which there appears to be a biphasic response whereby increases in MBV precede MFV (Mitchell et al. 2013), however given this theoretic underpinning it is likely that MBV is key to subsequent physiological effects (i.e., glucose clearance), irrespective of the speed of arrival. It may also be that given the inherent variation in MFV and the

relative contribution of this measure to the calculation of MBF that the study was underpowered to detect a change in MBF.

That we did not find any improvement in MBV following HIIT is more challenging to explain, given previous findings in studies in older adults following RET (Phillips et al. 2015), and it is possible that this is a type 2 error due to a lack of power, or a limitation of the study's 4-arm trial design.

To our knowledge, this is the first study to assess effect of either an IHG or RIPC training protocol using the upper limb on the microvascular function of the lower limb. The lack of any significant change in this study may imply that these protocols have no effect on the microvasculature of a remote site. However, in contrast to this suggestion a previous study utilising upper limb RIPC evaluated healing of lower leg ulcers in patients with diabetes mellitus and showed a significant improvement in wound healing (Shaked et al. 2015), with the authors of this study suggesting that this may be due to increased capillarisation. However, neither capillarisation nor microvascular blood flow was assessed in this study and as such the underlying mechanisms for this observed

improvement in wound healing remains unknown. It may also be that differences in the RIPC protocol between ours and the work of Shaked and colleagues (2015) explain these seemingly divergent responses to RIPC training; while our study employed a 3 x 3 min unilateral protocol, the study by Shaked *et al.* used 5-minute periods of bilateral upper limb ischaemia.

Of note, this study is one of the few to use CEUS to measure muscle microvascular volume and flow responsiveness before and after a chronic exercise intervention. Whilst this has been done in both the upper limb (Russell et al. 2017) and the lower limb (Phillips et al. 2015) in response to resistance exercise training, this is the first to measure the response following chronic HIIT. Previous chronic training studies using CEUS to measure muscle MBV have only compared the change in the microvascular responsiveness to an stimuli (e.g. feeding)(Phillips et al. 2015) due to CEUS assessment of baseline microvascular blood flow parameters over a chronic time-period not yet being validated. The primary concerns regarding this application of CEUS is ensuring the same region of the muscle is studied before and after an intervention period, as even if the probe position was marked based on static anatomical landmarks (i.e., the mid-point of the patella),

changes in the underlying tissue composition (i.e., due to hypertrophy and or adipose losses) would alter the measurement region. Baseline to baseline comparisons have previously been reported in one study, however this study made no comment as to the validity of this comparison and lacked any non-intervention control group to demonstrate repeatability of measurements (Russell et al. 2017). Of note, and promising for future work in this area, the similarity of the both the baseline and post-acute exercise data sets in the control group before and after the intervention period suggests that baseline MBV comparisons may be a valid in future studies.

This study demonstrated no effect of either HIIT, IHG or RIPC on brachial artery endothelial function, as measured by FMD. Previous studies that have measured changes in FMD following HIIT have shown significant benefits, however the majority of these studies have been carried out in younger adults, (Boff et al. 2019; Rakobowchuk et al. 2008; Sawyer et al. 2016), whilst vascular smooth muscle function has previously shown to be negatively correlated with advancing age (Montero et al. 2015). In addition to the large age discrepancy between the age of the studies cited above and our study, there were

significant differences in the HIIT protocols they utilised; the study by Rakobowchuk *et al.* employed 30s "all-out" Wingate style sprints for 6 weeks, the study by Sawyer *et al.* a 10 x 1minute protocol with training intervals set at 90-95% maximum heart rate for 8 weeks and finally the study by Boff *et al.* used a protocol of steadily increasing intensity intervals finishing at 85% maximum heart rate, however interspersed with 4 minute rest periods, again for 8 weeks.

Previous studies showing benefit in older adults have mostly been conducted with patients with significant cardiovascular impairments at baseline such as heart failure (Wisløff et al. 2007) or those who have recently had coronary artery stenting (Munk et al. 2009). Indeed, in the Wisløff heart failure study, baseline FMD values were approximately half (~3.5% (data only presented in graphical form)) of that in our participants (6.5%). The impaired baseline status of these individuals may have facilitated easier improvements to a physiologically "normal" value.

In addition, and likely of significance given that vascular remodelling (Green et al. 2017) and improvements in FMD (Campbell et al. 2019) are often seen with moderate-intensity

aerobic exercise training, the Wisløff study employed HITT sessions that were ~1 hour in duration with 4 minute working intervals. Thus, it may be that our reduced intensity 5x1 minute HIIT protocol provides an insufficient stimulus for improving endothelial function in older adults.

This finding, on the background of the largest systematic review to date of studies comparing improvements in FMD after either HIIT or moderate continuous aerobic exercise suggests that advancing age may be a limiting factor in the ability of HIIT to improve endothelial function. This systematic review included 7 RCTs with 182 participants, with most included studies reporting a mean age between 50-60 years. The analysis in this review demonstrated a mean increase of 4.31% in FMD after HIIT compared to an increase of 2.15% from moderate continuous aerobic exercise (Ramos et al. 2015), with the mean change in our HIIT group only +2.1%. As with almost all HIIT reviews, there is disparity between the protocols used and as outlined above in relation to the Wisløff study (Wisløff et al. 2007) this may explain some of divergent responses observed in response to a single 'type' of exercise training. Similarly, many of the studies included in the systematic review which showed a positive effect of HIIT on

endothelial function (Ramos et al. 2015) had a larger sample size that in our current study, and as such we may have been underpowered to detect such changes.

IHG has been previously demonstrated to improve FMD in both young normotensive adults (Badrov et al. 2016) and older hypertensives (McGowan et al. 2007c). However these studies have so far only shown beneficial changes in FMD in the trained arm (Badrov et al. 2016) and as such may be of limited use to changing whole-body endothelial function. The current study is the first RCT to our knowledge to assess changes in FMD in the contralateral limb following a unilateral IHG training protocol, and in older adults it appears to be ineffective, despite changes in resting systolic blood pressure (reported in chapter 5). The effect of our IHG protocol on the trained arm remains unknown as it was not measured, although this measure may have offered some insight into whether previously observed improvements were likely to have been confined to the training arm only. This suggestion is however already supported by the observation of McGowan *et al.,* who demonstrated changes in the FMD of both arms in subjects performing bilateral IHG protocols, and in the trained arm only in those conducting a unilateral protocol (McGowan et al.

2007c). These results also agree with the findings of a cohort study carried out in adults with a mean age of 81 years, where FMD was only improved in the trained limb (Dobrosielski et al. 2009). That IHG protocols appear to only be effective at changing FMD locally, whilst exerting a systemic effect on systolic blood pressure would imply that the two do not share a common mechanism with local IHG elicited improvements possibly due to local changes in vascular structure or smooth muscle function (McGowan et al. 2007c).

The effect of RIPC on FMD has previously been evaluated in one study using healthy 20-year old men, which demonstrated a unilateral, 4 x 5-minute, daily ischaemic preconditioning protocol conducted for 7 days was effective at improving FMD in both the trained and untrained arms (Jones et al. 2014a). Reasons for the discrepancy between our results and the results of Jones *et al.* may be due to differences in the RIPC protocols, with our protocol potentially containing insufficient ischaemic time per session or number of sessions, or may be due to an inability of RIPC to work in older adults potentially due to a smaller volume of muscle being rendered ischaemic .

Neither the HIIT, IHG or RIPC protocols employed in this study appear to change either resting or post-acute exercise LBF. That our HIIT protocol was unable to elicit any change in resting leg blood flow is contrary to a number of resistance exercise training studies (Anton et al. 2006; Egaña et al. 2010), and in disagreement with the only other study investigating leg blood flow following HIIT in older adults (Lim et al. 2017). However, the study by Lim *et al.* (which is only published in abstract form), used an elliptical trainer and a 4 x 4-minute, 90% heart rate max HIIT protocol, with sessions lasting 40 minutes in total. Therefore, it may be that our reduced intensity 5 x 1-minute protocol was not a sufficient stimulus for increasing resting limb blood flow. Of note, studies using moderate-intensity continuous aerobic exercise interventions in a similar age group to ours have also failed to increase resting limb blood flow (Seals et al. 2008). The lack of increase in resting limb blood flow in our study may be linked to the inability of our protocol to cause gains in lean mass in our participants (reported in chapter 6), given the known negative correlation between leg blood flow and lean leg muscle mass (Dinenno et al. 2001), however previous work has shown that decreases in limb blood flow in ageing are not entirely linked to reductions in muscle mass (Seals et al. 2008).

Interestingly the changes in exercise tolerance evident from both the HIIT and RIPC protocols (reported in chapter 5) do not appear to be linked to changes in either resting or exercising LBF. This may be because exercise leg blood flow is reported to not be a limiting factor in the cessation of a ramped incremental exercise test (Whipp and Ward 2009), or potentially due to a lack of sensitivity of our measurement technique to detect an increase in exercising leg blood flow. However this may have been due to our exercise stimulus (6 leg extensions at 50% 1RM) being not being sufficient to elicit a maximal blood flow response either before or after training, with our acute resistance exercise stimulus having significantly different metabolic and contractile demands to a ramp-incremental exercise test on a cycle ergometer (Egan and Zierath 2013), it may be that improvements in one modality may not benefit the other. There is also the potential for technical measurement error having played a part, with a loss of sensitivity to detect a change in exercise LBF (discussed further below in the limitations section).

### 7.5.1 Limitations

As discussed above, the aspects of this study discussed within this chapter were primarily limited by a small sample size with no *a priori* power calculation performed for any of the variables measured (as it was powered for outcomes measured in chapter 5), and a further loss of statistical power due to participant drop out. The study was also limited by its 4-arm trial design as previously discussed. Measurement of post-acute exercise LBF was limited by our inability to measure blood flow immediately post-exercise due to the need to move the participant to a couch for semi-supine measurements. Although the distance between the leg extension machine and the examination bed was short and standardised, and participants were given clear instructions and supervised throughout, any variation in the time taken for them to travel to the bed may have affected the results, and the delay in this movement may mean that we missed the true maximal post-exercise blood flow. The accuracy of measurement of FMD in this study may have been adversely affected by small inadvertent movements made by the participant during the 10-minute period of measurement. As the diameter of the brachial artery is less than 1 cm, even 1 mm movements in the artery can lead to proportionately large changes in diameter. Although attempts to minimise any inadvertent

movement were made by using a beanbag and mechanical probe holder, we cannot exclude the possibility of measurement errors contributing to our lack of a significant finding. Finally, although we have demonstrated an increase in MBV responsiveness following HIIT in the current study, we are currently unable to comment on the validity of any change in resting MBV as such a comparison is not yet validated. Nevertheless, the strong agreement between pre and post intervention resting MBV values in our control group indicate that in the future this comparison may be valid.

## 7.6 Chapter conclusions

This chapter has demonstrated that 6-weeks of training using either the HIIT, IHG or RIPC protocols described in this thesis has no effect on either microvascular blood volume responsiveness to exercise in older adults nor either resting or post-acute exercise whole leg blood flow or endothelial function.

# 8 The time-course of physiological adaptations to high intensity interval training in older adults

## 8.1 Chapter focus

As HIIT appears to have the greatest effect on the majority of variables studied in this thesis (in terms of resting blood pressure, CRF and microvascular measures), this chapter sought to try to understand the time course of the major physiological adaptations in CRF resting blood pressure over a training period. The study described in this chapter compares the effects of 6 weeks of HIIT, to those in participants completing HIIT protocols of shorter durations. The first 10 participants from the study described in chapters 5, 6 and 7 in both the HIIT and control groups are analysed in this study, in addition two other cohorts of participants who performed the HIIT protocol for either 2 or 4 weeks respectively, with the aim of determining the optimum duration for a HIIT protocol preoperatively.

## 8.2 Introduction

The UK, like the majority of the western world, has an ageing population with those aged over 65 years projected to represent over a quarter of the population within the next 50 years (Office for National Statistics 2018). With this, comes increasing prevalence of diseases associated with advancing age, many of which are treatable surgically (Smittenaar et al.

2016). However, cardiorespiratory fitness (CRF) is known to decline with advancing age (Milanović et al. 2013) and is associated not only with increased all-cause mortality *per se* (Ross et al. 2016), but also with a decreased ability to recover from a physiological insult (e.g. surgery (Older et al. 1999) or cardiovascular events (Barons et al. 2015)). Thus, there remains a need to identify well-tolerated exercise interventions to increase the CRF of older adults, with potential utility as both a population public health intervention (Taylor 2014) or as "prehabilitation" prior to major surgery (Wynter-Blyth and Moorthy 2017).

Aerobic exercise interventions in older adults are well-known to be effective at improving a variety of health-related variables, including CRF (Huang et al. 2005) and resting blood pressure (Herrod et al. 2018). However, one of the most commonly cited barriers to exercise uptake and adherence is "lack of time" (Withall et al. 2011); this is especially true for aerobic exercise training, as sessions of this nature commonly last for 30 minutes or more, and in older adults often need 12 or more weeks to show benefit (Herrod et al. 2018). However, a recent meta-analysis has demonstrated that high intensity interval training (HIIT), with a significantly reduced time-

commitment, is more effective than moderate continuous exercise training at improving CRF in older adults in less than 8-weeks (Blackwell et al. 2018).

Despite numerous studies in the field of HIIT assessing the efficacy of different protocols for improving CRF (Wen et al. 2019) and blood pressure (Costa et al. 2018), there is significant heterogeneity between the specific HIIT protocols used, with vast variations in intensity, exertion duration, number of exertions and recovery periods. Also, a large proportion of these studies are in young individuals, often utilising supra-maximal protocols (i.e. Wingate training) that would not be tolerable for the majority of older adults (Hardcastle et al. 2014). Therefore, there is a gap in the current evidence as to how HIIT can best be applied for older adults. Additionally, robust data on the time-course of physiological adaptions to HIIT in older adults is lacking. Considering temporal adaptations to HIIT, one recent meta-analysis did evaluate the effect of HIIT over different durations and demonstrated greater benefit from longer (>4-weeks) training durations. However, this analysis only included studies in younger adults, including athletic populations, and considered a variety of different HIIT regimes (Wen et al.

2019). Similarly, individual studies which have evaluated the time-course of adaptations to a single HIIT protocol have so far only been conducted in younger population groups (Astorino et al. 2017) and we are unaware of any studies of this nature in older adults. The studies that have been conducted in young individuals have suggested that HIIT may be able to expedite the rapidity of gains in CRF, when compared to lower intensity training regimes (Astorino et al. 2017).

Our research group has previously published a HIIT protocol that was both well-tolerated and effective at improving the CRF of older adults over a 4-week period (Boereboom et al. 2016b). Therefore, the primary aim of this study was to determine the effect of either shortening (to 2 weeks) or lengthening (to 6 weeks) this training programme on CRF adaptations in healthy older adults. We hypothesised that longer durations would lead to larger improvements, which may have clinical relevance given the mandated time-constraints for interventions in certain age-associated clinical conditions (Boereboom et al. 2016b).

## 8.3 Methods

Institutional research ethics approval (University of Nottingham Medical School Ethics Committee) was obtained (J14112013/A12092016 SoM MS GEM, see Appendix 6) to recruit healthy recreationally active subjects (defined as not participating in a regular formal exercise) aged 65-85 years, BMI >18 or <32kg/$m^2$ by local advertising. Thirty subjects were assigned to participate in an identical HIIT programme for either 2, 4 or 6-weeks, with 10 subjects allocated to a non-intervention control group. Clinicaltrials.gov registration was gained (NCT03019146/ 03331679/ 02188342) and all research activities complied with the 2013 Declaration of Helsinki. Before baseline assessments, all subjects provided written informed consent and were examined by a qualified medical doctor with exclusion criteria for further participation taken from the ATS/ACCP guidelines for Cardiopulmonary Exercise Testing (CPET) (Weisman et al. 2003).

### 8.3.1 Measurements

#### *8.3.1.1 Pre- and Post-Intervention Testing*

Baseline testing began with a whole-body dual-energy X-ray absorptiometry (DEXA) scan to assess body composition. Integrated DEXA software associated with the scanner (Lunar

Prodigy II, GE Medical Systems, Buckinghamshire, UK) provided automated data for whole-body body lean mass (LM), body fat percentage (FM%) and lean leg mass (LLM). A more detailed description of DEXA analysis is provided in chapter 4 (section 4.11)

Blood pressure was then measured in triplicate (mean value used) in the subject's right arm using oscillometry (Datascope trio patient monitor, Datascope, New Jersey, USA) and a blood pressure cuff (Welch Allyn, New York, USA) of appropriate size with the subject in a seated position, after having rested for 5-minutes (British and Irish Hypertension society 2017).

A ramp incremental CPET was then performed according to ATS/ACCP guidelines (Weisman et al. 2003) using a Lode Corival cycle ergometer (Lode Corival, Lode, Groningen) and inline gas analysis system (ZAN 680, nSpire Health, Colorado, USA) as previously described (Boereboom et al. 2016b). In brief, after 2-minutes of unloaded cycling, subjects were instructed to maintain a cadence of 50-60 revolutions per minute whilst being verbally encouraged to exercise to volitional exhaustion. The ramp protocol selected (10-20 Watts per minute) was based on the subjects body weight and self-reported level of physical activity to ensure a CPET duration

between 8 and 12-minutes (Weisman et al. 2003). Anaerobic threshold (AT) was determined using a combination of the V-slope and ventilatory equivalents methods (Wasserman et al. 1973) by two blinded, experienced assessors with disagreement resolved by consensus. $VO_2$ peak and exercise tolerance were determined as the oxygen utilisation and power output (directly related to test duration) at volitional exhaustion, respectively. All baseline testing procedures were repeated ~72hrs after the intervention period. A more comprehensive description of the conduct of CPET is provided in chapter 4 (section 4.8)

*8.3.1.2 Interventions*

Subjects assigned to a HIIT programme attended our exercise laboratory three times each week for either 2, 4 or 6-weeks to perform our previously published HIIT protocol (Boereboom et al. 2016b; Phillips et al. 2017a). In brief, each training session lasted 16.5 minutes, comprising a 2-minute warm-up of unloaded cycling followed by 5, 1-minute intervals at 90-110% of the peak power output achieved during the pre-intervention CPET. Exact training intensity was determined using an initial assessment session (Boereboom et al. 2016b). Working intervals were interspersed with 90-seconds active recovery of

unloaded cycling, with the final working interval followed by a 3.5-minute active recovery and monitoring period of unloaded cycling. Each training session was fully supervised with continuous 12-lead ECG monitoring throughout. At the half-way point of the intervention, subjects in the 4 and 6-week HIIT groups had their training intensity increased by 10% to account for expected training adaptations (Phillips et al. 2017a). Subjects in the control group attended only for pre- and post-intervention testing sessions, 6-weeks apart. All subjects were asked to maintain their habitual diet and levels of physical activity for the duration of the study.

#### *8.3.1.3 Statistics*

All calculations were performed using Graphpad Prism Version 7.02 (California, USA). Data are presented as mean (SD). Data were tested for normality using the D'Agostino & Pearson normality test and subsequently analysed appropriately. Participant demographics and outcome variables at baseline were compared using one-way ANOVA, whilst outcome data were compared using repeated measures two-way ANOVA with Sidak's multiple comparisons post-hoc testing. Significance was taken as an alpha of $p<0.05$.

## 8.4 Results

### 8.4.1 Subject characteristics

40 subjects (mean (SD age) 71(5) years; (mean (SD) BMI 25.8(2.6) kg/m$^2$) were recruited to this study. There were no significant differences in sex, age, baseline height or weight between the groups (Table 31).

*Table 31 Subject characteristics*

| **Group** | **Age (y)** | **Weight (kg)** | **Height (m)** | **Male/Female (n/n)** |
|---|---|---|---|---|
| **Control** | 73(6) | 74(7) | 1.70(0.06) | 6/4 |
| **2-Week** | 73(4) | 69(9) | 1.66(0.06) | 5/5 |
| **4-Week** | 68(3) | 71(10) | 1.68(0.12) | 5/5 |
| **6-Week** | 69(3) | 80(13) | 1.69(0.10) | 5/5 |

All 40 participants completed the entire study with none lost to follow-up. Training compliance was 100% with no adverse events throughout the study. There were no significant differences in any outcome variable between the groups at baseline (all $p>0.1$).

### 8.4.2 Anaerobic threshold

There was no significant difference in AT between the groups at baseline (*P*=0.42). A significant increase in AT was observed in both the 4-week (*P* <0.001) and 6-week groups (*P* <0.001), with no significant change in either the 2-week (*P* =0.08) or control groups (*P* >0.99) (Table 32, Figure 45). There was a main effect of time across groups (*P*<0.001) and a significant group x time interaction (*P* =0.01).

*Table 32 Anaerobic threshold before and after intervention (or control) (ml/kg/min)*

| | Pre | Post | Change | *P* |
|---|---|---|---|---|
| **2-week** | 12.9 (1.8) | 13.9 (2.3) | +1.0 (1.1) | 0.08 |
| **4-week** | 15.6 (2.8) | 17.6 (3.3) | +2.0 (1.1) | <0.001 |
| **6-week** | 14.0 (3.7) | 15.8 (4.3) | +1.9 (1.9) | <0.001 |
| **CON** | 15.2 (6.3) | 15.4 (6.7) | +0.1 (1.1) | >0.99 |

CON: control (no intervention). Data is presented as mean (SD).

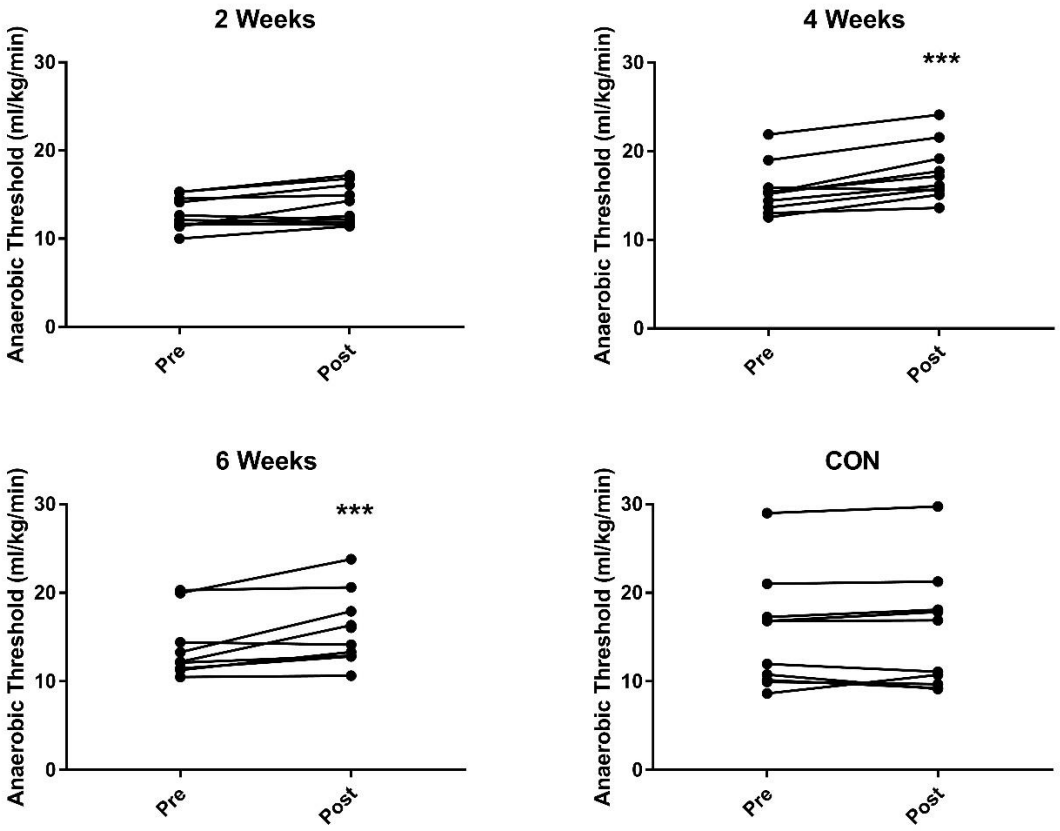


*Figure 45 Anaerobic threshold before (pre) and after (post) 2-weeks (n=10), 4-weeks (n=10) or 6-weeks (n=10) of high intensity interval training (HIIT) or a no intervention control (n=10) period.*** denotes P<0.001*

### 8.4.3 $VO_2$ Peak

There was no significant difference in $VO_2$ Peak between the groups at baseline (*P*=0.87). There was a main effect of time across the groups (*P* =0.002) but no group x time interaction (*P* =0.58).(Table 33, Figure 46).

*Table 33 $VO_{2peak}$ before and after intervention (or control) (ml/kg/min)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **2-week** | 23.3 (5.2) | 25.1 (5.4) | +1.7 (2.4) |
| **4-week** | 25.2 (3.6) | 27.2 (4.8) | +2.0 (2.5) |
| **6-week** | 23.8 (7.7) | 26.8 (5.4) | +3.0 (5.5) |
| **CON** | 25.6 (10.1) | 26.4 (9.8) | +0.8 (2.7) |

CON: control (no intervention). Data is presented as mean (SD).

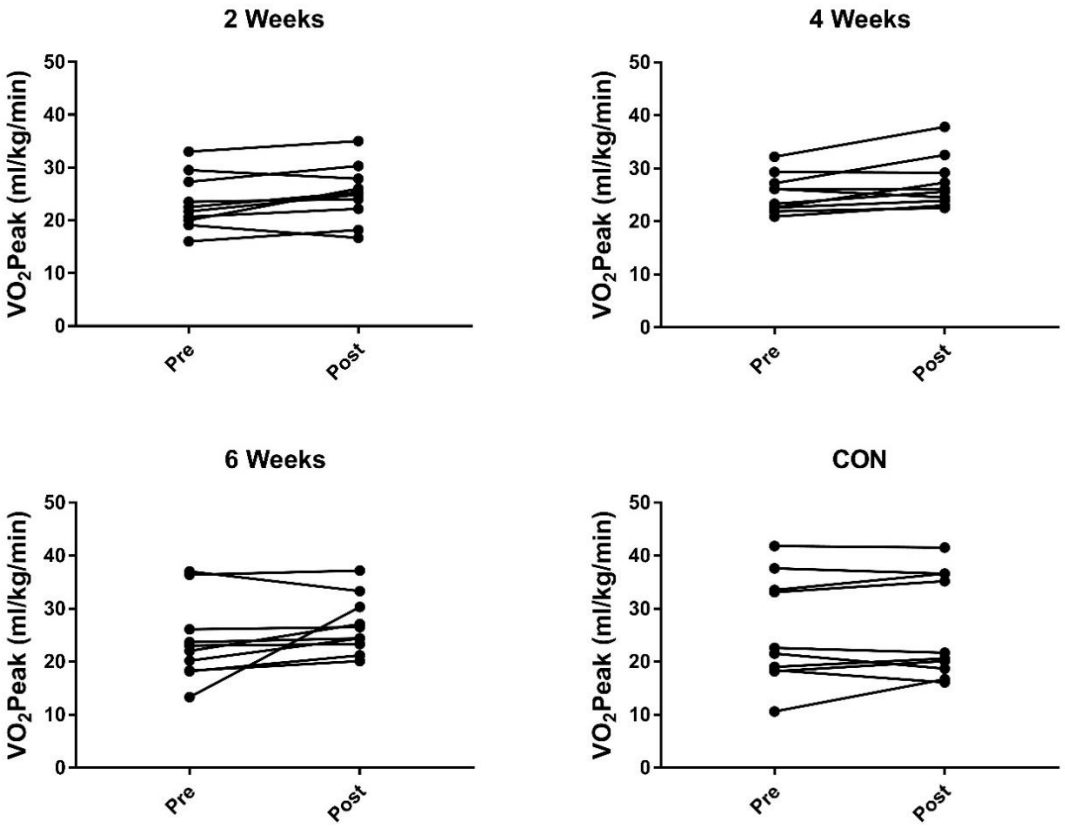


*Figure 46 Peak oxygen consumption before (pre) and after (post) 2-weeks (n=10), 4-weeks (n=10) or 6-weeks (n=10) of high intensity interval training (HIIT) or a no intervention control (n=10) period.*

### 8.4.4 Exercise tolerance ($W_{peak}$)

There was no significant difference in $W_{peak}$ between the groups at baseline ($P$=0.87). $W_{peak}$ increased in both the 2-week, 4-week and 6-week groups (all $P$ <0.001) after HIIT, with no significant change in the control group ($P$ >0.99), (Table 34, Figure 47). There was a main effect of time across groups ($P$ <0.001) and a significant group x time interaction ($P$ =0.007).

*Table 34 $W_{peak}$ before and after intervention (or control) (W)*

| | **Pre** | **Post** | **Change** | ***P*** |
|---|---|---|---|---|
| **2-week** | 124 (35) | 139 (47) | +15 (15) | <0.001 |
| **4-week** | 137 (37) | 154 (39) | +17 (11) | <0.001 |
| **6-week** | 157 (63) | 174 (61) | +16 (11) | <0.001 |
| **CON** | 144 (66) | 144 (66) | +1 (7) | >0.99 |

CON: control (no intervention). Data is presented as mean (SD).

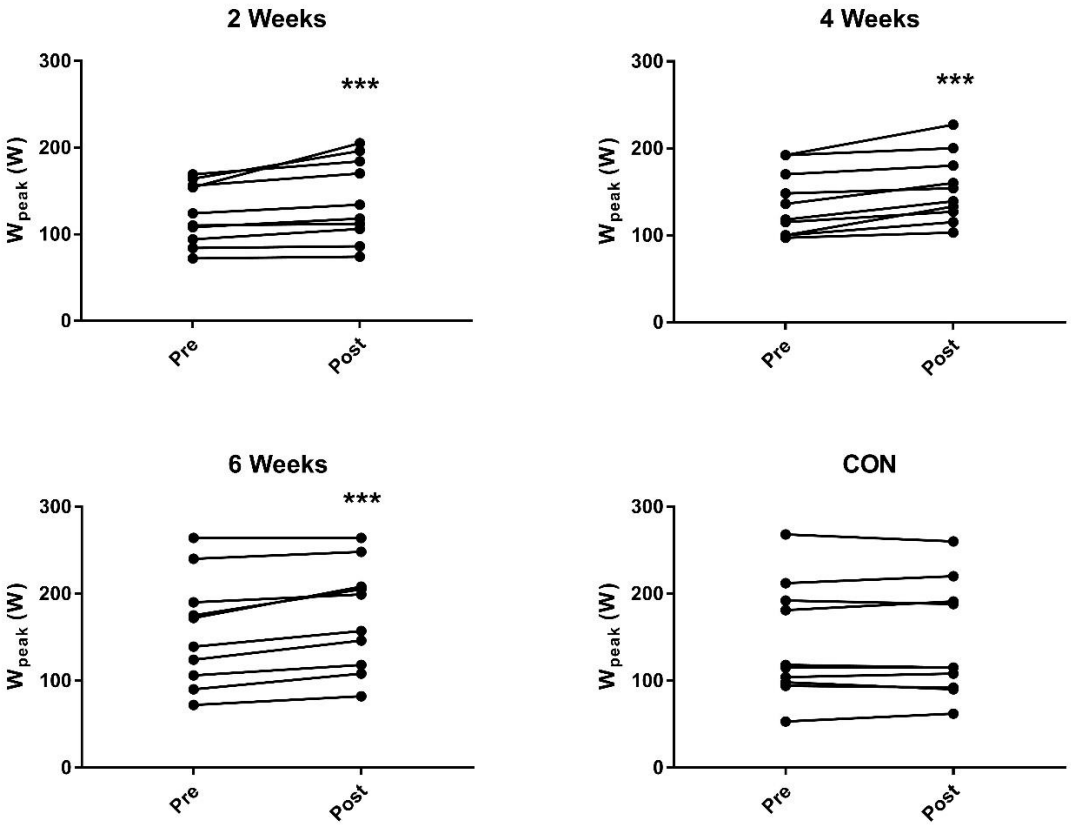


*Figure 47 Peak power output before (pre) and after (post) 2-weeks (n=10), 4-weeks (n=10) or 6-weeks (n=10) of high intensity interval training (HIIT) or a no intervention control( n=10) period. .*** denotes P<0.001*

### 8.4.5 Systolic blood pressure

There was no significant difference in systolic BP between the groups at baseline (*P*=0.10). There was a main effect of time across the groups (p=0.003) but no group x time interaction (p=0.13) (Table 35).

*Table 35 Systolic BP before and after intervention (or control) (mmHg)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **2-week** | 131 (12) | 129 (17) | -2 (9) |
| **4-week** | 140 (19) | 132 (18) | -8 (13) |
| **6-week** | 143 (18) | 134 (12) | -9 (10) |
| **CON** | 128 (10) | 128 (11) | 0 (6) |

CON: control (no intervention). Data is presented as mean (SD).

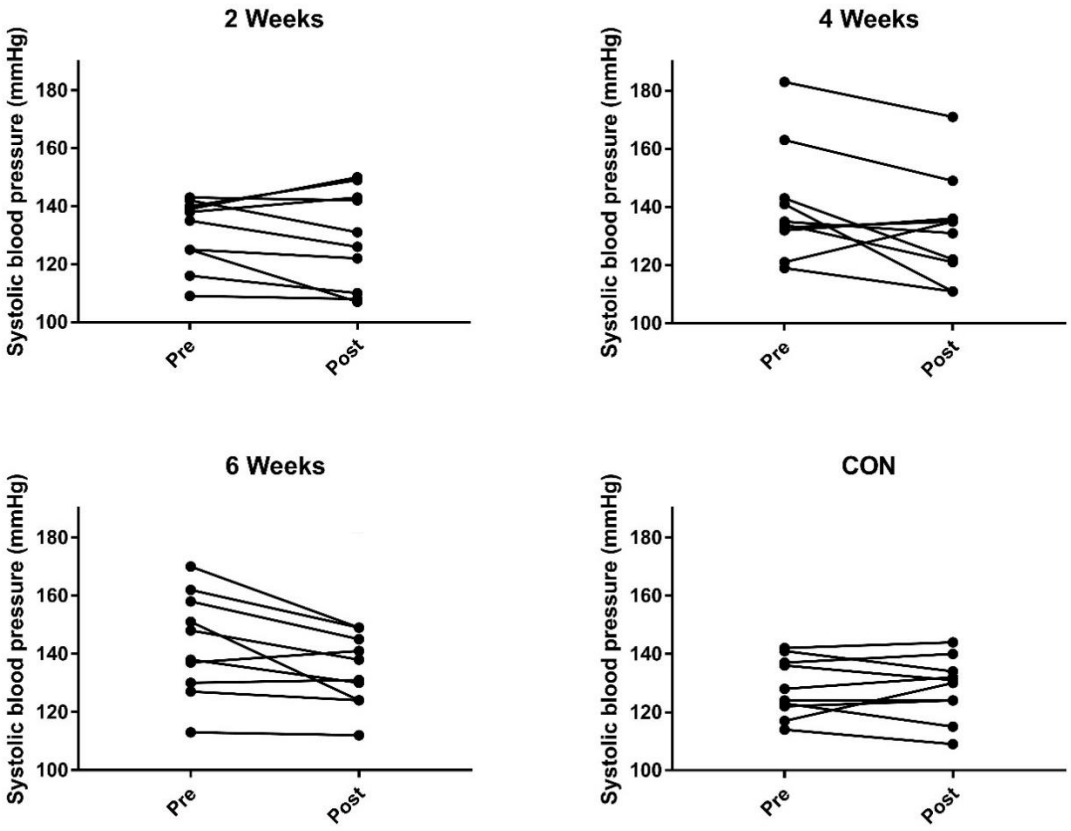


*Figure 48 Resting systolic blood pressure before (pre) and after (post) 2-weeks (n=10), 4-weeks (n=10) or 6-weeks (n=10) of high intensity interval training (HIIT) or a no intervention control (n=10)l period.*

### 8.4.6 Diastolic blood pressure

There was no significant change in diastolic BP in any group (Table 36). There was a trend towards a main effect of time across the groups (p=0.054) but no group x time interaction (p=0.59).

*Table 36 Diastolic BP before and after intervention (or control) (mmHg)*

| | **Pre** | **Post** | **Change** |
|---|---|---|---|
| **2-week** | 79 (9) | 78 (9) | -1 (6) |
| **4-week** | 82 (7) | 74 (12) | -8 (15) |
| **6-week** | 86 (14) | 83 (8) | -3 (11) |
| **CON** | 79 (10) | 77 (7) | -2 (12) |

CON: control (no intervention). Data is presented as mean (SD).

### 8.4.7 Body composition

There was no significant change in any of our body composition parameters (whole-body body lean mass, body fat percentage and lean leg mass), in any group (Table 37). There was no main effect of time across the groups for any body composition parameter nor any significant group X time interaction.

*Table 37 Body composition parameters before and after 2, 4 or 6-weeks of high-intensity interval training (HIIT), or a no intervention control period*

| Group | Before HIIT | After HIIT | Change |
|---|---|---|---|
| **Control LM (Kg)** | 49.9(5.4) | 49.9(5.0) | +0.0(1.2) |
| **2-Week LM(Kg)** | 43.9(8.6) | 44.5(2.8) | +0.5(0.7) |
| **4-Week LM(Kg)** | 44.6(10.9) | 44.7(10.8) | +0.1(1.1) |
| **6-Week LM(Kg)** | 48.3(11.2) | 48.4(11.3) | +0.2(0.8) |
| | | | |
| **Control FM%** | 29.5(7.3) | 29.5(6.8) | 0.0 |
| **2-Week FM%** | 32.7(7.1) | 32.2(7.0) | -0.5(0.9) |
| **4-Week FM%** | 32.0(10.1) | 31.7(9.6) | +1.7(0.6) |
| **6-Week FM%** | 38.0(6.3) | 37.4(6.2) | -0.5(0.7) |
| | | | |
| **Control LLM (Kg)** | 15.5(4.1) | 15.7(4.4) | +0.2(1.6) |
| **2-Week LLM(Kg)** | 14.3(3.1) | 14.6(3.1) | +0.3(0.4) |
| **4-Week LLM(Kg)** | 14.6(4.2) | 14.4(4.3) | -0.2(0.6) |
| **6-Week LLM(Kg)** | 13.4(2.0) | 14.0(3.4) | +0.7(2.7) |

LM: whole body lean mass, FM %: whole body fat mass %, LLM: leg lean mass. Data is presented as mean (SD).

## 8.5 Discussion

This study has demonstrated that a time-efficient, well-tolerated HIIT programme elicits appreciable improvements in exercise tolerance before any measurable changes in CRF in a cohort of older adults. Two weeks of HIIT appears to be insufficient to improve AT, but this can be achieved with 4 or 6-weeks of training. We did not show any significant change in $VO_2$ peak in any group, despite these changes in AT. It might be that our protocol requires longer than 6 weeks (the maximum time period tested in this study) to change $VO_2$ peak, or that our study was underpowered to detect a change at 6 weeks due to its small sample size and the limitations of its 4-parrallel arm design. However, we believe this is the first study to attempt to plot the time-course of CRF changes resulting from a single HIIT protocol in healthy older adults.

The findings of our study agree with other studies using HIIT in older adults which have demonstrated improvements in $VO_2$ peak of a similar magnitude to ours after 6 and 8 weeks of HIIT (Hwang et al. 2016), whereas longer duration studies (12 weeks) have demonstrated gains approaching 5ml/kg/min (Ahmaidi et al. 1998). Likewise for changes in AT, similar improvements to ours have been seen at 6 weeks (Grace et al.

2018), with longer studies demonstrating improvements of ~4ml/kg/min at 12 weeks (Ahmaidi et al. 1998). However, as far as we are aware, this is the first study to compare the changes in both $VO_2$ peak and AT from different durations of a single HIIT protocol in older adults.

The physiological basis behind our observation that significant improvements in AT occur before increases in $VO_2$ peak may reside at the mitochondrial level. Mitochondrial biogenesis pathways, potentially the main determinant of AT (Aunola et al. 1988), are seen to be upregulated after just a single HIIT session (Gibala et al. 2009) and therefore could be important earlier in a training regime. Conversely, central cardiovascular adaptions (i.e., blood volume and cardiac output), which are thought to be the main mechanisms through which HIIT may influence $VO_2$ peak, tend to occur later during a training programme (MacInnis and Gibala 2017). We were unable to find any previous studies evaluating HIIT over durations as short as 2 weeks in older adults, but those conducted in young men showed similar findings to ours; an increase in peak power without significant changes in CRF (Zwetsloot et al. 2014). It may therefore be that the peak power increases seen very early in training are a consequence of improved cycling

technique/efficiency and improved exercise tolerance (Zwetsloot et al. 2014), after which changes in AT related to mitochondrial upregulation become apparent before finally, central cardiovascular changes manifest.

Despite our HIIT programme eliciting CRF improvements in just 4-weeks and physical function gains in just 2 weeks, it did not lead to any significant changes in body composition over 2, 4- or 6 weeks. This is despite previous studies in older adults demonstrating gains in lean mass (Herbert et al. 2017) and reductions in body fat (Søgaard et al. 2018) with HIIT. This disparity may be due to differences in participant demographics and/or training modalities between these studies and ours. For example, Herbert and colleagues (Herbert et al. 2017) employed 30-second Wingate sprints in an exclusively male cohort, who were a decade younger than our cohort, including some masters athletes, but were able to demonstrate a ~3% increase in lean mass with 6-weeks of HIIT. Søgaard and colleagues (Søgaard et al. 2018) also studied a cohort that were a decade younger than our particpants who were overweight or obese with a minumum BMI for inclusion of 27 $kg/m^2$ and failed to significantly increase in lean mass, but were able to significantly reduce

their body fat percentage by ~0.6%. In support of the notion of exercise modality having a role to play in disparate body composition changes in response to HIIT, a recent systematic review of previous studies (albeit based on studies largely in younger adults) has demonstrated cycling-based HIIT to be far less effective than running HIIT for reducing body fat percentage (Wewege et al. 2017), likely due to the recruitment of more muscle groups when running.

One underlying reason why the participants in our study did not significantly alter their body composition may be due to the anabolic resistance to both exercise (Kumar et al. 2009) and nutrition (Atherton and Smith 2012) that is associated with advancing age. In relation to this we have previously shown that with resistance exercise training (RET; often considered the gold standard for eliciting hypertrophy), advancing age is negatively correlated with exercise-induced hypertrophic responses (Phillips et al. 2017b), and this may also be true for hypertrophic responses to HIIT. One final consideration as to the reason for the lack of body composition changes must be given to diet, which was not controlled or measured in this study; although participants were instructed to maintain their habitual pre-study dietary behaviour. There

is a possibility that our HIIT may have led to an increase in appetite and subsequent energy intake which may have diminished body fat reductions (King et al. 2012).

This study did not demonstrate any significant reduction in resting systolic or diastolic blood pressure from our HIIT protocol, despite a large numerical difference between the group mean changes in systolic BP of both the 4-and 6-week groups and the control group (-8 and -9 vs 0 mmHg). However, the lower bound of the 95% confidence intervals for these reductions was -0.1 mmHg the 4-week group and -1 mmHg for the 6-week group, highlighting the large uncertainty in the true effect size from this small study. This may have been a type 2 error, due to the study not having sufficient power due to its small sample size and its 4-arm trial design. These mean reductions of 8 mmHg and 9 mmHg systolic blood pressure however are greater than that previously observed in trials of both moderate continuous aerobic exercise and RET in older adults (Herrod et al. 2018), but similar to that observed in the few previous studies of HIIT in older adults that have measured this (Grace et al. 2018). A reduction of 8 mmHg approaches the reduction of 10 mmHg described by Law and colleagues (Law et al. 2009) in their vast meta-analysis of

46,4000 patients across 147 randomised controlled trials of antihypertensive treatment. In this meta-analysis a 10 mmHg reduction was associated with a 22% reduction in coronary heart disease events and a 41% reduction in strokes (Law et al. 2009), highlighting the potential of our HIIT protocol as a strategy for reducing CVD risk in older adults, if it were found to be effective in a larger, adequately powered study and could potentially decrease the need for pharmacological intervention in a growing sector of society.

One important feature of the training programme used herein is its tolerability; as was demonstrated by the 100% training compliance in a cohort with a mean age over 70 years. This demonstrates its suitability for use in older adults *per se* and in older patient groups, where adherence to previous exercise prehabilitation has been reported as low as 16% (Vermillion et al. 2018). Although some previous studies that have explored the temporality of physiological adaptations to HIIT in younger adults have demonstrated significant changes in CRF in very short time-frames (2-weeks or less) (Gholizadeh et al. 2017), these studies used supramaximal Wingate-type exercise, which based on the existing literature are likely not feasible in a general older population (Hardcastle et al. 2014).

That our HIIT protocol can increase AT in 4-weeks, would allow our HIIT protocol to be used as exercise prehabilitation before, for example, major surgical intervention for cancer where there is a limitation on the pre-operative time available; the 31-day time to first treatment cancer target in the UK (Cancer Research UK 2015). Based on epidemiological data, an increase in CRF prior to major surgery has the potential to reduce perioperative and postoperative morbidity and mortality (Older et al. 1999), however the results of large randomised clinical trials of exercise prehabilitation (i.e., NCT03509428) are still needed to fully determine the effects of exercise prehabilitation on direct clinical outcomes (e.g., complication rates, length of stay). targets beyond 4 weeks in order to achieve this.

### 8.5.1 Limitations

As with all studies, we must acknowledge some limitations to our study design. That we did not have a single intervention group with repeated measurements at baseline, 2, 4 and 6 weeks is our main limitation. However, incorporating repeated CPET assessments every 2 weeks would have negatively impacted delivery of our HIIT protocol (as these sessions

would have had to replace HIIT sessions), and based on anecdotal feedback from our participants was not something many of them would have been willing to undertake.

In addition, the relatively small sample size in this study negates our ability to assess inter-individual responses to any of our training durations (Phillips et al. 2017a).

We also acknowledge that prescribed exercise interventions interact with habitual physical activity and nutritional intake, aspects which were not measured in this study. We did however ask all participants to maintain their normal diet and levels of physical activity for the duration of the study.

## 8.6 Chapter conclusions

In conclusion, this study is the first to map the time-course of physiological adaptions to a single, well-tolerated HIIT protocol in older adults, demonstrating that a 5-by-1-minute HIIT protocol performed 3 times each week can elicit significant improvements in the exercise tolerance of older adults in just 2 weeks, whilst 4 weeks of this training regime can lead to significant improvements in CRF.

# 9 Overall thesis conclusions

The background literature review conducted in chapter 1 of this thesis established the challenges that an ageing population brings to healthcare settings in terms of both general cardiovascular disease risk, the growing population of older adults requiring major surgery and the increased risk that older adults bring in terms of poorer postoperative outcomes. This review elaborated on the importance of blood pressure and hypertension management for both general cardiovascular risk reduction and the importance of several other aspects of fitness for potentially reducing perioperative ramifications. Finally, this chapter highlighted the potential of exercise, or other brief lifestyle interventions, as strategies to target hypertension.

A subsequent systematic review and meta-analysis reported in chapter 2 of this thesis demonstrated the effectiveness of several non-pharmacological interventions at reducing blood pressure in adults aged over 65 years, with the magnitude of reductions approaching those achieved from the use of a single agent antihypertensive. However, the majority of interventions in the studies included in this review had durations of 3 months

or greater, which could limit both the usefulness and transferability to the preoperative setting, in which substantial time pressure often exists (Cancer Research UK 2015). Furthermore, the majority of intervention sessions in these studies lasted between 30 and 60 minutes, with some sessions lasting as long as 120 minutes. Previous work has demonstrated "lack of time" to be a key determinant of exercise adherence (Gothe and Kendall 2016; Moschny et al. 2011) and thus the likely adherence of the general population to such interventions remains questionable. This review did however identify early evidence from 2 small studies suggesting that IHG could be of use in lowering blood pressure.

Based on knowledge gained from the background literature review and the subsequent systematic review and meta-analysis, chapters 5-7 describe different endpoints from the main study reported in this thesis. This study set about to evaluate the impact of 6 weeks HIIT, IHG or RIPC on several indices of cardiovascular and metabolic health in 48-healthy-older adults, powered to detect a clinically relevant reduction of 10 mmHg in resting systolic BP as the primary outcome.

Chapter 5 describes the effect of the 3 interventions on the primary outcome of resting systolic BP, together with several secondary outcome measures. This study showed that 6-weeks of directly supervised training with either the HIIT or IHG protocol used in this study can significantly reduce resting systolic BP by a clinically meaningful magnitude that has been previously associated with vast reductions in both cardiovascular and cerebrovascular event risk (Law et al. 2009). That the IHG intervention achieved a reduction of the same magnitude as the HIIT protocol is largely surprising, given the significant difference between the level of physical exertion required by the participants completing these two very different interventions. This finding raises the possibility of employing IHG as a home-based, unsupervised intervention that patients could incorporate into their activities of daily living. This form of IHG implementation has been shown to be effective in younger adults (Blackwell et al. 2017; Gordon et al. 2018) and should now be investigated in older adults.

Despite similar improvements in systolic BP, 6 weeks HIIT provided additional benefits over IHG in terms of improved CRF. HIIT led to significant gains in AT , which could be expected to provide benefit if used as both short-term

prehabilitation for surgery or potentially as a longer-term lifestyle intervention. In support of this notion, a large body of observational research has demonstrated that both AT and $VO_{2peak}$ have utility to stratify surgical risk and predict those at most risk of perioperative mortality (Older et al. 1993, 1999) and morbidity (West et al. 2014; Lee et al. 2018a). Therefore, the ability of this HIIT protocol to increase AT by what is considered the minimum clinically important difference of 1.5ml/kg/min (Dunne et al. 2016) is supportive of its inclusion in prehabilitation programmes. Outside of the perioperative setting, large observational studies have demonstrated CRF to be a strong independent predictor of mortality (Clausen et al. 2018; Mandsager et al. 2018) and as such HIIT may have a role in improving the health and lifespan of older adults in general. Similarly, in cohort studies, low CRF is also associated with the loss of independence in activities of daily living (Paterson et al. 2007, 2004) and becoming dependent on external help.

However, despite these beneficial adaptations in response to both HIIT and IHG, there is currently no data as to the longevity of any benefits accrued from a period of HIIT or IHG, and it is largely unknown how rapidly detraining will occur,

especially in older adults. There is however a suggestion from work in young adults that for HIIT this may only be a matter of weeks (Bonsu and Terblanche 2016; Marles et al. 2007). Interestingly, a recent trial investigating IHG in younger adults demonstrated not only similar reductions in BP with home-based versus lab-based training (Gordon et al. 2018), but also assessed BP 6-weeks after the end of IHG and found that the majority of benefit persisted for this duration, even in the absence of continued training (Gordon et al. 2018).

Disappointingly, the reductions in BP elicited by both HIIT and IHG did not translate to reductions in ambulatory BP in this study, an observation that may limit the real-world applicability of these interventions to reduce cardiovascular risk. However, it must be acknowledged that this study was not adequately powered to detect changes in ambulatory BP, and as such, given recent recommendations on the use of ambulatory BP the gold standard outcome measurement for the management of hypertension (Hermida et al. 2015; National Institute for Health and Care Excellence 2011), future studies should consider making ambulatory BP their primary endpoint with a view to achieve increased adherence for this

measurement (a limitation of the current study) and be powered appropriately to assess this.

Unfortunately, the RIPC protocol in this study appeared to be ineffective at changing any measure of CRF or BP. It did however increase our measure of exercise tolerance ($W_{peak}$), however in the absence of changes in CRF or BP, it is unlikely that this alone would be clinically relevant. Future studies investigating RIPC should consider more intense protocols with either longer durations of ischaemia or a larger mass of muscle rendered ischaemic based on studies aimed at improving performance in competitive athletes (Sharma et al. 2015).

Chapter 6 of this thesis demonstrated that none of the interventions implemented in this study had any impact on any measure of insulin resistance or body composition. The lack of a significant effect of the HIIT protocol is particularly noteworthy due to the relatively sizable contrasting body contrasting these findings (Søgaard et al. 2018; Robinson et al. 2017). However, as discussed at length in chapter 6 there are a wide range of potential reasons for this, including dietary factors, a loss of statistical power due to drop outs, or the potential for participants performing HIIT to have reduced their

habitual activities of daily living due to fatigue from the training sessions. Any future studies investigating the effect of time efficient, submaximal HIIT on body composition or insulin resistance in older adults should include some form of dietary control and monitoring, in additional to physical activity assessment.

In the last of the three chapters outlining findings from the main study in this thesis, Chapter 7 described the effects of this studies interventions on markers of both macro- and microvascular function. This study did not demonstrate any effect of either the HIIT, IHG or RIPC protocols used in this study on the responsiveness of the muscle microvasculature of the *vastus lateralis*, nor any change in either macro vascular leg blood flow either at rest, or in response to a standardised exercise stimulus or any change in endothelial function as measured in the brachial artery.

Neither RIPC nor IHG appeared to have any effect macro- or microvascular function. In the case of RIPC this may again be due to our 15-minute upper limb protocol not providing a sufficiently ischaemic stimulus, and further studies with longer periods of ischaemia and/or larger muscle groups will be

required to exclude this possibility. Regarding IHG, this study agrees with previous work that has demonstrated that any change in endothelial function appears to be only local to the limb trained (Badrov et al. 2016; McGowan et al. 2007c), limiting its potential usefulness as a health-based intervention.

Finally, chapter 8 of this thesis attempted to map the time-course of the physiological adaptations of older adults to the HIIT protocol implemented throughout this thesis by comparing the adaptations made by groups old older adults completing either 2, 4 or 6 weeks of training. This study demonstrated that improvements in exercise performance precede improvements in CRF by several weeks, such that neither statistically significant nor clinically relevant improvements in measures of CRF are apparent after only 2 weeks of HIIT despite marked increases in exercise performance. This is of particular relevance in the field of surgical prehabilitation where significant time pressures and organisational targets may limit the amount of training time possible preoperatively (Boereboom et al. 2019). Importantly, both in relation to this clinical perspective, and in relation to questioning if longer intervention durations in this thesis would have led to greater benefit, extending training from 4-weeks

to 6-weeks did not lead to any greater improvement in AT suggesting that for this specific parameter, a prolonged HIIT regime would not confer additional benefit.

Considering the findings of the individual chapters in this thesis as a collective, there is considerable evidence to show the detrimental effects of hypertension in an ageing population, and the potential of lifestyle interventions to mitigate this. Both HIIT and IHG can effectively reduce the resting systolic BP of older adults in a time-efficient manner, hopefully offering interventions that can achieve better adherence than current government guidelines on physical activity. In addition, a combined approach of both of these interventions may lead to even greater reductions in systolic BP when compared to each being performed in isolation. Finally, both of these interventions have the potential to be delivered as unsupervised, home-based interventions which could allow their wider delivery to patients. In this context IHG could be delivered as in this study, with our HIIT protocol only requiring minimal modification, to make them both time-efficient and (almost for IHG) equipment free. In conclusion, both of these interventions have significant potential to improve measures of cardiorespiratory and vascular health of older adults.

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# 11 Appendices

## 11.1 Appendix 1

Search strategies used in systematic review

Search strategies were as follows:

**MEDLINE**

(exp "ARTERIAL PRESSURE"/ OR exp HYPERTENSION/ OR exp "MASKED HYPERTENSION"/ OR exp "WHITE COAT HYPERTENSION"/) NOT exp "HYPERTENSION, PULMONARY"/) AND (exp "LIFE STYLE"/ OR exp "SERVING SIZE"/ OR exp "PORTION SIZE"/ OR exp "DIET, SODIUM-RESTRICTED"/ OR exp "DIET, REDUCING"/ OR exp "DIET, DIABETIC"/ OR exp "DIET, CARBOHYDRATE-RESTRICTED"/ OR exp "DIET, FAT-RESTRICTED"/ OR exp "DIET, MEDITERRANEAN"/ OR exp "DIET, VEGETARIAN"/ OR exp "EXERCISE THERAPY"/ OR exp "WARM-UP EXERCISE"/ OR exp WALKING/ OR exp SWIMMING/ OR exp RUNNING/ OR exp "RESISTANCE TRAINING"/ OR exp "PLYOMETRIC EXERCISE"/ OR exp "PHYSICAL CONDITIONING, HUMAN"/ OR exp "PHYSICAL CONDITIONING, HUMAN"/ OR exp "MUSCLE STRETCHING EXERCISES"/ OR exp "COOL-DOWN EXERCISE"/ OR exp "CIRCUIT-BASED EXERCISE"/ OR exp EXERCISE/ OR exp MUSIC/)) NOT exp "DRUG THERAPY"/) AND (reduc* OR lower).ti,ab) AND exp AGED/) [Document type Meta-analysis OR Multicenter Study OR Randomized Controlled Trial OR Review] [Human age groups Aged] [Humans]"

**EMBASE**

((((exp "SYSTOLIC BLOOD PRESSURE"/ OR exp "DIASTOLIC BLOOD PRESSURE"/ OR exp "BLOOD PRESSURE

VARIABILITY"/ OR exp "BLOOD PRESSURE REGULATION"/ OR exp "BLOOD PRESSURE FLUCTUATION"/ OR exp "ARTERIAL PRESSURE"/) AND (exp "WHITE COAT HYPERTENSION"/ OR exp "SYSTOLIC HYPERTENSION"/ OR exp "RESISTANT HYPERTENSION"/ OR exp PREHYPERTENSION/ OR exp "METABOLIC SYNDROME X"/ OR exp "MASKED HYPERTENSION"/ OR exp "ESSENTIAL HYPERTENSION"/ OR exp "DIABETIC HYPERTENSION"/ OR exp "BORDERLINE HYPERTENSION"/)) NOT exp "PULMONARY HYPERTENSION"/) AND (exp "LIFESTYLE MODIFICATION"/ OR exp "VEGETARIAN DIET"/ OR exp "MEDITERRANEAN DIET"/ OR exp "LOW CARBOHYDRATE DIET"/ OR exp "LOW CALORY DIET"/ OR exp "HIGH FIBER DIET"/ OR exp PLYOMETRICS/ OR exp "STATIC EXERCISE"/ OR exp "MUSCLE EXERCISE"/ OR exp "LEG EXERCISE"/ OR exp "EXERCISE INTENSITY"/ OR exp "BREATHING EXERCISE"/ OR exp EXERCISE/ OR exp PILATES/ OR exp "DYNAMIC EXERCISE"/ OR exp "CIRCUIT TRAINING"/ OR exp "ARM EXERCISE"/ OR exp "AQUATIC EXERCISE"/ OR exp "ANAEROBIC EXERCISE"/ OR exp "AEROBIC EXERCISE"/ OR exp "MUSIC THERAPY"/ OR exp MUSIC/)) NOT (exp "ANTIHYPERTENSIVE THERAPY"/ OR exp "DRUG THERAPY"/)) AND (reduc* OR lower).ti,ab) AND exp AGED/) [Human age groups Aged 65+ years] [Humans] [Clinical trials Randomized Controlled Trial OR Controlled Clinical Trial OR Multicenter Study]"

**CINAHL**

((exp "ARTERIAL PRESSURE"/ OR exp "HYPERTENSIVE CRISIS"/ OR exp "HYPERTENSION, ISOLATED SYSTOLIC"/ OR exp "HYPERTENSION, REFRACTORY"/ OR exp "MASKED HYPERTENSION"/ OR exp "HYPERTENSION, WHITE COAT"/)

AND (exp "MIND BODY TECHNIQUES"/ OR exp AROMATHERAPY/ OR exp "LIFE STYLE CHANGES"/ OR exp "WEIGHT REDUCTION PROGRAMS"/ OR exp "WEIGHT CONTROL"/ OR exp VEGETARIANISM/ OR exp "MEDITERRANEAN DIET"/ OR exp "DIET, SODIUM-RESTRICTED"/ OR exp "DASH DIET"/ OR exp "DIET, REDUCING"/ OR exp "DIET, LOW CARBOHYDRATE"/ OR exp "DIABETIC DIET"/ OR exp "RESISTANCE TRAINING"/ OR exp EXERCISE/ OR exp "EXERCISE TEST, CARDIOPULMONARY"/ OR exp MUSIC/ OR exp YOGA/ OR exp "TAI CHI"/ OR exp "RELAXATION TECHNIQUES"/ OR exp MEDITATION/ OR exp "MUSIC THERAPY"/ OR exp "DIET THERAPY"/)) NOT exp "DRUG THERAPY"/) AND (reduc* OR lower).ti,ab) AND exp AGED/) [Publication types Meta Analysis OR Randomized Controlled Trial OR Systematic Review] [Human age groups Aged: 65+ years]"

**AMED**

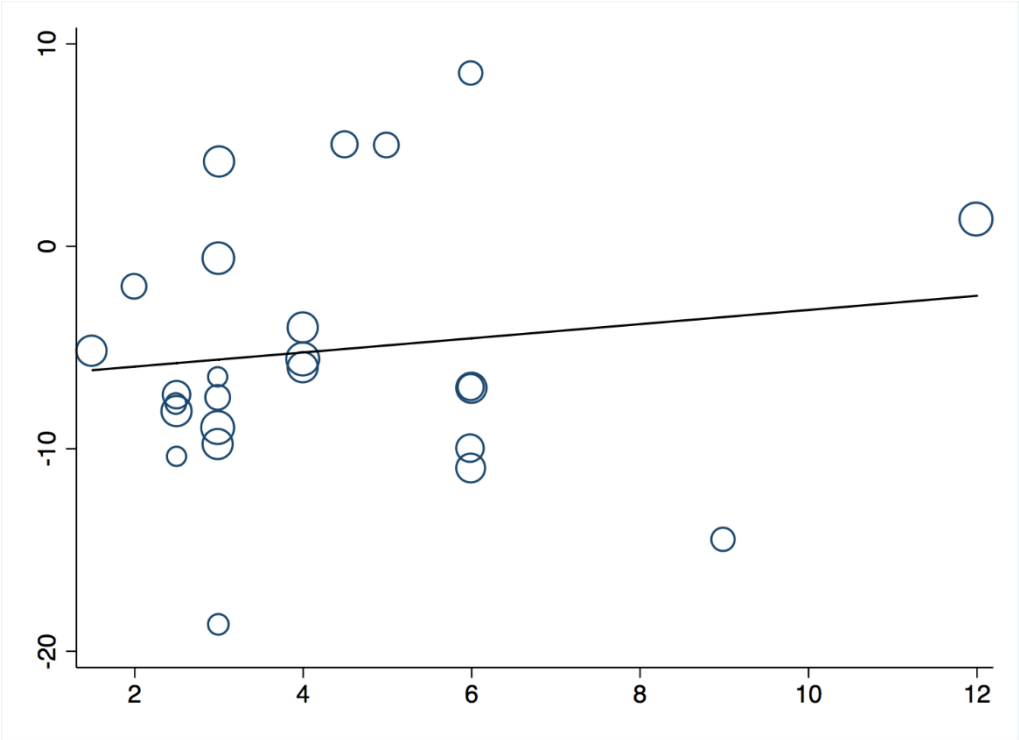
(exp "BLOOD PRESSURE"/ OR exp HYPERTENSION/ OR exp "WHITE COAT HYPERTENSION"/) AND (exp "LIFE STYLE"/ OR exp "DIET VEGETARIAN"/ OR exp "DIET MEDITERRANEAN"/ OR exp "CALORIC INTAKE"/ OR exp "WEIGHT TRAINING"/ OR exp EXERCISE/ OR exp SPORTS/ OR exp "PHYSICAL FITNESS"/ OR exp "LEISURE ACTIVITIES"/ OR exp MUSIC/ OR exp YOGA/ OR exp "TAI CHI"/ OR exp "LAUGHTER THERAPY"/ OR exp VISUALIZATION/ OR exp RELAXATION/ OR exp HYPNOSIS/ OR exp BIOFEEDBACK/ OR exp MEDITATION/)) NOT exp "DRUG THERAPY"/) AND (reduc* OR lower).ti,ab) AND exp AGED/) [Publication types Journal Article OR Review]"

**PubMed**

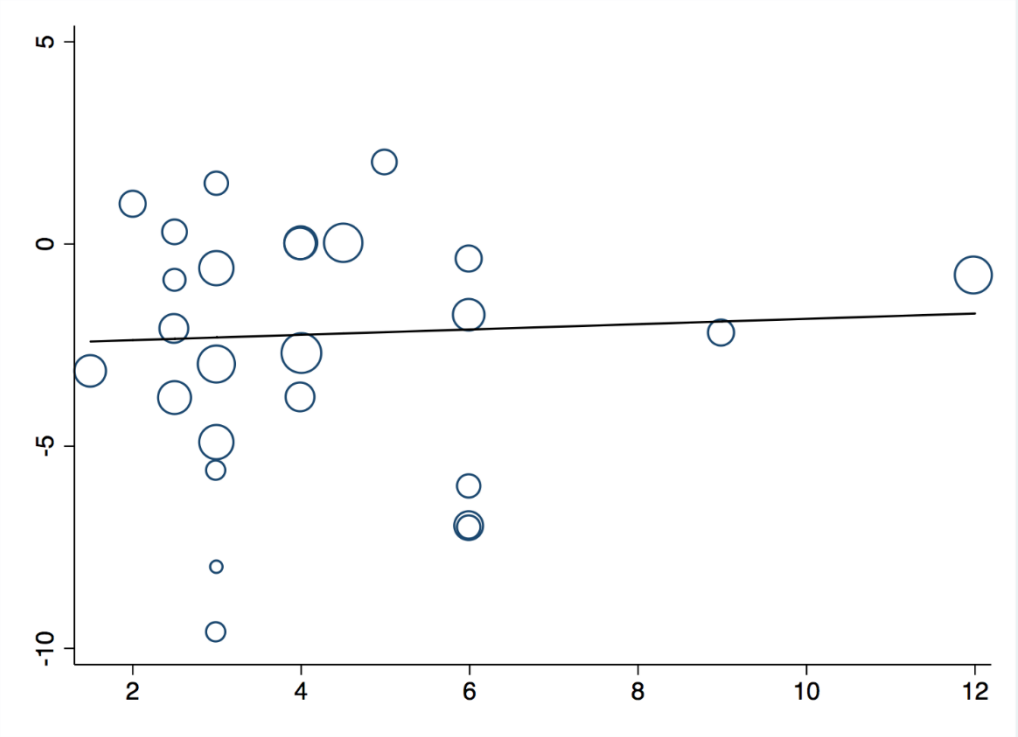
((("blood pressure" AND"hypertension"[Title/Abstract]) AND (reduc*[Title/Abstract] OR lower[Title/Abstract])) AND (diet[Title/Abstract] OR exercise[Title/Abstract] OR yoga[Title/Abstract] OR meditation[Title/Abstract] OR lifestyle[Title/Abstract])) AND (aged[Title/Abstract] OR elderly[Title/Abstract]) NOT ("drug therapy" OR medication [Title/Abstract])

## 11.2 Appendix 2

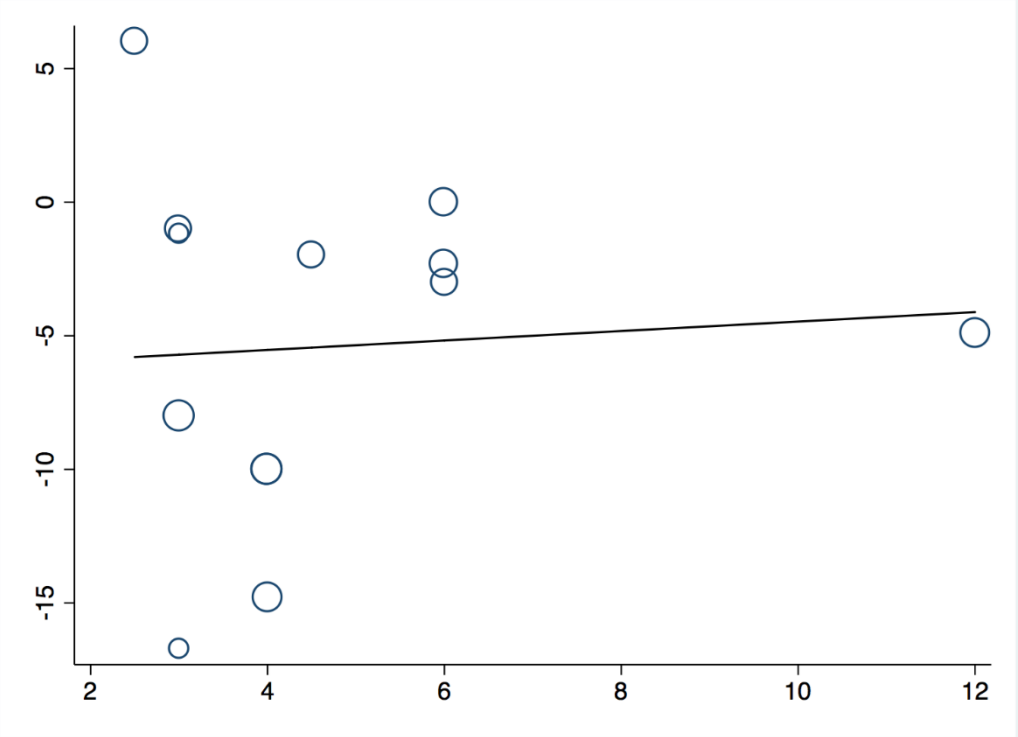
Supplementary tables and meta-regression plots for systematic review in chapter 2.



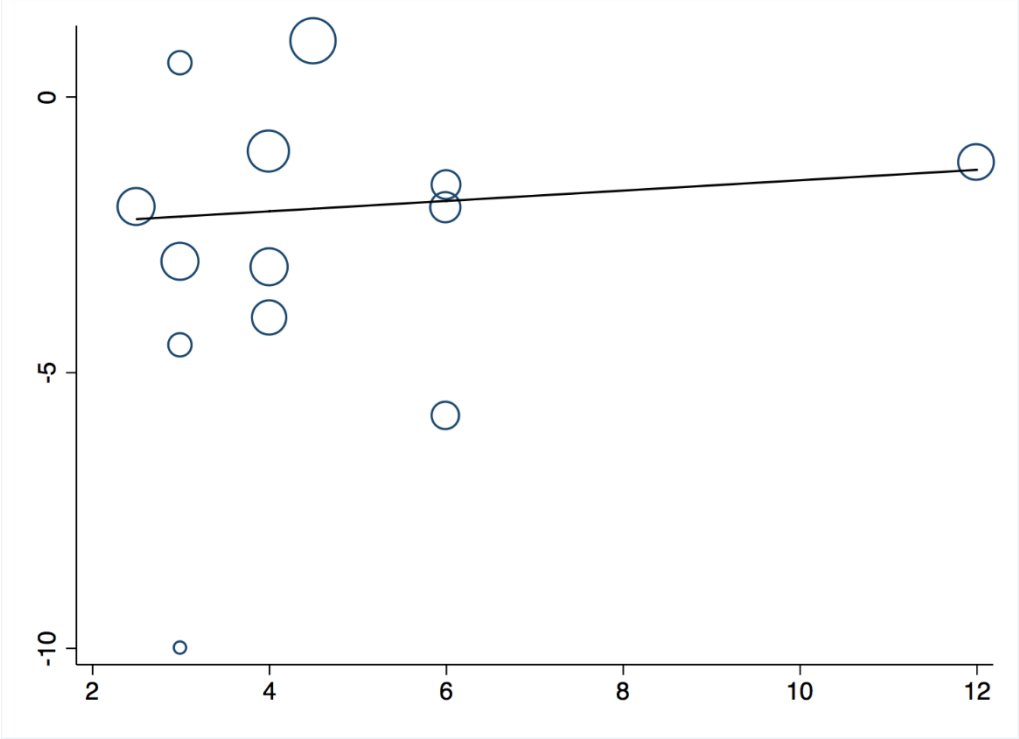

*Meta-regression plot for aerobic exercise systolic BP of change in BP (y-axis) against duration of intervention (x-axis)*



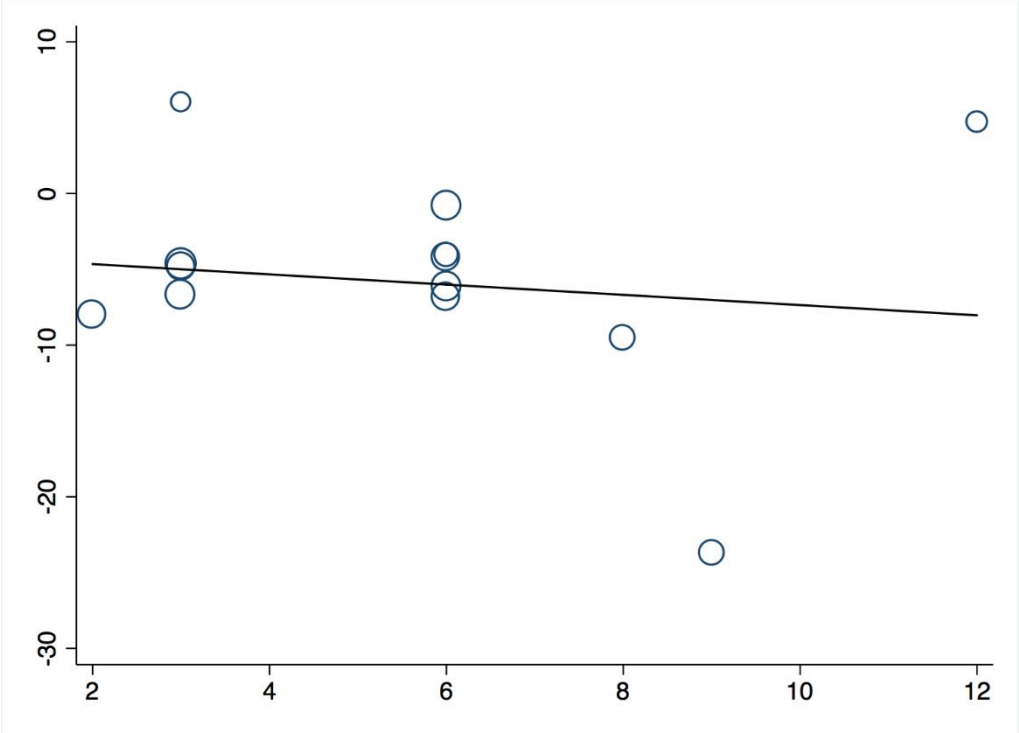

*Meta-regression plot for aerobic exercise diastolic BP of change in BP (y-axis) against duration of intervention (x-axis)*



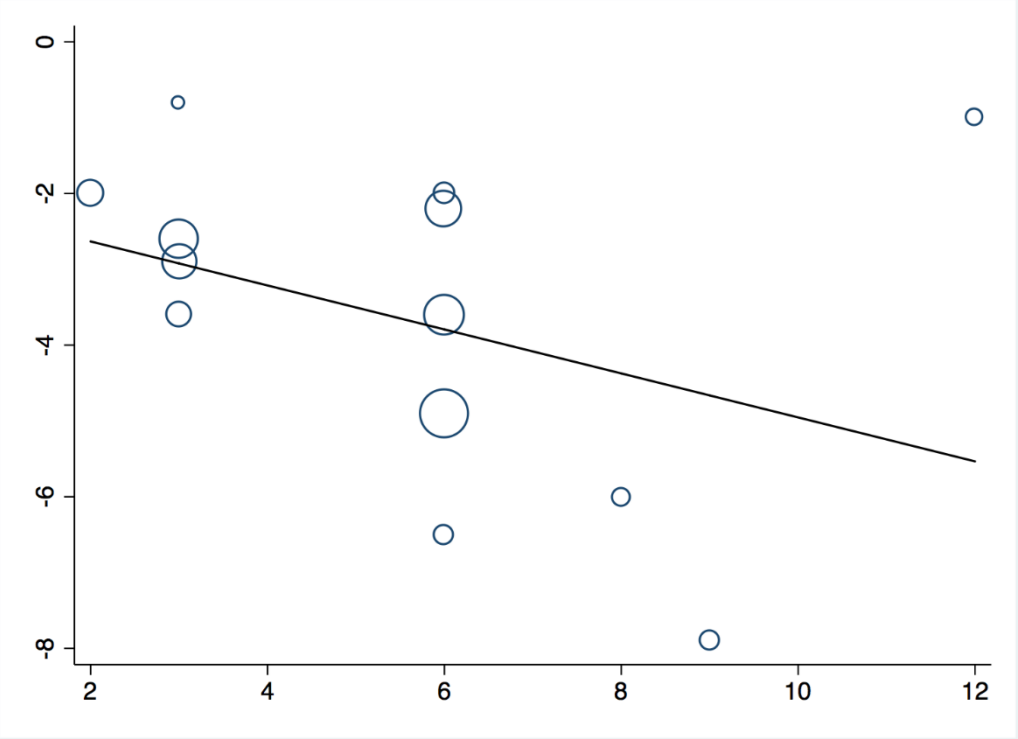

*Meta-regression plot for resistance exercise systolic BP of change in BP (y-axis) against duration of intervention (x-axis)*




*Meta-regression plot for resistance exercise diastolic BP of change in BP (y-axis) against duration of intervention (x-axis)*




*Meta-regression plot for combined exercise systolic BP of change in BP (y-axis) against duration of intervention (x-axis)*




*Meta-regression plot for combined exercise diastolic BP of change in BP (y-axis) against duration of intervention (x-axis)*

*Appendix 2 Table 1 Dietary interventions in studies included in systematic review in chapter 2*

*BP, Blood Pressure; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; COM, Combined Aerobic and Dynamic Resistance Exercise training;*

| Study | Intervention | Change in SBP (Mean +/- SD) mmHg | p (compared to control) | Change in DBP (Mean +/- SD) mmHg | p (compared to control) |
|---|---|---|---|---|---|
| Whelton 1998 | Sodium Restriction | -3.4 (14.6) | <0.001 | -1.9 (9.1) | <0.001 |
| | Calorie control – weight loss | -4.0 (15.6) | <0.001 | -1.1 (9.6) | <0.001 |
| | Sodium restriction with calorie | -5.3 (14.4) | <0.001 | -3.4 (9.6) | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| | control weight loss | | | | |
| | Non-intervention control | -0.8 (15.6) | | -0.8 (9.12) | |
| Nestel 1993 | Sodium Restriction | -2.5 | | -2.1 | |
| | Normal sodium | 4.2 | | 2.9 | |
| Bouchonville 2014 | COM | -1.2 (15.5) | 0.49 | -2.1 (6.1) | 0.60 |
| | Calorie control weight loss | -13.1 (15.1) | 0.23 | -6.7 (10.3) | 0.09 |
| | COM + calorie control weight loss | -15.9 (18.9) | <0.01 (compared to COM) | -4.9 (9.5) | 0.36 (compared to COM) |

| | | | |
|---|---|---|---|
| | Non-intervention control | -5.9 (23) | -1.1 (11.2) |

*Appendix 2 Table 2 other interventions in studies included in systematic review in chapter 2*

BP, Blood Pressure; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; AET, Aerobic Exercise Training; RET, Dynamic Resistance Exercise training

| Study | Intervention | Change in SBP (Mean +/- SD) mmHg | p (compared to control) | Change in DBP (Mean +/- SD) mmHg | p (compared to control) |
|---|---|---|---|---|---|
| Li 2003 | Cobblestone mat walking | -8.0 | 0.08 | -5.9 | 0.05 |
| | Non-intervention control | -1.0 | | -0.8 | |

| | | | | | |
|---|---|---|---|---|---|
| Li 2005 | Cobblestone mat walking | -9.0 | 0.01 | -5.8 | 0.08 |
| | Normal walking | -3.7 | | -2.6 | |
| Patil 2015 | Yoga | -13.2 (5.1) | <0.001 | -1 (5.3) | 0.38 |
| | Walking | 1 (4.6) | | -0.9 (6.3) | |
| Blumenthal 1989 | AET | Not reported | | -4 | |
| | Yoga | Not reported | | -2 | |
| | Control | Not reported | | -4 | |
| Thomas 2005 | Tai Chi | -1.0 (10.8) | 0.76* | 2.2 (8.4) | 0.43* |
| | RET | -5.2 (19.3) | 0.09* | -0.1 (9.0) | 0.40* |
| | Non-intervention control | -0.3 (15.8) | | 1.1 (8.1) | |

| | | | | | |
|---|---|---|---|---|---|
| Dusek 2008 | Relaxation therapy | -9.4 (11.4) | 0.73 | -1.5 (6.2) | 0.48 |
| | Lifestyle advice (control) | -8.8 (13) | | -2.4 (6.9) | |
| Venturelli 2015 | Relaxation therapy | -10 (3) | 0.08* | -7 (3) | 0.21* |
| | AET | -19.0 (9.5) | 0.006* | -8.0 (6.3) | 0.18* |
| | RET | -17.0 (15.8) | 0.03* | -10 (9.5) | 0.12* |
| | Non-intervention control | -0.3 (16.6) | | 0 (17.0) | |
| Teng 2007 | Classical music | -15.4 (14.1) | 0.03* | -4.3 (10.2) | 0.49* |

| | | | |
|---|---|---|---|
| | Non-intervention control | -3.6 (13.6) | -1.4 (10.6) |

* Values calculated using an unpaired t test from data presented in the manuscripts

## 11.3 Appendix 3

Main initial submission to University of Nottingham Medical School ethics committee




**Division of Medical Sciences and Graduate Entry Medicine**

**School of Medicine**

**Study Proposal**

**Title:** Exploring time-efficient strategies to improve fitness for surgery in older adults

**1. Background & Aims:**

The percentage of people aged >65 y in the UK increased from 15% in 1985 to 17% in 2010, an increase of 1.7 million people (1). One age-associated physiological change is the reduction in vascular function that is observed, both at the levels of the large arteries (2) and the muscle microvasculature (3). In itself this vascular dysfunction is associated with reduced aerobic performance (4). Cardiorespiratory fitness (marked by aerobic performance) has been shown to be an independent predictor of postoperative mortality, which provides more accurate prognostic information than age alone (5). In contrast, physical activity can reverse elements of pathophysiology associated with these conditions, including vascular dysfunction. Nonetheless, major roadblocks to exercise as a strategy to combat age-associated vascular dysfunction and associated conditions exist, namely: i) poor exercise tolerance, ii) "lack of time", iii) age-related mobility impairments, and iv) exercise resistance.

The aim of this study is to investigate whether if novel low-volume, time-efficient training strategies can improve indices of vascular health and cardiorespiratory performance in older individuals with a view towards improving their fitness for surgery. Numerous studies have demonstrated that periods of

supervised exercise training effectively improve indices of cardiorespiratory (blood pressure, aerobic capacity and blood lipids (6-9) and vascular function (10,11). However, the majority of these studies were conducted using high-volume continuous submaximal aerobic training (e.g. 50-65% $VO_2$max for 30-60 min) (12, 13) or moderate to high volume progressive weight training (14). This research group have recently shown the efficacy of a time-efficient exercise strategy known as HIIT - High Intensity Interval Training, for improving $VO_2$ max and muscle mass in young individuals with heightened metabolic disease risk (15) and also demonstrated significant improvements in $VO_2$ max comparable to classic aerobic exercise training using several different time-efficient HIIT protocols (15,16). However, despite the potential benefits of HIIT, not least its 70-80% reduction in required time-commitment compared to current WHO guidelines, it does have limitations, particularly for an older population where physical (mobility/joint) and/or socio-economic (transport/gym access/equipment purchase) barriers may render it ineffective and/or unachievable.

Alternative interventions for prevention or treatment of age-associated vascular dysfunction could be provided by isometric handgrip training (IHG) or remote ischaemic pre-conditioning (RIPC), both of which have a similar low time-commitment

compared to HIIT but are less strenuous, have potential as home-based interventions, and require only inexpensive equipment. IHG has been demonstrated to improve resting blood pressure in both normotensive and medicated hypertensive populations to a similar or greater extent as classic aerobic exercise training (17,18). However, the effects of IHG on other vascular (e.g. limb, brain and muscle microvascular blood flow) or cardio-respiratory parameters ($VO_2$ max, heart rate (resting/recovery), exercise tolerance) have not been assessed. Similarly, although RIPC has recently been shown to improve maximal athletic cardio-respiratory performance and vascular function in young subjects (19, 20), no work to date has explored the efficacy of chronic RIPC on indices of health or vascular function in older individuals.

Therefore, the aims of this project are to:

**(i)** Assess the efficacy of 6 weeks HIT, IHG and RIPC for improving indices of cardio-respiratory, vascular (brain, limb and muscle) and metabolic function in older subjects as a means of improving fitness for surgery.

**(ii)** Explore the concept of "exercise resistance" in relation to HIT, IHG and RIPC by:

- **a.** Assessing if the same degree of response heterogeneity exists for the three time-efficient training modes employed in this study as has been reported for classic resistance and aerobic exercise training

**b.** Assessing if a "non-responder" for one index (i.e., resting blood pressure or leg blood flow) is a non-responder for all other indices

**2. Overview of Study Protocol:**

This study will involve the recruitment of 60 healthy subjects (aged 65-85 y). 15 will be allocated to each group (HIIT, IHG, RIPC and control). All subjects will undergo a full medical screening before being enrolled into the study. This screening will involve measures of height and weight, a full medical history, blood tests (for FBC's, U&E's, LFT's, TFT's, lipid profiles and coagulation) and an ECG.

Subjects will be excluded if they display evidence of:

- Current participation in a formal exercise regime
- A BMI < 18 or > 32 $kg \cdot m^2$
- Active cardiovascular disease:
    - uncontrolled hypertension (BP > 160/100),
    - angina,
    - heart failure (class III/IV),
    - Significant arrhythmia,
    - right to left cardiac shunt,
    - recent cardiac event
- Taking beta-adrenergic blocking agents,
- Cerebrovascular disease:
    - previous stroke,
    - aneurysm (large vessel or intracranial)
    - epilepsy
- Respiratory disease including:

    - pulmonary hypertension,
    - Significant COPD,
    - Uncontrolled asthma,
- Metabolic disease:
    - hyper and hypo parathyroidism,
    - untreated hyper and hypothyroidism,
    - Cushing's disease,
    - type 1 or 2 diabetes
- Active inflammatory bowel or renal disease
- Malignancy
- Clotting dysfunction
- Significant Musculoskeletal or neurological disorders
- Family history of early (<55y) death from cardiovascular disease
- Known sensitivity to Sonovue

Abnormal screening results will be discussed with the subject and their GP will be informed.

If subjects are included on to the study then they will be assigned to one of the four groups (control, HIIT, IHG or RIPC).

On their first study visit (day 1) all subjects will attend after an overnight fast (from 10pm) and then undergo a DXA scan. This will be followed by an oral glucose tolerance test (3hrs) (11x 3ml blood samples). Following a standardised light meal volunteers will then undergo a supervised $VO_2$ peak test. They will finally undergo a trial trans-cranial Doppler ultrasound to ensure they have a window for measurement, measurement of their 1-rep max for a leg extension, measurement of their hand-grip strength and

maximum voluntary contraction (MVC) using a dynamometer and a short physical performance battery test.

Patients will be fitted with a continuous ambulatory blood pressure monitor at the end of day 1, which will be removed on attendance the following day.

On day 2 subjects will attend after an overnight fast (from 10pm) for 60 minutes of observed rest. They will then undergo a trans-cranial Doppler, measurement of forearm flow-mediated dilatation, measurement of leg blood flow and muscle microvascular blood flow. This will involve the placement of one 20G peripheral intravenous cannula and an injection of sonovue contrast. After these measurements, they will perform 6 unilateral leg extensions at 50% 1-rep max and then have repeat measurements of leg blood flow and muscle microvascular blood flow (totalling 3 injections of sonovue contrast). They will then be provided with a standardised light meal.

Subjects will then undergo a trans-thoracic echocardiogram and a repeat trans-cranial Doppler. Following this, subjects will

undergo a 60-minute cycling time trial at 50% $VO_2$ peak achieved the previous day. During the time trial continuous non-invasive blood pressure and heart rate monitoring will be carried out, together with a repeat trans-cranial Doppler at 30 mins. After the time trial the subjects will be observed for 30 minutes recovery time, during which a further trans-cranial Doppler and echo will be performed together with continuous non-invasive blood pressure and heart rate monitoring.

After these two 'acute' visits subjects will begin their intervention (or not if control group). This will consist of 6 weeks training for of 3 sessions per week, all of which will be fully supervised. HIIT sessions will involve 5x 1minute intervals (set at 95-125% Wmax achieved at baseline $VO_2$max test) with 90s unloaded cycling rest.

IHG sessions consist of 4 x 2minute contractions at 30% MVC with 2-minute rest periods

RIPC sessions will involve 3 x 3minute cuff inflations at 200mmHg with 3-minute rest periods

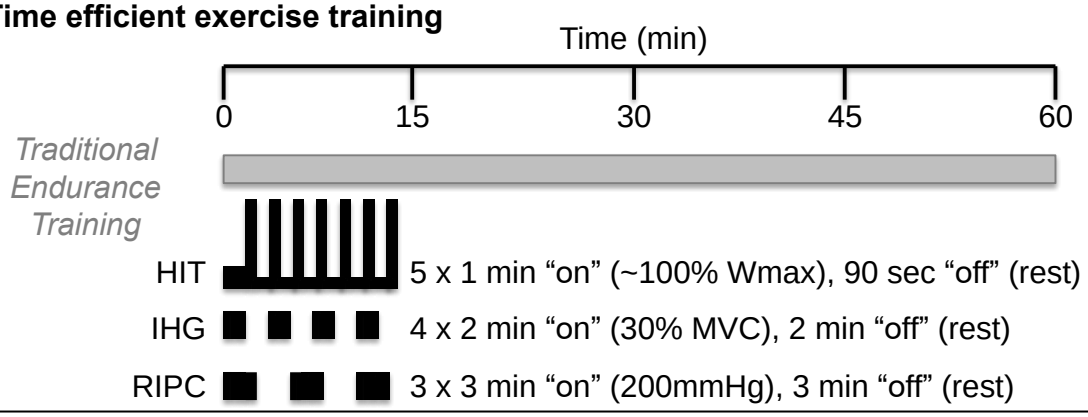
All subjects will have measurements taken of their blood pressure at two weekly intervals (either before the 3$^{rd}$ exercise session of that week, or at a separate visit for control subjects).

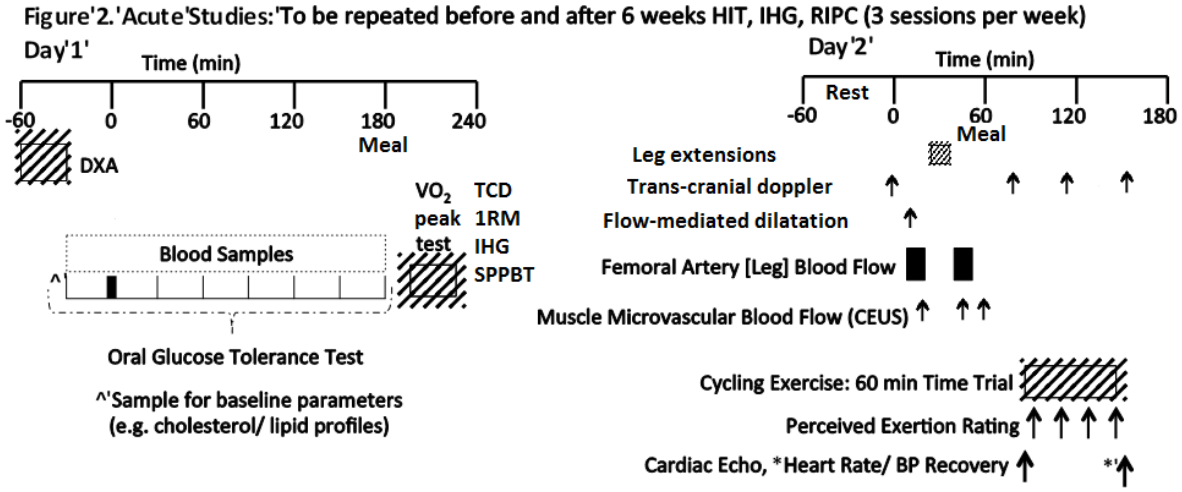
Medical cover will always be available during training sessions for all subjects and all direct supervision of exercise will be performed by an Advanced Life Support (HLS) trained individual. For the HIIT sessions, a second member of staff (in addition to the direct supervision) will always be in close proximity to the exercise training facility and a direct-access phone has been installed in the exercise training room so that additional staff can be contacted. Due to the nature of the building in Derby, all staff members of the Clinical Physiology group, could reach the exercise training facility within one minute from their standard place of work (office and/ or lab). There is access to a full resuscitation trolley, an automated defibrillator and oxygen. Similar exercise regimes of this intensity have been delivered for subjects in this age range without incident. Exercise training will be terminated immediately if a subject complains of:

- Chest pain or tightness,
- Faintness,
- Sudden pallor,
- Loss of co-ordination,
- Confusion,
- Dizziness,
- Signs of respiratory failure, or
- Palpitations

After 6 weeks intervention, all subjects will undergo the same two "acute" study days to assess for impact on the measured parameters.

**Figure 1. Time efficient exercise training modes**



**3. Measurable end points:**

The measurable end points of this study are:

i. Resting systolic blood pressure
ii. $VO_2$ peak
iii. Anaerobic threshold
iv. Body composition measured by DXA
v. Leg, muscle microvascular and brain blood flow
vi. Endothelial function via flow-mediated dilatation
vii. Resting heart rate and HR/BP recovery post exercise,
viii. Glucose handling via oral glucose tolerance test
ix. Blood cholesterol and lipid status
x. Exercise tolerance and performance via 60 min cycling time-trial
xi. Cardiac function
xii. 1-Rep max for unilateral leg extension
xiii. Handgrip strength
xiv. Short physical performance battery test
All in response to 6 weeks of exercise intervention (or control)

**4. Power calculation:**

Based on the primary endpoint of this study, reduction in resting systolic blood pressure, a power calculation suggests that we need 12 per group to detect with $\alpha$ of 0.05 and $\beta$ of 0.9 a clinically relevant difference in change in SBP vs control of 10mmHg. We will thus recruit (to allow for potential non-completion) 60 subjects.

**5. Key references:**

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# 11.4 Appendix 4

## Participant information sheet

University of Nottingham
UK | CHINA | MALAYSIA

Participant Information Sheet
(Draft Version 1.1 / Final version 1.1: 22.05.2018)
University of Nottingham REC REF: A12092016

Title of Study: Exploring time-efficient strategies to improve fitness for surgery in older adults

Name of Chief Investigator: Dr Beth Phillips
Local Researcher(s): Mr Philip Herrod, Mr Jonathan Lund

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

**Background**

One change associated with human aging is a reduction in "fitness", both in terms of how far or quickly a person can run/cycle/swim and also in how well their blood vessels work. Previous studies have shown that as people become less fit, they are at increased risk of suffering a complication whilst undergoing surgery. We also know that measuring how fit someone is, is better at predicting the risk of a complication than purely using a person's age alone. Lots of research has shown that exercise can potentially reverse some of the age-related decline in fitness, however most work so far has been using long periods of continuous exercise over a long period of time. We have recently shown at the University of Nottingham that "interval training", where people cycle on an exercise bike very hard in order to get their heart pumping very fast for as short a time as a minute, followed by a short rest and then repeated over the course of 15 minutes can more rapidly improve a person's fitness. However this kind of exercise is quite strenuous, might not be suitable for people with arthritis and can require a trip to the gym to access specialist equipment.

Alternative exercise types that could possibly be used to improve fitness in a short period of time (such as before an operation), which do not have these limitations are Isometric handgrip training and remote ischaemic preconditioning. These have been shown previously to have some effects on fitness but have not yet been properly evaluated. Isometric handgrip training involves squeezing a hand grip trainer very hard for 2 minutes, followed by a 2 minute break and then repeated three times. Remote ischaemic preconditioning involves having a blood pressure cuff blown up on an arm for 3 minutes, followed by a 3 minute break and then repeated twice. Through an effect on the blood vessels in the arm, we hope that these two forms of "static" exercise will have similar effects to that of training on the exercise bike. The hope is then that patients could be given these exercises to do in the weeks before an operation, to make them fitter for it.

We aim to recruit 60 older volunteers (age 65-85) and study the effects of 6 weeks of training using these methods on their fitness. 15 volunteers will be in a control group who will not enter a training programme. Volunteers will undergo 2 days of fitness testing and measurements of how their body functions on entering the trial. These days will involve an Xray scan of their body makeup, blood tests and two fitness tests on an exercise bike whilst measuring blood flow through the brain and legs with a scanner. These two days will then be repeated after the volunteers have undergone 6 weeks of training (3 sessions a week for 15 minutes).



**Why have I been invited?**

In order for this study to be successful, volunteers have been chosen who f
not have significant on-going medical problems. We are inviting 60 particip
take part.

**Do I have to take part?**

It is up to you to decide whether or not to take part. If you do decide to t
this information sheet to keep and be asked to sign a consent form. If yo
are still free to withdraw at any time and without giving a reason. This w
rights.

**What will happen to me if I take part?**

The location for this study is The Medical School at the Royal Derby Hospit
screening visit and 4 acute study visits. If you are selected to be in an exer
also be required to attend 3 exercise-training sessions each week for 6 weel
sessions will last approximately 15 minutes.

*Screening Visit*

Prior to be enrolled onto the study you will be asked to attend the Medica
fasted (from 10pm the night before), for a screening visit to assess your su
you the opportunity to ask questions about the study and ask you to provid
participation in the study. This visit will last approx. 60 min, during this tim

i. take a small amount of blood to measure health parameters, incl
and kidney function
ii. measure your height and weight
iii. conduct a health assessment, including an ECG to monitor your
check
iv. Measure the strength of your leg muscles

*Baseline Study Day 1*

Once we have the received the results of your blood tests and on the prem
are within normal range we will enrol you on to the study and assign you to
or control groups.

On your first study day we will ask you to attend at approx. 0900h fasted fr
for a DXA scan. DXA is short for dual-energy x-ray absorptiometry. The D
information about the type of tissue under the skin (fat, muscle etc.) and a
additional leaflet providing more information about DXA is enclosed with th

After the DXA scan, measurements will be made of the blood flow in you
machine. For one of these measurements a small intravenous cannula will
so that you can be given a small dose of an ultrasound dye, in order to mo
blood flow through your leg muscles. You will then be asked to lift some we
machine, and then the ultrasound scans will be repeated

After the blood flow measurements you will undergo an oral glucose tolera
information about how your body produces insulin and manages the sugar
small cannula will be inserted into a vein in your hand or arm and from th
blood will be drawn. This cannula will then be connected to an intravenous
the cannula patent and your hand will be placed in a warm box. You will th
sugary drink and over the following 3 hours small samples of blood pe
cannula. You will then be provided with a light meal.

After this you will be asked to undergo a CPET test. CPET is short for cardio-pulmonary exercise test. This involves cycling on an exercise bike as the difficulty is slowly increased until you cannot continue. During the test your heart, blood pressure and oxygen levels will be measured. You will also have a tight fitting face mask applied in order to measure how much oxygen your lungs are using. After the CPET we will measure your grip strength and finally asked you to perform a series of gentle exercises against the clock.

At the end of Day 1, we will fit you with a device which will monitor your blood pressure continuously over the next day until you return for Day2. This blood pressure cuff will intermittently become tight around your arm and take measurements of your blood pressure as you go about your normal activities.

*Baseline Study Day 2*

On your second day we will again ask you to attend at approx. 0900h for 40 minutes of observed rest, fasted from 10pm the night before. During this time measurements will be made of the blood flow in your arm before and after inflating a blood pressure cuff, using an ultrasound machine. Your blood pressure will also be measured several times.

After your meal you will be asked to cycle on an exercise bike for up to 60 minutes at a gentle pace, whilst your blood pressure and heart rate are measured.

*Exercise Training*

If you have been assigned to one of the exercise training groups you will start your training ~5 days after Baseline Study Day 2. The training sessions will all be fully supervised and take place at the Medical School or in the hospital. HIIT training will consist of a warm up followed by 5x 1 minute sprints on an exercise bike with 90s gentle cycling between sprints. Isometric handgrip training involves squeezing a hand grip trainer hard for 2 minutes, followed by a 2 minute break and then repeated three times. Remote ischaemic preconditioning involves having a blood pressure cuff blown up on an arm for 3 minutes, followed by a 3 minute break and then repeated twice. You will need to attend 3 training sessions each week for 6 weeks. You will be excluded from the study if you:

- Are unable to attend for more than 3 consecutive sessions,
- Miss more than 6 sessions,
- Fail to complete the set exercise regime on 6 sessions or more.

*Final study Days*

After your 6 weeks of exercise training (or control) you will be asked to return to the Medical school to repeat the measurements taken on Baseline study days 1 and 2.

**What do I have to do?**

We are studying the effects of three different types of exercise training on blood pressure and general fitness. We are studying these aspects in healthy older adults, so we need volunteers who are eating and exercising as they would normally.

With the exception of fasting on the night before your health screening and acute study days please do not modify your usual diet or activity levels for this study.

**Expenses and payments**

Participants will not be paid an inconvenience allowance to participate in the study.

**What are the possible disadvantages and risks of taking part?**

Research studies often involve some risks, not all of which may be currently known. Blood tests and the insertion of cannulae can result in bruising around the area where the needles have been.

Healthy volunteers have been chosen for this study; however, it is possible that the routin
detect unknown health problems. Examples of these include diabetes and high blood press
are common and often undiagnosed. Should this be the case, you will be informed an
attend your GP practice for further management. Your GP will also be notified. Be advised
insurance status e.g. for life insurance or private medical insurance, could be affect
diagnoses.

**What are the possible benefits of taking part?**

We cannot promise the study will help you, but if you are assigned to one of the exercis
would expect to see improvements in blood pressure control and physical fitness. Partici
study would provide you with a basic medical check-up, including clinical examination, m
of your blood pressure and blood tests for fasting glucose and functions of the kidney a
will also receive a pack at the end of the study containing data about your fitness and h
responded during the trial. In addition, the information we get from this study will give
understanding of the cardiovascular responses to static exercise, which may help us d
guidelines for exercise training in older people and potentially help improve a patient
undergo surgery. Finally, all of our volunteers are invited to our annual research celebrat

**What happens when the research study stops?**

Data collected during the study will be published in the scientific literature enabling
professionals to use the information. You will not be identified in any publication. There
delay of up to one year from the study completion before this occurs.

**What if there is a problem?**

If you have a complaint on your treatment by a member of staff or anything to do with th
can initially approach the lead investigator Dr B Phillips . If this achieves no satisfactory o
should then contact the Ethics Committee Secretary, Mrs Louise Sabir, University of
Faculty of Medicine & Health Sciences Research Ethics Committee c/o School of Medicin
Centre, B Floor, Medical School, QMC Campus, Nottingham University Hospitals, NG7 2
louise.sabir@nottingham.ac.uk. In the unlikely event that you suffer injury to yourself o
your property as a result in taking part in this research, the University does have an insu
to cover harm arising as a result of the defect in the design of the study. In addition
practitioners taking part in the research have personal medical negligence cover.

In the event that something does go wrong and you are harmed during the research an
to someone's negligence then you may have grounds for a legal action for compensation
University of Nottingham but you may have to pay your legal costs.

**Will my taking part in the study be kept confidential?.**

We will follow ethical and legal practice and all information about you will be handled in c

If you join the study, we will use information collected from you and your medical record
course of the research. This information will be kept **strictly confidential**, stored in a
locked office, and on a password protected database at the University of Nottingham. Un
Protection laws the University is the Data Controller (legally responsible for the data secu
Chief Investigator of this study (named above) is the Data Custodian (manages access t
This means we are responsible for looking after your information and using it properly. Y
access, change or move your information are limited as we need to manage your informati
ways to comply with certain laws and for the research to be reliable and accurate. To saf
rights we will use the minimum personally – identifiable information possible.

You can find out more about how we use your information and to read our privacy notice

https://www.nottingham.ac.uk/utilities/privacy.aspx.

The data collected for the study will be looked at and stored by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people from regulatory organisations to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

Where possible information about you which leaves the School of Graduate Entry Medicine and Health will have your name and address removed and a unique code will be used so that you cannot be recognised from it, however sometimes we need to ensure that we can recognise you to link the research data with your medical records so in these instances we will need to know your name and date of birth.

Your contact information will be kept by the University of Nottingham for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies (unless you advise us that you do not wish to be contacted). This information will be kept separately from the research data collected and only those who need to will have access to it. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team given permission by the data custodian will have access to your personal data.

In accordance with the University of Nottingham's, the Government's and our funders' policies we may share our research data with researchers in other Universities and organisations, including those in other countries, for research in health and social care. Sharing research data is important to allow peer scrutiny, re-use (and therefore avoiding duplication of research) and to understand the bigger picture in particular areas of research. Data sharing in this way is usually anonymised (so that you could not be identified) but if we need to share identifiable information we will seek your consent for this and ensure it is secure. You will be made aware then if the data is to be shared with countries whose data protection laws differ to those of the UK and how we will protect your confidentiality.

Although what you say to us is confidential, should you disclose anything to us which we feel puts you or anyone else at any risk, we may feel it necessary to report this to the appropriate persons.

**What will happen if I don't want to carry on with the study?**

Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw we will no longer collect any information about you or from you but we will keep the information about you that we have already obtained as we are not allowed to tamper with study records and this information may have already been used in some analyses and may still be used in the final study analyses. To safeguard your rights, we will use the minimum personally-identifiable information possible.

**Involvement of the General Practitioner/Family doctor (GP)**

Healthy volunteers have been chosen for this study; however, it is possible that the routine tests could detect unknown health problems. Examples of these include diabetes and high blood pressure, as they are common and often undiagnosed. Should this be the case, you will be informed and advised to attend your GP practice for further management. Your GP will also be notified. Be advised, that future insurance status e.g. for life insurance or private medical insurance, could be affected by these diagnoses.

**What will happen to any samples I give?**

The samples you give will be analysed in laboratories in either the Royal Derby Hospital or the Division of Medical sciences and Graduate Entry Medicine and Health at the University of Nottingham, Derby



We would also like to seek your consent so that any remaining s
possible future research – this is optional (please indicate you agre
samples will be stored with a code unique to you and securely at t
the University's Human Tissue Research Licence (no 12265).
Some of these future studies may be carried out by researchers ot
first study, including researchers working for commercial compan
be anonymised, and you will not be identified in anyway. If you
samples will be disposed of in accordance with the Human Tissue

**Will any genetic tests be done?**

In the current study we will not be using your samples for genetic

**What will happen to the results of the research study?**

Data collected during the study will be published in the scientif
professionals to use the information. You will not be identified in
delay of up to one year from the study completion before this occu
of publications resulting from this study, please inform the study t

**Who is organising and funding the research?**

This research is being organised by the University of Nottingham a
College of Surgeons of England and the Dunhill Medical Trust

**Who has reviewed the study?**

All research in healthcare is looked at by independent group of
Committee, to protect your interests. This study has been reviewe
University of Nottingham Medical School Research Ethics Committ

**Further information and contact details**

Thank you for your interest in this study. For further information
01332 724731/ beth.phillips@nottingham.ac.uk or Philip Herro
alternatively contactable at The University of Nottingham, Division
Entry Medicine, School of Medicine, Royal Derby Hospital, Derby,

# 11.5 Appendix 5

## Ethical approval

The University of Nottingham

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

**Faculty of Medicine and Health Sciences**

Research Ethics Committee
C/o Faculty PVC Office
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

14th October 2016

Mr PJJ Herrod
Research Fellow
Division of Medical Sciences and Graduate Entry Medicine
School of Medicine
Royal Derby Hospital
Uttoxeter Road
Derby DE22 3DT

Dear Mr Herrod

**Ethics Reference No:** A12092016 – **please always quote**
**Study Title**: Exploring time-efficient strategies to improve fitness for surgery in older adults.
**Chief Investigator/Supervisor:** Mr J N Lund, Associate Professor and Consultant Surgeon, Division of Medical Sciences and Graduate Entry Medicine (MS GEM), School of Medicine, Derby Royal Campus
**Lead Investigators/student**: Mr PJJ Herrod, Research Fellow, MSGEM
**Other Key Investigators:** Dr B E Phillips, Assistant Professor, MSGEM
**Type of Study:** Physiology, exercise monitoring, DXA scan
**Proposed Start Date:** 01/10/2016 **Proposed End Date:** 30/04/2019
**No of Subjects:** 60 + 15 controls **Age:** 65-85 years, Controls

**School:** Medicine MSGEM

Thank you for submitting the above application which was considered by the Committee at its meeting on 12th September 2016 and the following documents were received:

- FMHS Research Ethics Application form dated 4/8/2016
- DEXA Scan Signed off by Aline Nixon IRMER Radiation Practitioner UoN dated 12/9/2016.
- Study Proposal dated 4/8/2016
- Recruitment poster v1 dated 4/8/2016
- Participant Information Sheet v1.0: 04/8/2016
- Consent Form v1.0: 04/8/2016
- DEXA Scan information Leafletv1.1 May 2014.

These have been reviewed and are satisfactory and the study is approved.

Approval is given on the understanding that the conditions set out below are followed:

1. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).

2. You must notify the Chair of any serious or unexpected event.

The University of Nottingham

3. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

4. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

pp

**Professor Ravi Mahajan**
**Chair, Faculty of Medicine & Health Sciences Research Ethics Committee**

# 11.6 Appendix 6

## Approval for 2-week HIIT study

University of Nottingham
UK | CHINA | MALAYSIA

Email: FMHS-ResearchEthics@nottingham.ac.uk

**Faculty of Medicine & Health Sciences**
**Research Ethics Committee**
c/o Faculty PVC Office
School of Medicine Education Centre
B Floor, Medical School
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13 September 2017

**Mr PJJ Herrod**
Research Fellow
Division of Medical Sciences and Graduate Entry Medicine
School of Medicine
Royal Derby Hospital
Uttoxeter Road
Derby DE22 3DT

Dear Mr Herrod

**Ethics Reference No:** A12092016 – **please always quote**
**Study Title**: Exploring time-efficient strategies to improve fitness for surgery in older adults.
**Chief Investigator/Supervisor:** Mr J N Lund, Associate Professor and Consultant Surgeon, Division of Medical Sciences and Graduate Entry Medicine (MS GEM), School of Medicine, Derby Royal Campus.
**Lead Investigators/student**: Mr PJJ Herrod, Research Fellow, MSGEM
**Other Key Investigators:** Dr B E Phillips, Assistant Professor, MSGEM
**Type of Study:** Physiology, exercise monitoring, DXA scan
**Proposed Start Date:** 01/10/2016 **Proposed End Date:** 30/04/2019
**No of Subjects:** 45 + 15 controls+ 2 groups of 16 for HMB +12 for 2 wk HIIT **Age:** 65-85 years, Controls, 18-35y – HMB group
**School:** Medicine MSGEM

Thank you for notifying the Committee of amendment no 4: 25.08.2017 as follows:

- To recruit a further 12 subjects (aged 65-85 years) to undergo HIIT training for just 2 weeks. Subjects in this additional group would attend for only 1 study visit at each time where they would have their physical fitness and body composition assessed via CPET, SPPBT, hand-grip, DXA and muscle ultrasound. This is in order to assess the benefits of HIIT in older people and to maximise the potential translation of the work via improved exercise uptake and adherence if the required duration was shorter.
- Notice of amendment and Participant Information sheet 2 week HIIT dated 25.08.17 were received.

These have been reviewed and are satisfactory and the study amendment no 4:25.08.2017 has been given a favourable opinion.

A favourable opinion is given on the understanding that the conditions set out below are followed:

1. You should follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).
2. You must notify the Chair of any serious or unexpected event.
3. An End of Project Progress Report is completed and returned when the study has finished (please request a form).

Yours sincerely

**Professor Ravi Mahajan**
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee