Exploring the efficacy of novel environmental stimuli to improve cardiorespiratory fitness and muscle health in older adults

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

November 2020

Declaration of Authenticity

I hereby declare that the work presented in this thesis is my own and has been completed under the supervision of Mr Jonathan Lund and Dr Bethan Philips.

Tanvir Singh Sian November 2020

Acknowledgements

I would like to thank my supervisors Mr Jon Lund and Dr Bethan Phillips for their guidance, support and patience during my research.

Preya Sian is an amazing wife and without her tremendous support and understanding, I simply could not have completed this body of work. My wonderful little boy Reyan has grown so much during these four years and I thank him for his “help” when typing on the laptop.

I have to thank the other inhabitants of “The Prof Off”: Hannah Boyd-Carson, Edward Hardy and Phillip (PJ) Herrod for their fantastic friendship, humour and shoulders when times were difficult. And of course, where would I be without the help of Amanda Gates, who was vital for keeping my studies (and me) organised and on track.

My thanks to Laura Dawson, Caoimhe Dignam, Ushnah Din, Kate Hession, Isobel Kotler, Annette Kwakye and Paula Scaife for their invaluable help in running study days and HIT sessions. My thanks to Joe the Bass for (trying) to teach me stats and Brett Doleman for completing the final statistical analyses. Finally, Thomas Inns was an excellent padawan and priceless in helping me to complete my studies.

I reserve my last thanks to our incredible volunteers for their time as well as their bodies. It was a privilege getting to know each of you and I am eternally grateful to you all for your patience, soreness but particularly your commitment to helping improve health for future generations.

“This is how you do it: You sit down at the keyboard and you put one word after another until it is done. It’s that easy and that hard.”

- Neil Gaiman

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Thesis synopsis

The UK population is increasing, and people are living longer than ever before, which has implications for the whole of society. Increasing rates of comorbidity and ill-health associated with age is, and will continue to have, significant socioeconomic consequences when considering the treatment of chronic disease and comorbidity, and their implications for hospitalisation and social care for the older adult. The benefits of regular exercise for older adults have been extensively studied and are known to protect against and even reverse the effects of comorbidity. However, rates of exercise uptake amongst older but also younger adults are poor, with a worrying shift to more sedentary lifestyles in an increasingly technological age. The ageing population has also resulted in a generation of comorbid older adults for whom exercise is not possible and as such are prone to muscle wasting. In addition, low cardiorespiratory fitness, which results from prolonged inactivity, is also a risk factor for many cardiovascular and cardiometabolic diseases, and even premature mortality.

Higher-intensity exercise interventions with reduced time commitment have been shown to dramatically improve cardiorespiratory fitness and cardiometabolic health in a short timeframe and as such may be more effective in maintaining adherence to regular exercise. However, the health benefits of such regimens have not been extensively studied outside of a supervised, laboratory environment or without utilising expensive gym equipment. This thesis will explore the efficacy of a home-based, high intensity exercise regimen for improving indices of cardiorespiratory fitness in older and younger adults. It shall also explore the effects of supervision on achieving these improvements. Finally, for those who cannot exercise due to older age or co-morbidity, the effects of plant-based treatments on improving blood flow to increase muscle nutrient and oxygen delivery will also be explored.

Outputs arising from this thesis

Publications

Cocoa Flavanols Adjuvant to an Oral Nutritional Supplement Acutely Enhances Nutritive Flow in Skeletal Muscle without Altering Leg Glucose Uptake Kinetics in Older Adults. **Tanvir S Sian**, Ushnah Din, Colleen S Deane, Ken Smith, Amanda Gates, Jonathan N Lund, John P Williams, Ricardo Rueda, Suzette L Pereira, Bethan E Phillips, Philip J Atherton. *Nutrients*. 2021 May;13(5):1646.

Short-term, equipment-free high intensity interval training elicits significant improvements in the cardiorespiratory fitness of young adults irrespective of supervision.
Tanvir S Sian, Thomas Inns, Amanda Gates, Brett Doleman, Nima Gharahdaghi, Philip J Atherton, Jon N Lund, Bethan E Philips. *Frontiers in Sport and Active Living*. 26 July 2021.

Podium presentations

Four-weeks, equipment-free, high-intensity interval training (HIT) elicits improvements in the cardiorespiratory fitness and body composition of older adults irrespective of exercise supervision. **Sian TS**, Kwakye A, Kotler I, Lund JN, Philips BE
Physiology, Aberdeen, UK, July 2019

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

A randomised control trial to assess the efficacy of short-term, home-based, high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness. **Sian TS**, Blackwell JEM, Lund JN, Phillips BE
Society of Academic and Research Surgery (SARS), Nottingham, UK Jan 2018
Shortlisted for Future Work Prize
Abstract published British Journal of Surgery

Poster presentations

Four-weeks equipment-free high-intensity interval training does not improve the cardiorespiratory fitness of young healthy adults, but does elicit gains in muscle mass

Sian TS, Inns T, Lund JN, Philips BE

Europhysiology, London, UK September 2018

Abbreviations

ADP	Adenosine diphosphate
AP-1	Activator protein-1
ATP	Adenosine triphosphate
AVB	Arterio-venous balance
BMI	Body Mass Index
CF	Cocoa flavanols
CHD	Coronary heart disease
CNS	Central nervous system
COPD	Chronic Obstructive Pulmonary Disease
CPET	Cardio-pulmonary exercise test
CRF	Cardiorespiratory fitness
CT	Computer Tomography
CU	Curcumin
CVD	Cardiovascular disease
CVE	Cardiovascular event
D2O	Dueterium Oxide
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DXA	Dual-energy x-ray absorptiometry
ECGg	Epigallocatechin 3-gallate
FL	Fascicle length
FMD	Flow mediated dilatation
G6P	Glucose-6-phosphate
GDP	Gross Domestic Product
GLUT	Glucose transporter type
GTE	Green tea extract
HGS	Hand grip strength
HIT	High-Intensity interval training
HOMA-IR	Homeostatic model assessment of insulin resistance
IPAQ-SF	International Physical Activity Questionnaire - Short Format
IR	Insulin resistance
LBF	Limb blood flow
LMM	Lean muscle mass
MetS	Metabolic Syndrome
MM	Muscle mass

MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MPT	Muscle protein turnover
mRNA	Messenger ribonucleic acid
MRI	Magnetic Resonance Imaging
MT	Muscle thickness
NF-KB	Nuclear factor kappa B
NO	Nitric oxide
OGTT	Oral Glucose Tolerance Test
PA	Pennation angle
PIS	Patient information sheets
PNS	Peripheral nervous system
RCT	Randomised control trial
SBP	Systolic blood pressure
SPPBT	Short Physical Performance Battery Tests
SNS	Sympathetic nervous system
T2DM	Type 2 Diabetes Mellitus
TUG	Timed up and go
US	Ultrasound
UK	United Kingdom
V1	Visit 1
V2	Visit 2

Chapter 1: Introduction

1.1 Chapter overview

This chapter will begin by discussing the improvements in health and social care in the United Kingdom (UK) which has led to an increased life expectancy and hence an ageing population. This has implications for the future of healthcare expenditure which is set to rise dramatically in the next two decades. The chapter will then review the biological processes and structures which contribute to maintaining cardiorespiratory fitness (CRF), muscle health and energy production and the response to insult, such as ill health. Importantly, this chapter will discuss how these basic processes can falter in the older adult, leading to an increased risk of morbidity and even mortality. Finally, this chapter will conclude with an overview of strategies to improve and maintain CRF and muscle health in the older adult, including exercise training and alternatives for those who cannot regularly exercise, such as the elderly and infirm, with an introduction to three novel, plant-based treatments each with postulated health benefits.

1.2 The future of an ageing population

The UK population is increasing and people are living longer than ever before which has implications for the whole of society. The nature of this population shift needs to be understood to be able to cope with the ever-increasing demands on healthcare and to ensure the elderly live fuller and longer working lives.

1.2.1 An older UK population

In 2017, the population of the UK was 66 million, the largest ever recorded in official government statistics. By 2041, this is projected to grow to over 73 million (Office For National Statistics, 2018) (Figure 1.1). Ongoing advances in technology, healthcare and lifestyles, especially for those aged 65 and above, has led to an increase in life expectancy with the UK population consequently getting older (Office For National Statistics, 2018). In 1997, 1 in every 6 people (15.9%) were aged 65 years and over which has already increased to 1 in every 5 people (18.2%) aged 65 and over and 2.4% aged 85 and over (Office For National Statistics, 2018).

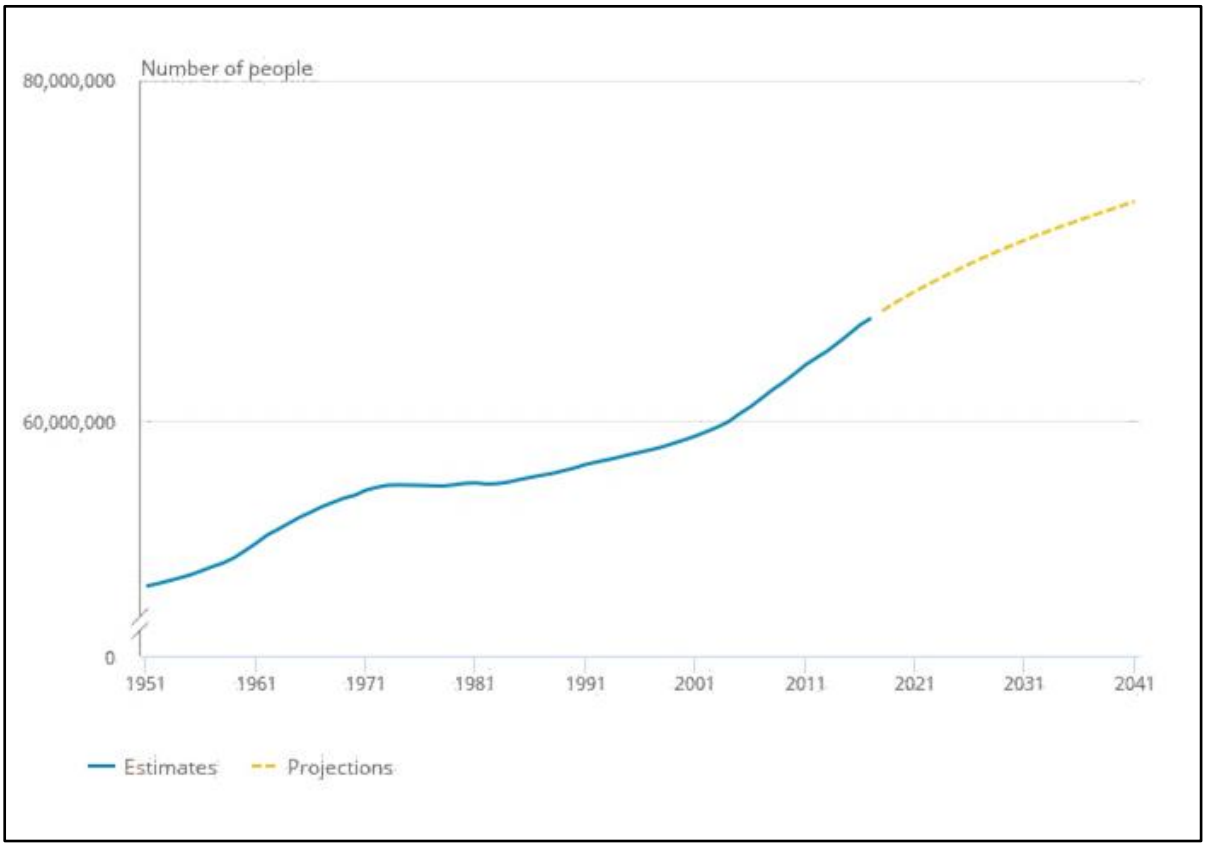


Figure 1.1: UK population estimates and projections, 1951 to 2041 (Office for National Statistics, 2018)

In mid-2014, the median age of the UK population exceeded 40 years for the first time, an increase from 33.9 years in 1974 (Government Office for Science, 2016). Compared to those born in 1991, females born in 2015 are expected to live until 82.8 years, an increase of 4 years and males to 79.1 years, an increase of 5.7 years (Office For National Statistics, 2017a). It is projected that by 2041 over 26.5% of the UK population (around 20.4 million people) will be aged 65 or above and 5% greater than 85 years old, creating significant socio-economic and health concerns from an increasing number of comorbid patients (Government Office for Science, 2016).

1.2.2 Chronic disease and the clinical impact of an ageing society

Although increasing longevity is often assumed to be accompanied by an extended period of good health, little evidence exists that older people today are experiencing better health than their parents did at the same age (Beard *et al.*, 2016). Ageing is a complex, natural phenomenon, characterized by decline in structure and function and diminished adaptability and resistance, ultimately ending in death (Hui, 2015). Chronic diseases are defined as long-term conditions that usually have a latent onset and are considered to be related to ageing, because of their high incidence in the elderly (Hui, 2015). However, it is not clear whether ageing is a cause or result of chronic disease, since chronic diseases found in the elderly are also found in young and middle-aged individuals (Beard *et al.*, 2016).

An ageing population will mean a greater prevalence of age-related conditions and an increase in the amount of ill health. Consequently, this will mean an increased overall demand for health and care services, with a projection that users of these services will grow by 86% to 393,300 in 2035 and expenditure on health to grow from 7.3 to 8.3% of Gross Domestic Product (GDP) and on long-term care from 1.1 to 2.2% of GDP (Government Office for Science, 2016) (Figure 1.2).

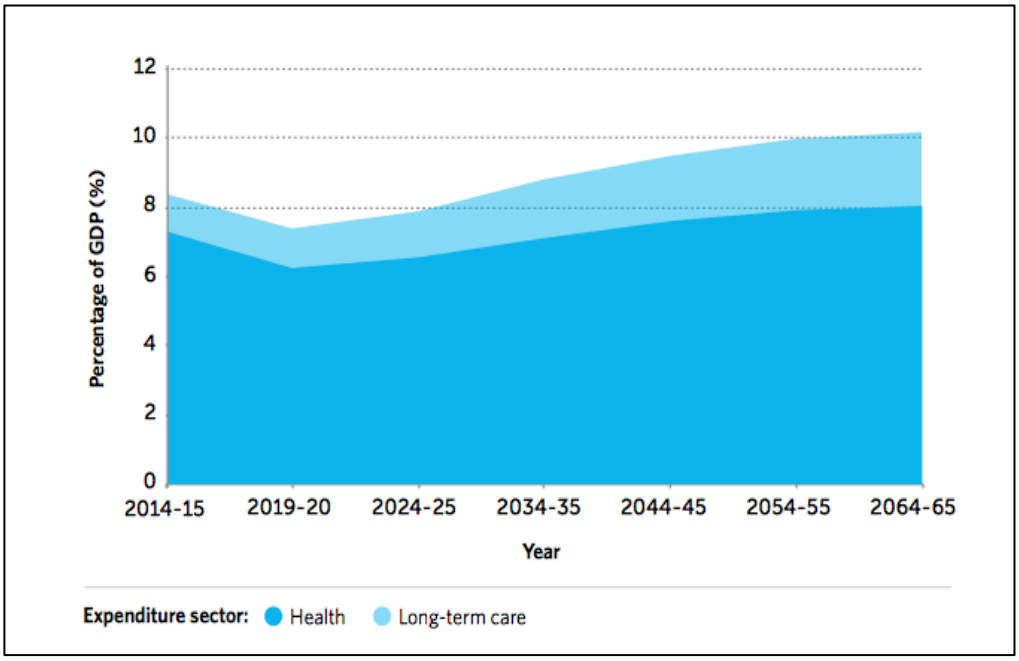


Figure 1.2: Projected public expenditure on health and long-term care as a percentage of UK GDP (Government Office for Science, 2016)

Increasing age is associated with a longer average length of stay in hospital and patients over the age of 65 account for over 35% of all hospital admissions (Evans, 2010). Cardiovascular disease (CVD) is the leading cause of death worldwide (World Health Organisation (WHO), 2017) accounting for nearly one third of deaths per year (17.3 million of 54 million total deaths per year) (Ryan, Heath and Cook, 2018). In the UK, complications of CVD remains the second most common cause of death behind cancer, and include cerebrovascular disease such as stroke, peripheral arterial disease and coronary heart disease (Office For National Statistics, 2017b).

Globally three clinical conditions dominate mortality in the over-60 age group; ischaemic heart disease (IHD), stroke and chronic obstructive pulmonary disease (COPD) with the burden from these diseases far greater in lower and middle socio-economic classes (Beard *et al.*, 2016). The greatest causes of years living with disability in this age group are attributed to back and neck pain, sensory impairments such as deafness and cataracts, depressive disorders, falls, diabetes, dementia and osteoarthritis (Beard *et al.*, 2016).

Over the last 20 years there has been a shift of the management of chronic disease from secondary to primary and community care (National Institute for Health and Care Excellence (NICE), 2015). Individuals with multi-morbidity (having several chronic health conditions at the same time) require a more complex care environment, with increased physician and specialist visits, and are likely to have higher prescription costs, use multiple medications and have a poorer quality of life with a greater mortality risk (Beard *et al.*, 2016). Physiological decline and reduced functional reserve due to ageing is likely to affect several systems leading to multi-morbidity. Amongst these are the immune, endocrine, cardiovascular, respiratory, renal, cognitive and musculoskeletal systems whilst nutritional status (Beard *et al.*, 2016) can also be a mediating factor.

1.2.3 The geriatric syndromes

Ageing is associated with an increased risk of multi-morbidity from chronic disease. Clinical conditions in older persons which do not fit into discrete disease categories are captured by the term “geriatric syndromes” (Mitchell *et al.*, 2012). These include many of the most common conditions cared for by geriatricians, such as frailty, falls, delirium, syncope, urinary incontinence and pressure ulcers (Inouye *et al.*, 2007). These syndromes are multifactorial, share many risk factors, are highly prevalent in older adults and have significant impact on their quality of life and disability, increasing their risk of needing institutional care (Inouye *et al.*, 2007).

1.2.3.1 Frailty

Between 25 and 50% of people aged over 85 are estimated to suffer with the clinical condition known as frailty, a multifactorial syndrome that represents a reduction in physiological reserve and an extreme vulnerability to environmental stressors and is foremost amongst the geriatric syndromes (Song, Mitnitski and Rockwood, 2010; Beard *et al.*, 2016). It is generally recognized to be age-associated (increasing significantly with age), being more common in older adults and related to adverse health outcomes, disability and admissions to hospital (Song, Mitnitski and Rockwood, 2010). Frailty can be quantified by the degree of impairment in functional reserves across multiple organ systems and is often associated with fatigue, reduced muscle strength and high susceptibility to disease and comorbidity (Rodríguez-Mañas *et al.*, 2013). There is a growing consensus that markers of frailty include age-associated declines in lean body mass, strength, endurance, balance, walking performance, and low activity (Beard *et al.*, 2016). In particular, frailty is more

prevalent amongst older adults who exhibit lower levels of physical activity combined with a great amount of time spent in sedentary behaviour (da Silva *et al.*, 2019).

Those aged over-65 in lower socio-economic groups are more likely to suffer from physical, psychological and cognitive health issues and therefore have the highest rates of frailty (Fried *et al.*, 2001; Beard *et al.*, 2016). A frail older person, following a minor stressor such as a urinary tract infection, experiences a larger deterioration than when they were not frail and may not return to their normal baseline functionality and as such may become dependent on services for recovery hence losing their independence (Clegg *et al.*, 2013).

1.2.3.2 Falls

Balance and gait impairment are important risk factors for falls (Clegg *et al.*, 2013), another geriatric syndrome again closely linked to frailty. Falls pose a serious health problem for the elderly and occur in 30% of adults aged over 65 and 40% over age 80 (Inouye *et al.*, 2007). It is the leading cause of unintentional injury, which is the sixth leading cause of death among the elderly worldwide, whilst also leading to functional decline, hospitalisation and rehabilitation thereby increasing healthcare costs (Inouye *et al.*, 2007).

1.2.3.3 Delirium

Delirium or acute confusion is another common presentation of a geriatric syndrome seen in secondary care, characterised by the rapid onset of fluctuating confusion and impaired awareness (Clegg *et al.*, 2013) often due to infection. Occurring in 14 to 56% of patients, delirium is associated with hospital mortality rates of 22 to 76% (Inouye *et al.*, 2007).

1.2.4 The socio-economic impact of an ageing population

Active ageing is concerned with facilitating the rights of older people to remain healthy (reducing the costs of health and social care), remain in employment longer (reducing pension costs), while also participating in community and political life (Foster and Walker, 2015). On average, men can expect to live around 15 years and women around 20 years after they stop working (Office For National Statistics, 2017b). This means that the population aged 65 years and over is for the first time growing faster than the working age population aged 16 to 64 years (Office For National Statistics, 2017b). There is also an associated increase in the average number of people in the UK spending time in retirement (Office For National Statistics, 2017b).

Working until later in life has benefits for individuals, employers and the state. Extending working life brings many positive personal benefits, such as financial stability, better health, well-being and cognition and positive social networks (Fung, 2013). Retaining older workers is likely to be increasingly important for employers; the intangible industry-specific knowledge, networks and skills developed are not readily available from new workers (Government Office for Science, 2016). Longer working lives could mean more people paying taxes for longer, thereby reducing the welfare bill; in 2014 there were 2.9 million people out of work aged over 50 to state pension age which amounted to £7 billion on out-of-work benefits for those in this age bracket (Government Office for Science, 2016). The UK economy is however still likely to face significant challenges in financing the state pension in the future (Office For National Statistics, 2017b). If current workforce exit rates continue for over-60s, the UK will face a severe labour shortage with only 7 million new workers set to enter the workforce over the next decade (Government Office for Science, 2016).

Health is an important factor that determines the employment and effectiveness of older workers. Forty four percent of those aged 50 to 64 years who would like to work are unable to because they are sick or disabled and it is estimated that if the employment rate of this age range matched that of those aged 35 to 49, it would add more than 5% to UK GDP, or £88 billion (Office For National Statistics, 2017b).

Rather than channelling frail older people into institutional care, an “ageing-in-place” policy emphasises support and resources to help the individual remain living in their own home and community settings (Means, 2007) whilst also reflecting the preferences of most older people, who generally wish to “stay put” in their own homes (Wiles, 2004). Yet the home may also have negative connotations, including poor housing conditions, insecure tenancies and inappropriate design (Sixsmith *et al.*, 2014). These issues can undermine the skills and abilities of the older person, creating an environment that is isolating and excluding. Furthermore, the home can be a source of tension when the requirements of the older person conflict with family members or others involved in the decision-making processes concerning care provision (Sixsmith *et al.*, 2014).

Social isolation and loneliness are particularly problematic in old age due to decreasing economic and social resources, functional limitations, the death of relatives and spouses and changes in family structures and mobility (Courtin and Knapp, 2017). Social isolation has been identified as a risk factor for poor health, reduced well-being, depression, cognitive decline and even mortality (Courtin and Knapp, 2017).

1.3 Skeletal muscle and body composition

Locomotion is a critical ability for animals, allowing the finding of food and escaping from predators. Even in modern day life where food is in relative abundance and predators are scarce, we still rely on skeletal muscle for almost every interaction with our environment and activities of daily living. Skeletal muscle, is the most abundant tissue type in humans, making up approximately 40% of the total body weight and containing 50 – 75% of all body proteins (Hoppeler and Fluck, 2002). The human body has more than 500 skeletal muscles which connect and support the skeleton and when combined, this permits movement (Tieland, Trouwborst and Clark, 2018). They have crucial roles in generating mechanical energy for force and power during exercise as well as being a metabolically active tissue type, maintaining core temperature and being an important source of storage for carbohydrates and particularly amino acids when responding to stress and infection (Frontera and Ochala, 2015).

1.3.1 Skeletal muscle structure

Skeletal muscle is mainly composed of water (75%) and protein (20%), with the remaining 5% made up of inorganic salts, fat, carbohydrate and minerals (Frontera and Ochala, 2015). As seen below in Figure 1.3 from Frontera and Ochala (2015), skeletal muscle architecture is characterised by a specific arrangement of muscle fibres called myofibers which are encased in a layer of connective tissue called the epimysium. Groups of myofibers which make up an individual muscle are arranged in bundles and surrounded by a second layer of connective tissue called the perimysium. Individual muscle fibres have their own cell membrane covering it called the sarcolemma and is comprised of thousands of smaller units called myofibrils which in turn contain billions of myofilaments (Figure 1.3).

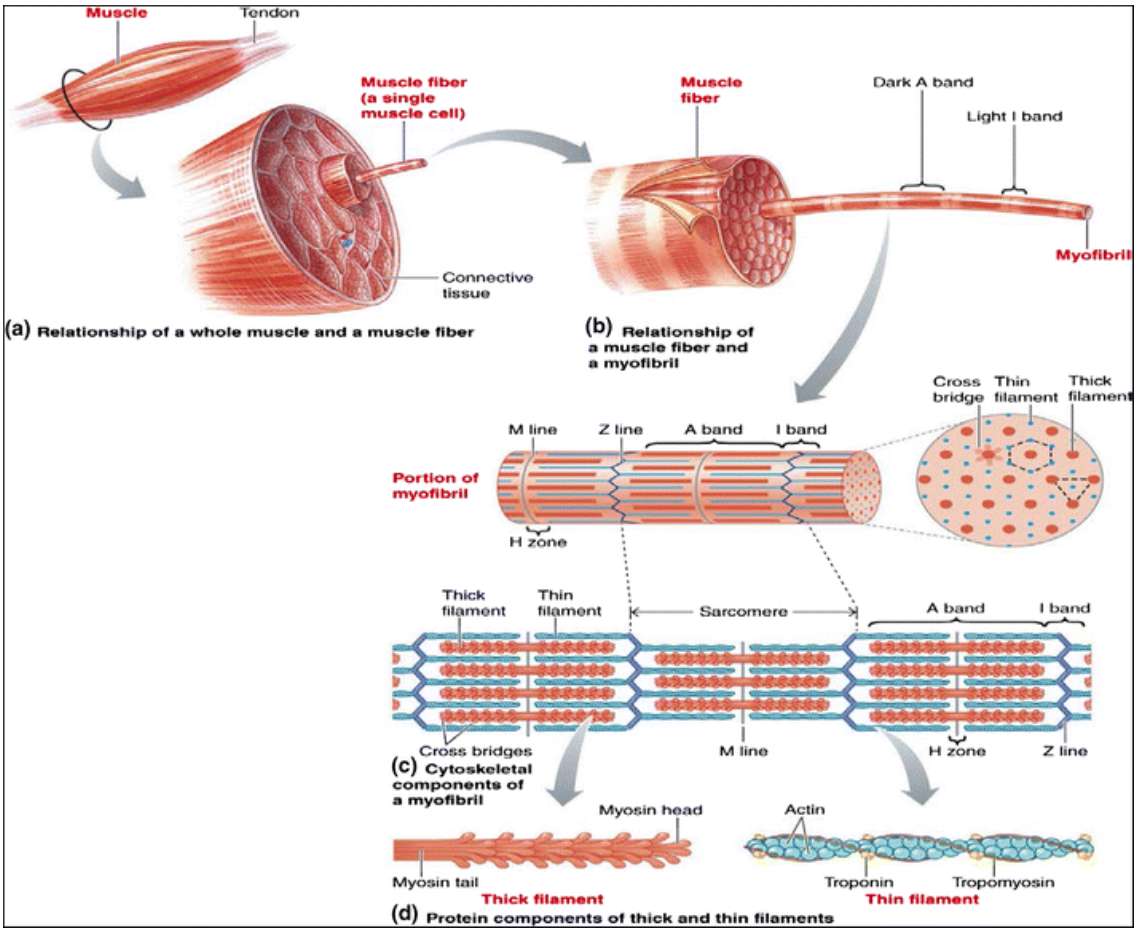


Figure 1.3: Macro and microscopic structure of skeletal muscle (Frontera and Ochala, 2015)

It is these myofilaments that form the basic contractile unit of skeletal muscle called sarcomeres (Frontera and Ochala, 2015). The two main myofilaments, which make up approximately 80% of the total protein content of a single muscle fibre, are actin and myosin and the “cross-bridges” that these proteins form are responsible for the generation of contraction and force and therefore movement (Frontera and Ochala, 2015). Ageing, as discussed later, results in the loss of integrity of these sarcomeres, resulting in decreased strength and muscle contractility, as well as reducing the potential amount of amino acids available for times of stress, illness or injury (Mitchell *et al.*, 2012).

1.3.2 Skeletal muscle function

The functions of skeletal muscle can be broadly classified into two main categories: mechanical, where the conversion of chemical energy into mechanical energy allows locomotion and participation in social settings, maintains health and contributes to functional independence and metabolic, where it serves as an important reservoir for amino acids,

carbohydrates and the maintenance of core temperature by creating heat (Frontera and Ochala, 2015). Skeletal muscle therefore plays a pivotal role in providing the skeleton with support, movement, proprioception and endurance, but more than just having the role of "moving the body", is the primary reservoir of amino acids for other tissues, the nitrogen component being a vital component of DNA (Weinert, 2009). As discussed later in section 1.4, skeletal muscle is crucial for maintaining glucose homeostasis, glycogenesis and fatty acid metabolism (Tieland, Trouwborst and Clark, 2018).

Specific environmental challenges require muscle tissue to be able to adapt to variables such as exercise, temperature and disease (Hoppeler and Fluck, 2002). Skeletal muscle is dynamic and displays great plasticity or the ability to change its function (Frontera and Ochala, 2015) in response to these environmental stimuli. The response to these external stressors can be rapid and extensive, being able to make adaptions in as little as 28 days (Hoppeler, 2016).

1.3.3 Quantifying body composition

Historically, quantifying skeletal MM was markedly difficult compared to fat and bone mineral. This now seems trivial given the wealth of imaging techniques available today that provide accurate and repeatable measurements of skeletal MM such as computer tomography (CT) and magnetic resonance imaging (MRI) (Heymsfield *et al.*, 2014; Franchi *et al.*, 2018). CT was the first to gain acceptance into clinical medicine and gives an excellent global evaluation of all tissue types, being able to accurately distinguish between adipose tissue, skeletal muscle and bone (Heymsfield *et al.*, 2014). Even with the latest advances in CT scanners there remains a high radiation exposure, especially for whole-body measurement, and when considered with the cost implications of CT, it does not form a good modality for research purposes. MRI is considered the "gold standard" for clinical and research imaging of skeletal muscle (Franchi *et al.*, 2018), however it remains a very expensive and less readily available modality in the field of research with the need for expert review of the final images.

Dual-energy x-ray absorptiometry (DXA) has emerged as a relatively low cost, reliable and low radiation modality for assessing whole-body and regional soft-tissue composition (Silva, Heymsfield and Sardinha, 2013). Originally developed to evaluate bone mineral density (Ponti *et al.*, 2019), it is now accepted worldwide for whole-body density and regional body composition assessment for both clinical and research purposes, by providing instant feedback in the form of numerical data for the user (Heymsfield *et al.*, 2014). DXA divides

the body into three components, namely bone mineral content, lean mass and fat mass, and by assessing these three components at a molecular level, DXA is widely considered to be the gold standard for assessment of body composition in clinical practice (Ponti *et al.*, 2019). DXA is however less precise in morbidly obese patients owing to the relatively little radiation involved (Silva, Heymsfield and Sardinha, 2013), is affected by hydration status during repeat scans and variation exists between the different models of scanner (Cruz-Jentoft *et al.*, 2019).

Figures 1.4 and 1.5 show ultrasonography (US) being used to assess muscle architecture, and this has also emerged as a repeatable and reliable, non-invasive modality for characterising the changes in muscle architecture in local or single muscle groups (Franchi *et al.*, 2018). Adaption and changes to muscle structure and function can be assessed using three measurements of muscle architecture. The first is the pennation angle (PA, Figure 1.4), the angle between a fascicle's orientation and the attached deep aponeurosis (Franchi *et al.*, 2015) which plays a significant role in determining force and contractility (Lee *et al.*, 2015) and is therefore an indicator of muscle strength. The larger the pennation angle, the more contractile material can be packed within a certain volume and thereby increasing the muscle's capacity to produce force (Strasser *et al.*, 2013). Secondly, the change in the muscle thickness (MT, Figure 1.5) which estimates the cross-sectional area at the point of interest. It is a reliable representation of body MM (and indeed an indicator of lean muscle wasting) and increases post-training are a representation of muscle hypertrophy (Franchi *et al.*, 2018). Finally, we consider the change in the length of the muscle fascicle (FL, Figure 1.4) which corresponds to the number of sarcomeres arranged in a myofilament and seen as another measure of contractility (Randhawa and Wakeling, 2015). It is measured by the distance from the intersection of the fascicle with the superficial aponeurosis to the intersection with the deep aponeurosis, with a longer length suggestive of greater contractility (Randhawa and Wakeling, 2015).

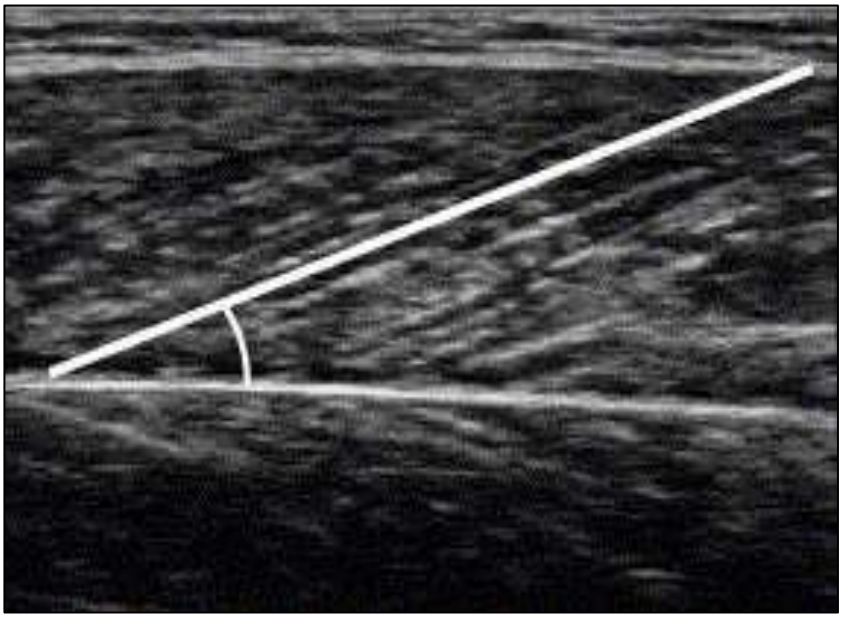


Figure 1.4: Fascicle length and Pennation Angle as measured on ultrasound (Lee et al., 2015). Using computer software, a line is drawn over a fascicle which is seen to be connected to both the superficial and deep aponeuroses and the angle with the deep aponeurosis

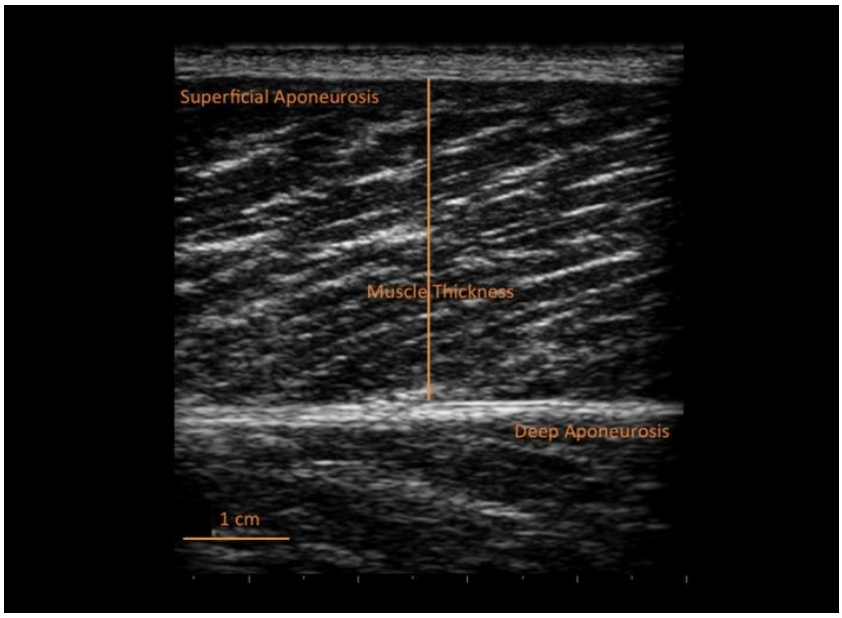


Figure 1.5: Ultrasound image of the vastus lateralis at 50% of femur length with muscle thickness demonstrated (Franchi et al., 2018)

1.3.4 Skeletal muscle mass and its relation to whole-body health

Maintenance of skeletal muscle protein turnover (MPT) and therefore MM is governed by a balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB); to gain MM, MPS must exceed MPB (Wolfe, 2006a). Clinicians have long held the view that a higher lean MM is usually considered as a strong indicator of better physical functioning and also CRF (Hoppeler and Fluck, 2002). The stressed state, seen in illness such as sepsis, cancer, trauma, chronic disease and post-surgery imposes greater demand for amino acids from MPB (Wolfe, 2006b). These illnesses are therefore often associated with a rapid and extensive loss of MM, strength and metabolic function (Wolfe, 2006b), which creates a significant problem for those with pre-existing deficiencies in MM such as the elderly.

MM is lost in the elderly due to an imbalance of MPS and MPB. The relationship of reduced MM to increased morbidity and mortality is strong (Barbat-Artigas *et al.*, 2014). Decreased MM and function make it more difficult for cells to repair themselves following a stressor to the body as muscle cannot provide the amino acids needed to facilitate tissue repair. An example of this is during hospitalisation; if there is pre-existing deficiency such as frailty, discussed in section 1.2.3, further acute loss of MM and function may push an individual over a threshold that makes adequate recovery unlikely to occur, resulting in morbidity or even mortality (Wolfe, 2006b).

In a recent study, Boo *et. al* demonstrated that skeletal MM was directly correlated to peak oxygen consumption or VO_2peak, an important marker of CRF, in community-dwelling elderly men (Boo *et al.*, 2019), further suggesting that MM is closely associated to CRF. Williams *et. al* demonstrated an association between MM and anaerobic threshold (AT) (another marker of CRF which will be discussed later) in colorectal cancer patients and healthy control subjects (Williams *et al.*, 2014), again highlighting the importance of maintaining MM for CRF.

1.3.5 Sarcopenia, skeletal muscle and fat mass alterations with ageing

Ageing affects all species and substantially influences the scope of our activities and quality of life (Nair, 2005). The loss of MM with ageing represents a major shift in whole body protein distribution and a partial loss of a primary protein reservoir that can be utilized to supply amino acids for synthesis of new body proteins (Evans and Campbell, 1993). From as early as the age of 20 up to 80 years of age, there is an estimated 30% loss of lean MM (Evans, 2010). It has also been well documented that as individuals age, along with losses

in lean MM, there is a preference to replace muscle tissue with less metabolically active adipose tissue, which increases the risk of cardiometabolic diseases (St-Onge and Gallagher, 2010).

Age-related declines in MM and function are first noticeable at the age of 50 and are similar to those that are seen in reduced physical activity (Faulkner, Brooks and Zerba, 1995), a process referred to as sarcopenia. For years there was no established universal definition of sarcopenia and a commonly accepted definition was “the involuntary, age-associated loss of skeletal MM and function” (Cruz-Jentoft *et al.*, 2010; Siparsky *et al.*, 2014). It is a multifactorial process caused by the combined effects of age-associated decreases in physical activity, nutritional intake, hormonal and neural stimulation of the muscle and chronic illness (Dennis *et al.*, 2008; Walston, 2012; Papa, Dong and Hassan, 2017). Peak changes are seen in those aged 70 and above and this group should be targeted for early intervention to prevent severe, irreversible MM loss (Siparsky *et al.*, 2014). Sarcopenia is a worldwide issue and has huge personal and socioeconomic implications (Cruz-Jentoft *et al.*, 2010) with future estimates for healthcare budgets in the billions of pounds.

In 2018 the European Working Group on Sarcopenia in Older People (EWGSOP) put forward a new definition, expanding on their work in 2010 above (Cruz-Jentoft *et al.*, 2019). This included defining sarcopenia as a disease of muscle constituting muscle failure (Cruz-Jentoft *et al.*, 2019). Low muscle strength is now defined as the primary parameter of sarcopenia as muscle strength is presently the most reliable measure of muscle function and sarcopenia is probable when low muscle strength is detected (Cruz-Jentoft *et al.*, 2019). Furthermore in the new definition, a sarcopenia diagnosis is confirmed by the presence of low muscle quantity or quality; when low muscle strength, low muscle quantity/quality and low physical performance are all detected, sarcopenia is considered severe (Cruz-Jentoft *et al.*, 2019).

1.3.6 Potential mechanisms of sarcopenia

Muscle is a highly vascular and metabolically active tissue requiring a high rate of muscle protein turnover to prevent degradation (Siparsky *et al.*, 2014). As discussed previously, the balance of MPS to breakdown must favour synthesis to maintain and improve MM, but this ability is lost in older age, leading to losses in lean MM. Sarcopenia leads to a reduction in the complement of motor neurons and muscle fibres and significant reduction in the reserve capacity of the neuromuscular system (Vandervoort, 2002). Sarcopenia is complex and likely multifactorial, however most studies would suggest it is governed by age-related

influences on two key mechanisms of muscle function; the functioning of motor units and muscle protein turnover (Dennis *et al.*, 2008). Various risk factors related to ageing have an impact on these two mechanisms, including reduced activity, nutritional intake and chronic diseases (Santilli *et al.*, 2014).

As activity and the amount of lean muscle decreases, there is a preferential increase in total body mass fat and visceral fat deposition as fat is less metabolically active than muscle (Evans and Campbell, 1993), requiring less turnover and maintenance which would otherwise consume energy. For example, limb muscles from older individuals are 25-35% smaller and are comprised of increased fat and connective tissue compared to younger individuals (Koopman and van Loon, 2009) (Figure 1.6).



Figure 1.6: CT scan of the upper leg, mid-thigh in a 25 and 85-year-old male subjects. Of note is the increased subcutaneous fat, reduced muscle area and increased fat and connective tissue infiltration into the muscle in the 85-year-old subject (Koopman and van Loon, 2009)

1.3.6.1 Reduced activity levels

A natural, age-related decline in activity levels has previously been well documented (Evans, 2010). The underlying theory remains that a natural decline in physical activity as we age leads to reduced metabolic activity in general and an overall decrease in muscle protein turnover (Evans, 2010). The imbalance between the rate of MPS and breakdown in sarcopenia is thought to cause an overall negative balance in protein turnover and hence muscle protein wasting (Yarasheski, 2003).

1.3.6.2 Reduced limb blood flow

Studies have consistently shown that ageing is associated with a 20-30% reduction in supine resting leg blood flow leading to an increase in vascular resistance (Donato *et al.*, 2006). This age related decrease in blood flow, and subsequent reduction in oxygen and nutrient delivery, is hypothesized to be a major factor in the aetiology of sarcopenia, as again the balance of protein turnover is disturbed in favour of negative balance, leading to muscle wasting (Dinenno *et al.*, 2001).

1.3.6.3 Neuromuscular stimulation

Studies have concluded that there is a reduction in the number of alpha motorneurons from the spinal cord and degeneration of their axons above the age of 60, in some cases up to a 50% loss (Porter, Vandervoort and Lexell, 2007). Motorneuron death and denervation, with reduced regenerative abilities, leads to decreased stimulation of motor units (Sions *et al.*, 2012). As neural innervation is crucial to the maintenance of muscle fibres (Janssen and Ross, 2005), the resulting decline in the number of neuromuscular junctions leads to reduced function and number of fast-twitch (type II) muscle fibres, which produce large tension output and therefore reduced function of motor units (Faulkner *et al.*, 2007). Those fibres which do not become re-innervated undergo severe atrophy and are lost.

The effects are compounded by a decrease in the number and action of skeletal muscle satellite cells which are responsible for forming new myofibres after muscle damage (Dennis *et al.*, 2008; Snijders, Verdijk and van Loon, 2009). Their role in muscle repair is greatly decreased in elderly muscle, the loss of regenerative function again resulting in atrophy of type II muscle fibres and hence less force for muscle contraction (Mitchell *et al.*, 2012).

1.3.6.4 Mitochondrial dysfunction

Maintenance of skeletal MM is further influenced by the action of mitochondria which are responsible for providing energy for all tissue types. Inactivity and ageing are associated with the loss of mitochondrial dynamics and proteins which are critical for the turnover and maintenance of healthy mitochondria (Wyckelsma *et al.*, 2017). There are significant changes in the mitochondrial ultrastructure of aged skeletal muscle, appearing microscopically enlarged and more rounded in shape with shorter cristae than in younger mitochondria (Seo *et al.*, 2016). Impairments of mitochondrial biogenesis mediated by increases in age related mitochondrial DNA oxidative damage results in reduced messenger RNA abundance and expression of mitochondrial proteins, leading to mitochondrial dysfunction and reduced regeneration (Nair, 2005). This in turn causes a reduction in the maximal ATP production and aerobic respiration rate which results in reduced CRF and directly reduces muscle protein turnover, therefore reducing motor unit quality and function (Seo *et al.*, 2016). Maintaining healthy mitochondrial quality is therefore essential for defeating age-related muscle dysfunction and weakness (Kim, Triolo and Hood, 2017).

1.3.6.5 Endocrine changes

Hormonal changes during the ageing process have an important role in changes to muscle physiology. Testosterone is the most potent anabolic steroid, stimulating skeletal MPS and hence increasing MM whilst also inhibiting protein degradation (Volpi, Nazemi and Fujita, 2004). In males, endogenous testosterone production begins to decrease around the age of 35-40 years, with circulating concentration levels decreasing by 1-3% per year (Horstman *et al.*, 2012). This may contribute to the “anabolic resistance” seen with increasing age. It therefore follows that an age-related decrease in testosterone results in a decrease in MPS and ultimately loss of lean MM (Volpi, Nazemi and Fujita, 2004; Horstman *et al.*, 2012). Reduced testosterone levels in males may also have a role in decreased levels of physical activity (Siparsky *et al.*, 2014), which as discussed earlier in this section, can lead to a higher risk of morbidity.

Similarly for women, 80% of oestrogens are lost in the first year following menopause (Horstman *et al.*, 2012). Oestradiol in women is known to play a key role in muscle and bone health. Following menopause, there is an accelerated decline in MM and strength which is directly related to the loss of oestradiol (Carville, Rutherford and Newham, 2006). Following the menopause, the average levels of testosterone in women is approximately 15% of premenopausal women (Horstman *et al.*, 2012). Although the biological role of

testosterone in women remains unclear, the sharp and rapid decline in androgen levels that accompany aging in women may play a critical role in the functional limitations seen in aging and may increase morbidity (Horstman *et al.*, 2012).

1.3.7 Clinical consequences of sarcopenia

Age-related changes in skeletal muscle lead to increasing difficulties for elderly people to conduct most everyday tasks. As a consequence of the ageing UK population, it is estimated that up to nearly half of men and women over 65 years old are likely to be sarcopenic, which will be a serious consideration for future healthcare costs as discussed above. Pronounced muscle atrophy, muscle weakening and an apparent slowing are inevitable followers of increasing age (Andersen, 2003). Sarcopenia is highly correlated with frailty and risk of falls in the elderly, and represents an important risk factor for disability and mortality (Santilli *et al.*, 2014). The relationship to functional impairment and disability also leads to a loss of independence in the elderly (Goodpaster *et al.*, 2006).

The quantitative loss in muscle cross-sectional area seen in sarcopenia is a major contributor to the decrease in muscle strength seen with advancing age (Frontera *et al.*, 2000). Despite the loss of type II muscle fibres and decrease in the number of active motor units, the overall size of the motor units increases due to new collaterals from more active type I fibres (Brunner *et al.*, 2007; Siparsky *et al.*, 2014). The cross-sectional area and mass of muscle in the elderly is smaller because of the loss of type II fibres which are responsible for providing strength in the motor unit (Siparsky *et al.*, 2014). It is this reduction in strength which is associated with an increased frailty and mortality risk (Newman *et al.*, 2006).

Metabolic changes in muscle, such as disruption of mitochondrial function, contribute to the overall physical fitness capacity of the elderly and are an important component of the reduction of around 30% of VO_2max (Volpi, Nazemi and Fujita, 2004) therefore there is a correlation between sarcopenia and reduced CRF with loss of lean MM (Boo *et al.*, 2019). Furthermore, the reduction in the amount of muscle available for gluconeogenesis and lipid oxidation increases the likelihood of developing a range of chronic disorders such as hypercholesterolaemia, atherosclerosis, hypertension and insulin resistance leading to type 2 diabetes (Lancha *et al.*, 2017). There are also potentially huge socioeconomic consequences of an increasing population age arising from the implications of sarcopenia on health as discussed above.

1.3.8 Loss of muscle strength and dynapaenia

More emphasis is being placed on the muscle quality which is also seen to reduce in ageing and correlates to reduced muscle strength which appears to be lost more rapidly as we age than MM (Mitchell *et al.*, 2012), leading to reduction in physical ability in the elderly. Although a loss of MM is associated with a decline in strength in older adults, this strength decline is much more rapid than the concomitant loss of MM, again suggesting a decline in muscle quality (Goodpaster *et al.*, 2006). This loss of skeletal muscle strength which is not associated with disease is termed dynapaenia (Mitchell *et al.*, 2012) and whilst holding some clinical significance (median MM loss in longitudinal studies per annum is 0.8 - 0.98% in men and 0.64% - 0.70 % in women and strength lost at 3 - 4% in men and 2.5 - 3% in women), it is still a relatively new concept needing more study (Mitchell *et al.*, 2012).

1.3.9 Fat mass and distribution

Lipids are fundamental building blocks of all cells being key components of the plasma membrane and other cellular components and play many important and varied roles (Muro, Atilla-Gokcumen and Eggert, 2014). They are small molecules that are insoluble in water (hydrophobic) which are classified into eight categories based on their chemical properties, the most important pertaining to CVD being fatty acids and sterol lipids, such as cholesterol (Zhang *et al.*, 2018), which will be discussed in more detail in section 1.5. Lipids are regarded as potent signalling molecules that regulate a multitude of cellular responses such as ion channels, cell receptors, growth and apoptosis and metabolic pathways, such as energy storage sources, transfer and inflammatory regulation (Fahy *et al.*, 2011; Zhang *et al.*, 2018).

Adipose tissue is the main lipid storage in the body and as such has a crucial role in buffering the daily influx if dietary fat entering the circulation (Goossens, 2017). These various fat depots in the body have unique characteristics ranging from smaller, specific depots that track with visceral fat such as pericardial and buccal to larger depots such as subcutaneous and intra-abdominal (Jensen, 2008). Subcutaneous white adipose tissue depots under the skin store 80-90% of total body fat mainly in the abdominal, subscapular, gluteal and femoral areas (Karastergiou *et al.*, 2012). Intra-abdominal depots include visceral adipose tissues, such as omental and mesenteric, which account for 6-20% of total body fat and drain into the portal venous system with retroperitoneal adipose tissue, such as perinephric fat, accounting for approximately 7% (Jensen, 2008; Karastergiou *et al.*, 2012). Sex differences in fat distribution are well established, with women when compared to men

having higher percentage body fat of around 10% in general and relatively more adipose tissue in the gluteal-femoral or gynoid distribution (Jackson *et al.*, 2002). Conversely, men are more likely to carry adipose tissue around the trunk and centrally around the abdomen, known as an android distribution, and are more likely to store intra-abdominal and visceral fat (Peterson *et al.*, 2015).

1.3.10 Consequences of excess fat mass

Body fat mass is determined by the balance between triglyceride storage and removal in adipocytes by either enzymatic hydrolysis (lipolysis) and subsequent fatty acid oxidation and/or ectopic deposition in non-adipose tissues (Arner *et al.*, 2011). Excess adiposity leads to classifications of overweight or obese and carries serious implications for cardiometabolic health (Peterson *et al.*, 2015). Obesity is currently a serious public health concern and a major contributor to the global burden of chronic diseases, including CVD, T2DM, non-alcoholic fatty liver disease and certain types of cancer (Kopelman, 2000). It is well established that excess adiposity is a key factor in the development of insulin resistance and T2DM by reducing the sensitivity of tissues to insulin and raising blood glucose levels (Goossens, 2017) and this will be discussed in more detail in section 1.4.5. Excess android fat is associated with increased CVD and all-cause mortality by increasing the number of serum lipids (Sari *et al.*, 2019) and this will again be discussed in more detail in section 1.5. Finally, there is clear evidence that increased central adiposity is linked to development of the Metabolic Syndrome (Eckel, Grundy and Zimmet, 2005) and this will again be discussed in more detail in section 1.6.

1.4 Glucose Homeostasis

As discussed in section 1.3, skeletal muscle is a highly metabolically active tissue which needs a constant energy supply to maintain its function. Glucose is the most readily available and preferred substrate for the production of energy by cells, especially the central nervous system (CNS), with the brain responsible for approximately 50% of total body glucose utilization (Kowalski and Bruce, 2014). As the stored form of glucose, glycogen, is neither accumulated nor produced in the CNS, it is essential for survival that plasma glucose levels are maintained at the precise range of 4-6 millimoles per litre (mmol/L) (La Fleur, Fliers and Kalsbeek, 2014; Röder *et al.*, 2016).

This is accomplished by a highly sophisticated network of various hormones and neuropeptides, organs, as well as adipose and muscle tissue (Röder *et al.*, 2016). They increase glucose output by glycogenolysis, the breakdown of glycogen and gluconeogenesis, the formation of glucose from non-carbohydrate substrates when plasma levels are low (hypoglycaemia), as well as increasing tissue glucose uptake (glycogenesis, the formation of glycogen) when plasma levels are high (hyperglycaemia). The maintenance of a steady glucose range is achieved by the opposing and balancing actions of these systems which is referred to as glucose homeostasis (La Fleur, Fliers and Kalsbeek, 2014). Disturbances in the interactions between these systems may lead to metabolic disorders such as obesity, insulin resistance and type 2 diabetes mellitus (T2DM) whose prevalence, comorbidities and economical costs take on a significant scale (Röder *et al.*, 2016; Jouvet and Estall, 2017).

1.4.1 Dietary glucose

Circulating glucose is derived from 3 sources; intestinal absorption during the fed state, glycogenolysis and gluconeogenesis (Aronoff *et al.*, 2004). In the fed state, carbohydrates ingested from the diet are digested by various glucosidases (enzymes which breakdown starch) from the mouth and gut into simple sugars (monosaccharides) of which the main form is a hexose (six carbon atom structure) glucose called glucose-6-phosphate (G6P) (Han *et al.*, 2016). These are then absorbed via the gut into the bloodstream causing a cascade of hormone activity which begins in the pancreas. The pancreas has key roles in the regulation of macronutrient digestion by releasing various digestive enzymes in an exocrine (via a duct into a structure) manner and metabolism/energy homeostasis in an endocrine (directly into the bloodstream) manner through pancreatic hormones (Röder *et al.*, 2016).

1.4.2 Pancreatic hormones and glucose homeostasis

The endocrine cells of the pancreas are clustered together as little island-like structures within the exocrine tissue, forming the Islets of Langerhans which account for only 1-2% of the entire pancreatic tissue (Röder *et al.*, 2016). The chief hormones of glucose homeostasis are insulin, coded on the short arm of chromosome 11 and released from β-cells, which acts to decrease plasma glucose levels and glucagon, released from α-cells, which acts to increase it (Wilcox, 2005) (Figure 1.7). Plasma glucose concentration and hence glucose homeostasis are strictly regulated by the opposing actions of each of these hormones.

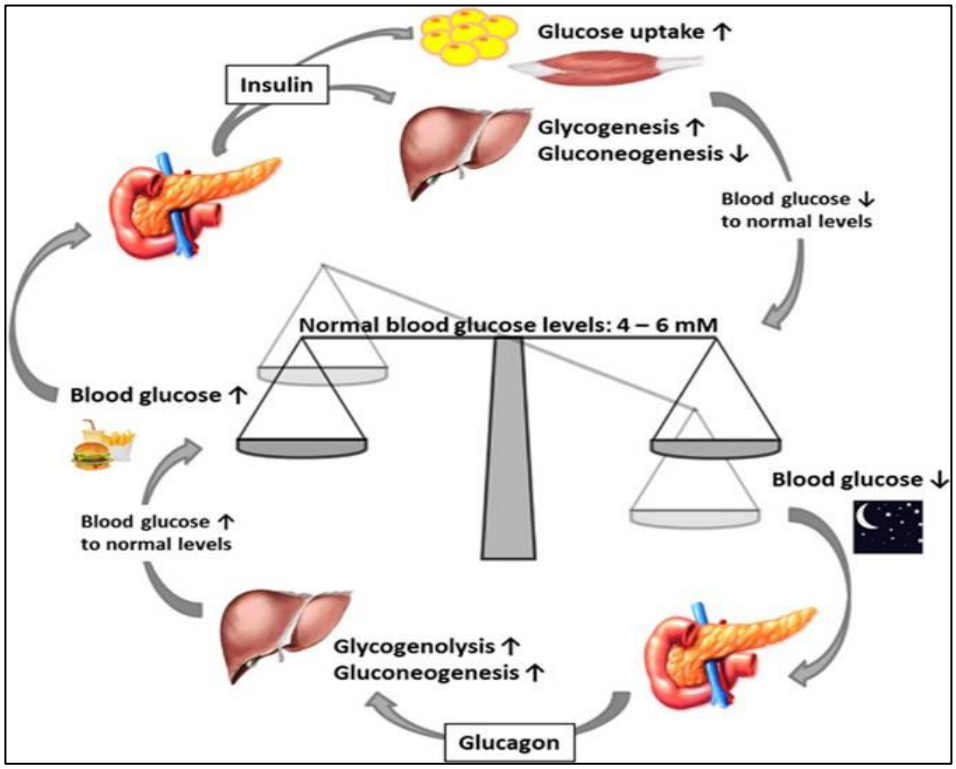


Figure 1.7: Maintenance of blood glucose levels by insulin and glucagon (Roder et al 2016)

1.4.3 The fed state and the insulin secretory pathway

β-cells are the chief glucose detectors of the pancreas and their presence and correct functioning are vital for proper glucose homeostasis. In a healthy individual, following a meal, glucose enters β-cells through facilitated diffusion via the glucose transporter GLUT-2 (Jouvet and Estall, 2017). The glucose undergoes phosphorylation by the enzyme glucokinase which creates the hexose form glucose-6-phosphate (G6P).

G6P is metabolised during glycolysis into the energy source ATP and then pyruvate, increasing the ratio of ATP to its broken-down form adenosine diphosphate (ADP). This increase in ratio causes closure of potassium-ATP (K-ATP) channels in the β-cells and depolarization of the cellular membrane (Röder *et al.*, 2016; Jouvet and Estall, 2017). This leads to the opening of voltage-gated calcium channels within the β-cells to increase intracellular calcium, promoting fusion of insulin granules with the plasma membrane for hormone release into the bloodstream (Jouvet and Estall, 2017). Insulin is stored in large vesicles close to the plasma membrane such that insulin is readily available after stimulation (Röder *et al.*, 2016). This secretory pathway is biphasic, with the first phase being rapid,

reaching a peak at around 5 minutes after the initial glucose stimulus and most of the insulin being released followed by a slower second phase where the remaining insulin is secreted (Aronoff *et al.*, 2004; Röder *et al.*, 2016).

Insulin is a key anabolic hormone whose main action is to lower plasma glucose levels by removing it from the bloodstream. When secreted in response to increased plasma glucose postprandially, insulin first binds to specific receptors in striated muscle cells to increase their uptake of glucose in the stored form of glycogen (glycogenesis) (Aronoff *et al.*, 2004) to prevent hyperglycaemia and organ damage. It then binds to the liver to promote further glycogenesis and simultaneously inhibits glucagon secretion from pancreatic α-cells, thus signalling the liver to stop producing glucose via glycogenolysis and gluconeogenesis (Aronoff *et al.*, 2004). Insulin also promotes anabolic protein formation from circulating amino acids postprandially for storage again in striated muscle and the liver (Röder *et al.*, 2016) as well as stimulation of fat synthesis and triglyceride storage in fat cells (Aronoff *et al.*, 2004).

1.4.4 The fasted state, glucagon and gluconeogenesis

Stored glycogen is critical for maintaining glucose homeostasis in mammals during an overnight fasted period. Being fasted stimulates glycogenolysis and this is the primary mechanism by which glucose is produced for the first 8 hours (Aronoff *et al.*, 2004). This catabolic process is regulated by the hormone glucagon produced from α-cells in the Islets of Langerhans. Glucagon essentially acts to oppose the effects of insulin, sustaining plasma glucose by primarily stimulating hepatic glycogenolysis using the enzyme glycogen phosphorylase (Han *et al.*, 2016) and inhibiting glycogenesis.

Prolonged fasting or starvation essentially makes use of the majority of glycogen in the liver and glucose must be synthesized from non-carbohydrate sources (gluconeogenesis) (Han *et al.*, 2016). The main precursors for this pathway are lactate transported from skeletal muscle or glycerol released from adipose tissues following stimulation of lipolysis by glucagon in the fasted state (Han *et al.*, 2016). This secondary process is again essential to ensure a maintained basal level of plasma glucose for utilisation by the brain in the starved state.

1.4.5 Consequences of insulin resistance

When there are chronically higher than normal levels of circulating glucose and lipids, such as in the pre-diabetic state, β-cells increase in mass to compensate for the higher insulin requirements, changing their structure (Jouvet and Estall, 2017). Glycaemia becomes

increasingly more difficult to control owing to the inefficiency of insulin to inhibit glucagon secretion, likely due to reduced α-cell insulin sensitivity (Jouvet and Estall, 2017) and inefficiencies of the insulin receptor in adipose tissue compared to skeletal muscle to bind to insulin (Carnagarin, Dharmarajan and Dass, 2015). By the time hyperglycaemia develops, reductions in both insulin sensitivity and β-cell function have already occurred (Kahn, 2003).

When hyperinsulinaemia becomes necessary to maintain glucose tolerance, a state of reduced insulin sensitivity, also known as peripheral insulin resistance (IR) is created (Tomlinson *et al.*, 2008). IR is defined as decreased sensitivity and responsiveness to insulin-mediated glucose uptake and decreased inhibition of hepatic glucose production (Sampath Kumar *et al.*, 2019). Sedentary lifestyles and western diets are major contributors to obesity and the growing incidence of IR (Lebovitz, 2001). IR is the central pathogenesis of major metabolic, endocrine and cardiovascular disorders (Gluvic *et al.*, 2017).

When insulin secretion fails to meet demands, T2DM is manifest, with consequent hyperglycaemia and eventual pancreatic β-cell failure (Tomlinson *et al.*, 2008). T2DM affects almost all populations in both developed and developing countries and its prevalence and complications therefore constitute a major worldwide public health problem (Wu *et al.*, 2014). Globally the number of people with diabetes mellitus has quadrupled in the past three decades, with around one in eleven adults being affected, of which over 90% have T2DM (Zheng, Ley and Hu, 2018). By 2030 the global number of people affected is set to reach 552 million (Aune *et al.*, 2015). Diabetes mellitus is now the ninth major cause of death worldwide and the cardiovascular complications associated with T2DM are a leading cause of morbidity and mortality (Zheng, Ley and Hu, 2018). Genetics (Kahn, 2003) and increased abdominal and visceral adiposity play a significant pathophysiological role in the development of T2DM (Kumar, Selby, *et al.*, 2009). Macrovascular complications include CVD and increased risk of stroke and microvascular complications include diabetic neuropathy, nephropathy and retinopathy (Wu *et al.*, 2014).

1.4.6 Diagnosing insulin resistance and T2DM

Two main methods exist to measure insulin action that differ in sensitivity, limitation and sophistication of technical procedures. Glucose tolerance tests examine the effects of exogenous glucose administration on the systemic clearance of glucose. Whilst glucose tolerance directly correlates with insulin sensitivity, it does not account for endogenous insulin secretion and therefore is limited in subjects with altered pancreatic function (Kim, 2009). Insulin tolerance tests examine the effects of administration of insulin on systemic

glucose clearance, with insulin tolerance also correlating with insulin sensitivity, however severe hypoglycaemia is a common side effect (Kim, 2009). Application of these tests in a "real-world" setting therefore determines their usefulness in diagnosis of IR and T2DM.

1.4.6.1 Hyperinsulinaemic-euglycaemic insulin clamp

The insulin clamp method is considered the gold standard to assess insulin sensitivity (Tam *et al.*, 2012). First described in 1979, this very technical procedure involves administering insulin to supress endogenous hepatic glucose production, whilst glucose is infused to maintain euglycaemia (a steady state of serum glucose concentration) using a negative feedback mechanism (DeFronzo, Tobin and Andres, 1979). The glucose infusion rate needed to maintain euglycaemia is a reflection of insulin action and hence glucose metabolism (Ayala *et al.*, 2006). Despite the wealth of information provided by this method and it being a very accurate measure of insulin sensitivity, its sophisticated and technical nature, as well as cost and invasiveness means it has limited it use (Stumvoll *et al.*, 2000).

1.4.6.2 The Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) (World Health Organisation (WHO) and International Diabetets Federation, 2006) has been the gold standard method for diagnosing T2DM for decades (Bartoli, Fra and Schianca, 2011). Whilst also diagnosing T2DM, it is currently the only method of detecting impaired glucose tolerance (IGT), another precursor to T2DM (Rydén *et al.*, 2016) and therefore identifies another demographic to target early for lifestyle modification. It is especially useful as a screening tool in individuals with a borderline fasting plasma glucose of 6.1 - 6.9 mmol/L to determine glucose tolerance status (World Health Organisation (WHO) and International Diabetets Federation, 2006).

When compared to an insulin clamp study, the OGTT simply involves measurement of fasting plasma glucose and then oral administration of 75g glucose, followed by a two-hour post-administration plasma glucose measurement (Hulman *et al.*, 2018). IGT is defined as a two-hour plasma glucose concentration between 7.8 and 11.1 mmol/L and a two-hour plasma of 11.1 mmol/L or greater is diagnostic of T2DM (World Health Organisation (WHO) and International Diabetets Federation, 2006). The OGTT therefore provides a consistent, repeatable method for diagnosis and also response to treatment for T2DM.

1.4.6.3 Calculating insulin resistance from the OGTT

Whilst the OGTT can provide excellent diagnostic information for IGT and T2DM, it provides little information on an individual's IR. The insulin response during an OGTT is composed of early and late phases and patterns of insulin concentration during an OGTT strongly predict the development of T2DM (Hayashi *et al.*, 2013). Measurements of plasma insulin at regular intervals during an OGTT can increase the power of a test to study IR (Hayashi *et al.*, 2013). One study reported that the two-hour area under the curve (AUC) for insulin response to oral glucose during an OGTT was the best surrogate measure of IR (Yeni-Komshian *et al.*, 2000). Late rises in insulin concentration during an OGTT are associated with higher risk of the development of T2DM and higher all-cause mortality rates (Hulman *et al.*, 2018).
One of the most commonly used tools to assess B-cell function and IR from fasting plasma glucose and insulin concentrations is the homeostatic model assessment of insulin resistance (HOMA-IR) (Wallace, Levy and Matthews, 2004). Since it was first described in 1985 (Matthews *et al.*, 1985), it has been reported in over 500 publications and extensively tested against models such as the insulin clamp method and is one of the most commonly reported measures of IR, with values greater than two indicating IR (Wallace, Levy and Matthews, 2004). The model is based on the following equation:

$$\frac{[\text{Fasting glucose (mmol/L)}] \times [\text{Fasting insulin (μU/L)}]}{22.5}$$

1.4.7 Exercise and improvements in insulin resistance

Whilst pharmacological therapies are the most commonly prescribed method to treat T2DM (Wu *et al.*, 2014), poor adherence to these treatments and rising financial costs of the complications of T2DM warrant new, efficient therapy strategies to control this condition. Early reversal of causative factors such as obesity and poor diet is crucial to avoid persistent hyperglycaemia and the onset of T2DM (Carnagarin, Dharmarajan and Dass, 2015). Many cases of T2DM could be prevented with lifestyle changes such as maintaining a healthy body weight, consuming a healthy diet, staying physically active, avoiding smoking and drinking alcohol in moderation (Zheng, Ley and Hu, 2018; Sampath Kumar *et al.*, 2019). It certainly seems more prudent to attempt to improve IR before the onset of T2DM and its complications (Hawley and Lessard, 2007).

There is irrefutable evidence that exercise training is an effective therapeutic intervention to increase insulin sensitivity in skeletal muscle (Hawley and Lessard, 2007; Gibala and McGee, 2008). Just one acute bout of exercise can improve increase insulin sensitivity for at least 16 hours post exercise (Borghouts and Keizer, 2000). The most popular theory of this dramatic improvement is that exercise increases skeletal muscle uptake by insulin stimulated translocation of the GLUT-4 glucose transporter, the main transporter of glucose in striated muscle (Sampath Kumar *et al.*, 2019), thereby reducing the amount of circulating blood glucose (Hawley and Lessard, 2007; Aune *et al.*, 2015). In addition, it has been hypothesized that the depletion of muscle glycogen stores with exercise plays a role herein (Borghouts and Keizer, 2000).

The weight-loss benefits of exercise also act to reduce central and visceral adiposity as well as BMI, further reducing the risk of developing IR (Sampath Kumar *et al.*, 2019). Whilst not only improving IR, exercise has beneficial effects on a number of other CVD risk factors such as improving lipid metabolism, hypertension and increasing muscle mass, thereby making exercise a potent tool in the reduction of IR and development of T2DM (Aune *et al.*, 2015).

Improvements in insulin sensitivity and reductions in blood glucose are directly related to the duration and intensity of exercise, with greater effects of more prolonged and intense exercise than for non-vigorous exercise (Aune *et al.*, 2015). Whilst all subtypes of physical activity appear to be beneficial, the optimum modality for maximal improvement of IR is not yet known and given the effects of exercise on improving IR and other CVD risk factors, further research is warranted (Sampath Kumar *et al.*, 2019).

1.5 Cholesterol and dyslipidaemia

1.5.1 Cholesterol biosynthesis

Cholesterol is a lipid, as discussed in section 1.3.5, and is a major component of cell membranes, playing a vital role in the modulation of membrane trafficking, signal transduction and host-pathogen interactions by regulating killer T cell blood cells (Luo, Yang and Song, 2020). A complex feedback regulatory system meditated by transcriptional mechanisms ensure homeostatic control of cholesterol and fatty acids (DeBose-Boyd, 2018). Synthetic cholesterol is derived from acetyl-CoA and involves a complex cascade of

more than 20 enzymatic steps, producing many intermediates which also play essential roles in cellular functions themselves (DeBose-Boyd, 2018).

Dietary cholesterol is absorbed by the small intestine and transported to the liver, the main site of cholesterol biosynthesis, through lymphatic channels as chylomicrons (Altmann *et al.*, 2004). Chylomicrons are converted by the liver into very-low density lipoproteins (VLDLs) which are then processed into circulating low-density lipoproteins (LDLs) and released into the bloodstream to be taken up by peripheral cells (Goldstein and Brown, 2009). Surplus cholesterol in the form of LDLs is converted into high-density lipoproteins (HDLs) using apoliprotein A-1 (apoA-1) produced by the liver (Luo, Yang and Song, 2020) and are finally transported back to the liver and used to generate steroid hormones and bile acids (DeBose-Boyd, 2018). HDLs also have an important role in transporting lipids back to the liver for recycling and disposal, therefore whilst high levels of circulating LDLs indicate a surplus of lipids in the blood, high levels of HDLs indicate a healthier cardiovascular system (Mann, Beedie and Jimenez, 2014).

1.5.2 Consequences of dyslipidaemia

Dysregulation of the mechanisms which control cholesterol can have serious implications for health. Dyslipidaemia has long been recognised as an independent risk factor for atherosclerosis and associated CVD and peripheral vascular disease (PVD) and is a target for numerous interventions to attenuate the associated large disease burden (Kopin and Lowenstein, 2017). Furthermore, disturbed cholesterol balance underlies not only atherosclerosis and CVD but also a number of other diseases such as obesity, type 2 diabetes, neurodegenerative diseases and cancer (DeBose-Boyd, 2018; Luo, Yang and Song, 2020).

High levels of non-HDL cholesterol (non-HDLc), the difference between total and HDL cholesterol (National Institute for Health and Care Excellence (NICE), 2019a), promote vascular smooth muscle disturbances, stimulating platelet aggregation and the development of atherosclerotic plaques, a precursor to CVD and stroke (Helkin *et al.*, 2016). Individuals with elevated non-HDLc have approximately twice the CVD risk than those with normal levels (Roger *et al.*, 2012). In a meta-analysis of 170,000 individuals, it was reported that a 1mmol/L reduction in LDL cholesterol is associated with a 10-20% reduction in the risk of cardiovascular events and all-cause mortality (Cholesterol Treatment Trialists' (CTT) Collaboration *et al.*, 2010). In contrast, high levels of HDLs have been independently shown to be cardioprotective by directly opposing the effects of non-HDLc (Helkin *et al.*, 2016). For

every 1 mmol/L increase in HDLs, the risk of a CVD event is 2% lower in men and 3% in women (Leon and Sanchez, 2001).

1.5.3 Strategies to control dyslipidaemia

Prevention and sensible management of dyslipidaemia can markedly alter cardiovascular morbidity and mortality. The best studied of the components of cholesterol are the change in total cholesterol, pro-atherosclerotic effects of non-HDLc and the cardioprotective effects of HDLs (Helkin *et al.*, 2016). Non-HDLc in particular has become a metric for diagnosis and treatment success (Ryan, Heath and Cook, 2018).

1.5.3.1 QRISK® calculator

The QRISK®3-2018 web calculator is an important online tool used by UK general practitioners developed to calculate a person's risk of developing a heart attack or stroke in the next ten years (ClinRisk Ltd, 2018). The tool is used primarily to identify those who would benefit from pharmacological modulation of dyslipidaemia following a consultation with their doctor. The tool uses many screening measures, such as age, blood pressure, smoking status, diabetes risk, renal function and cholesterol levels (ClinRisk Ltd, 2018).

1.5.3.2 Dyslipidaemia screening

In the UK, the NHS Health Check was introduced for healthy individuals aged 40 to 74 to identify early signs of cardiovascular and renal disease, as well as T2DM and dementia (National Health Service (NHS), 2019b). The screen includes measurement of lipids, the most important being HDL and non-HDL cholesterol and triglycerides.

1.5.3.3 Statins

Statins are a class of medication which have long used for dyslipidaemia, lowering cholesterol levels whilst also providing cardioprotective effects against CVD, stroke, hypertension and diabetes for those without dyslipidaemia (Mohammadkhani *et al.*, 2019). The primary action of statins is to decrease cellular cholesterol by selectively inhibiting the enzyme hydroxymethylglutaryl-CoA (HMG Co-A) reductase (Sirtori, 2014), the rate limiting enzyme in the production of LDLs. They increase the rate of catabolism of LDLs in the liver by increasing expression of LDL receptors (Sirtori, 2014), reducing their conversion into vLDLs, thereby reducing plasma concentrations of cholesterol (Mohammadkhani *et al.*,

2019). A secondary action of statins is to lower the production of apo-B containint lipoproteins, again reducing the amount of total cholesterol (Sirtori, 2014). Aside from lipid modulation, statins have been shown to have a beneficial effect on vascular tone by increasing upregulation of nitric oxide (NO), which is an important in reducing platelet aggregation, a hallmark of vascular plaque formation (Almeida and Budoff, 2019). To gauge the response to treatment with statins, NICE recommend repeating blood lipids in three months, looking for a reduction of 40% in non-HDLc (National Institute for Health and Care Excellence (NICE), 2019a).

Whilst being very effective for reducing cholesterol, statins also carry potentially serious side effects for a number of patients. Chief amongst these is muscular aches and pains or myalgia in up to 10% of users (Ryan, Heath and Cook, 2018) and in severe cases break down of muscle tissue or rhabdomyolysis, although thankfully this is rare (National Institute for Health and Care Excellence (NICE), 2019a). There has also been reports of decreased muscle mass and strength independent of rhabdomyolysis with extended statin use (Gui *et al.*, 2017). Having effects on hepatic function, a small number of patients have experienced a rise in liver enzymes, however the risk of serious hepatotoxicity remains low (Mohammadkhani *et al.*, 2019). These side effects have meant poor uptake in patients with borderline QRISK scores or cholesterol levels below the treatment threshold who do not want to start pharmacological therapy (Cholesterol Treatment Trialists' (CTT) Collaborators *et al.*, 2012).

Outside of statin therapy, there is limited clinical trial data for medication for primary prevention of CVD for individuals with or without dyslipidaemia (Ryan, Heath and Cook, 2018). Whilst the risk to benefit is overwhelmingly in favour of benefit of statin use for those with established CVD (Almeida and Budoff, 2019), it is again the group of borderline individuals who need to be targeted before they develop CVD and other methods of cholesterol reduction need to be investigated.

1.5.3.4 Exercise for improving dyslipidaemia

Exercise has consistently been shown as a strategy for improving markers of dyslipidaemia (Gordon, Chen and Durstine, 2014). In particular, HDL cholesterol can be modestly increased by regular aerobic exercise levels, with the effects being more pronounced in those with already high total cholesterol levels (Kodama *et al.*, 2007). A recent review found that both aerobic and resistance exercise are equally effective at reducing cholesterol, however the repetitions for resistance training must be high and moderate-intensity aerobic

exercise was not as effective as high-intensity aerobic exercise, implying that more intensity is needed for exercise to be effective at reducing cholesterol levels (Mann, Beedie and Jimenez, 2014). Another study of nearly 25,000 participants showed higher efficacy of resistance training over aerobic exercise for improving HDL cholesterol, although both forms of exercise still showed an improvement in HDLc (Hsu *et al.*, 2019). It has been suggested that exercise may result in increased production of apoA-1 (Hsu *et al.*, 2019), thereby stimulating the production of more HDLc from increased LDL breakdown (Luo, Yang and Song, 2020).

1.6 The metabolic syndrome

Disorders of glucose and lipid metabolism give rise to the condition known as The Metabolic Syndrome (MetS), a complex disorder defined by a cluster of interconnected factors that directly increase the risk of CHD, CVD and T2DM (Kassi *et al.*, 2011). It is now considered to be a pandemic by the WHO with high socioeconomic repercussions (Kahn *et al.*, 2005), not least because of the higher mortality risk for those with MetS when compared with healthy individuals (Gluvic *et al.*, 2017). The risk for CHD and stroke is a third higher in individuals with MetS than those with no risk factors (Tjønna *et al.*, 2008), with the risk of cardiovascular mortality also rising to 12% in those affected (Isomaa *et al.*, 2001).

The condition was first described by Reaven in 1998, then referring to it as “Syndrome X” and later MetS, hypothesizing that it was a central feature in the development of CVD and T2DM through the resistance of insulin action by target tissues (Reaven, 1988). Since that seminal paper, there have been many studies and reviews into MetS, 3,948 in humans according to one review (Kahn *et al.*, 2005), which has led to much confusion regarding its actual definition (Eckel, Grundy and Zimmet, 2005; Kahn *et al.*, 2005). This led to the intervention of the WHO, International Diabetes Federation (IDF) and the Third Report of the National Cholesterol Education Program’s Adult Treatment Panel (ATP III), to come to a consensus, albeit with still slightly differing definitions (Kahn *et al.*, 2005; Kassi *et al.*, 2011) (Figure 1.8).

ATP III definition (27,28)
Any three or more of the following criteria:
1) Waist circumference >102 cm in men and >88 cm in women
2) Serum triglycerides ≥1.7 mmol/l
3) Blood pressure ≥130/85 mmHg
4) HDL cholesterol <1.0 mmol/l in men and <1.3 mmol/l in women
5) Serum glucose ≥6.1 mmol/l (≥5.6 mmol/l may be applicable)
WHO definition (26)
Diabetes, IFG, IGT, or insulin resistance (assessed by clamp studies) and at least two of the following criteria:
1) Waist-to-hip ratio >0.90 in men or >0.85 in women
2) Serum triglycerides ≥1.7 mmol/l or HDL cholesterol <0.9 mmol/l in men and <1.0 mmol/l in women
3) Blood pressure ≥140/90 mmHg
4) Urinary albumin excretion rate >20 µg/min or albumin-to-creatinine ratio ≥30 mg/g

Figure 1.8: Definition of the Metabolic Syndrome (Kahn et. al, 2005)

Whilst these definitions of MetS may help to identify those at risk of CVD, its use is limited as there is little evidence in the form of randomised control trials to suggest pharmacological treatments (Kahn *et al.*, 2005). What has emerged however, is that insulin resistance continues to explain most if not all of the Metabolic Syndrome (Isomaa *et al.*, 2001). In fact, no other mechanisms have emerged that come close to justifying the individual components or their clustering, with evidence now indicating that MetS all begins with excess central adiposity, a precursor to IR (Eckel, Grundy and Zimmet, 2005). This in itself at least provides some strategy for management as follows:

- Weight loss: There is clear evidence that loss of central adiposity reduces IR and this can be achieved by calorific intake reduction and exercise (Eckel, Grundy and Zimmet, 2005).

- CVD risk factors: All patients identified as having one CVD risk factor should be investigated for the others in Figure 1.8. All factors should be aggressively treated as early as possible (Kahn *et al.*, 2005).

- Early recognition of MetS in adolescents: There is increasing evidence that subclinical changes in obese adolescents such as atherosclerosis and insulin resistance lead to early features of MetS causing morbidity in early adulthood (Magnussen *et al.*, 2010; Kassi *et al.*, 2011). One study concluded that whilst there is no adolescent equivalent to the criteria set out in Figure 1.8, using the BMI as measure of risk in this age group is valid, thus avoiding high cost and other common barriers to testing for MetS (Magnussen *et al.*, 2010).

The obesity epidemic is undoubtedly a primary cause of the prevalence of MetS and with at least 1.1 billion people worldwide classed as overweight (Tjønna *et al.*, 2008), the incidence of MetS is expected to continue to rise. Use of the MetS classification system can identify those at risk of developing the syndrome and early modification of these risk factors is key. Exercise remains an excellent methodology to decrease the risk of all the discussed risk factors for MetS, especially in adolescents (Misra and Khurana, 2008) and more studies need to be undertaken to find optimum exercise strategies to combat the rise in obesity and MetS.

1.7 Physiological changes during exercise and adaptation

Virtually all tissues in the human body rely on aerobic metabolism for energy production and therefore remain dependant on a continuous supply of oxygen. Exercise represents a major challenge to whole-body homeostasis and in an attempt to meet this challenge, a myriad of acute and adaptive responses take place at the cellular and systemic levels that function to minimize these widespread disruptions (Hawley *et al.*, 2014). The necessary energy which needs to be generated depletes the energy reserves within the body, which when coupled with the production of muscle waste metabolites and lactate, will result in fatigue and reduced intensity and eventually cessation of the exercise (Ament and Verkerke, 2009). Repeated exercise leads to changes in metabolic pathways and physiological adaptations such as improvements in cardiorespiratory capacity and strength (Rivera-Brown and Frontera, 2012). The extent to which a person can maintain exercise and increase adaptation is governed by their gender, genetics, CRF and barriers to perform exercise, such as the pre-existence of cardiorespiratory disease or loss of skeletal MM (Rivera-Brown

and Frontera, 2012). This is particularly important when considering exercise in the elderly as their responses may be blunted and possible improvements in their CRF and MM less striking (Ament and Verkerke, 2009).

1.7.1 Defining exercise

Physical activity and exercise are expressions often used to describe the same activity, but they are in fact fundamentally different. Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure; exercise is a subset of physical activity that is planned, structured and repetitive and has as a final or an intermediate objective of the improvement or maintenance of physical fitness and/or health (Caspersen, Powell and Christenson, 1974). This section will focus on exercise, as using the former definition, it falls into better context when we consider improvements in fitness and health which are more applicable for this thesis.

1.7.2 Aerobic respiration

Glucose is the most readily available and preferred substrate for the production of energy by cells. Metabolised glucose, as discussed in section 1.4, cannot be used directly by body cells and must instead be converted into the energy "currency" of the body adenosine triphosphate (ATP; adenosine with 3 phosphate bonds) (Akram, 2014). This process is reliant on oxygen and the energy organelles or "powerplant" of the cell called mitochondria which convert glucose to carbon dioxide and water, the basic equation for cellular respiration (Nazaret *et al.*, 2009). These organelles contain a gel-like solution, known as the matrix, which contains crucial proteins for ATP and thus energy production (Nazaret *et al.*, 2009). This energy production is maintained by a complex constant cycle of glycolysis and ATP production termed the Tricarboxylic Acid (TCA) Cycle, also known as the Citrate Cycle or the Krebs Cycle after its discoverer, the Noble Prize-winning biochemist Sir Hans Adolf Krebs (Wainwright, 1993). In the presence of adequate oxygen, this cycle can continue, producing a net gain of 36 ATPs per molecule of glucose (Sherwood, 2004) as per the equation below:

Glucose + Oxygen → Carbon dioxide + Water + Energy

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_20 + 36ATP}$$

Whilst glucose is the preferred principal nutrient of most cells and the only source for the brain, fatty acids and even amino acids can enter the cycle at specific points to eventually produce energy, albeit much less than from glucose.

1.7.3 Anaerobic conditions, lactate and the anaerobic threshold

At very high intensity exercise or during illness, when the oxygen demand cannot be met or the source of glucose is insufficient, the cells must use an alternative, anaerobic pathway which results in the build-up of the toxin lactic acid or lactate and thus cannot be sustained long term (Scott, 2005). If insufficient oxygen is available, such as at maximal exercise or during illness, then the pyruvate cannot be broken down into acetyl CoA and enter the TCA cycle. Glycolysis still yields 2 ATPs but there is no progression past this stage and the anaerobic pathway therefore produces a much lower yield of ATP when compared to the aerobic pathway using the TCA cycle (Sherwood, 2004). Pyruvate is instead broken down into lactic acid by the enzyme lactate dehydrogenase (LDH). Lactic acid is more than 99% dissociated into lactate and hydrogen ions, resulting in a metabolic acidosis at higher concentrations, leading to fatigue (Gladden, 2004) and this pathway is therefore finite.

This acid-base balance is governed by the Henderson-Hasselbalch equation seen below. At rest and during low intensity exercise, aerobic respiration is sufficient to provide ATP and there is no lactate accumulation. During intense exercise or illness, when the anaerobic pathway predominates, the equation must be pushed to the right and increased ventilation "blows off" the excess hydrogen ions as CO_2 from HCO_3^- buffering of lactic acid (Wasserman, Cox and Sietsema, 2014):

$$\mathbf{H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow CO_2 + H_2O}$$

Eventually lactate production exceeds excretion and the point at which the rate of change of concentration of plasma lactate rapidly increases (due to dependence on the anaerobic pathway) at the maximum rate of oxygen consumption during incremental exercise (termed the VO_2max) is termed the anaerobic threshold (AT) (Weisman *et al.*, 2003; Levett and Grocott, 2015), also called the lactate or ventilatory threshold, which will be discussed later in section 1.8.6 as an important indicator of CRF.

1.7.4 The cardiovascular response to exercise

Acute exercise raises metabolic activity and demand for oxygen and therefore blood flow in active muscular regions to ensure adequate oxygen and nutrient delivery and removal of muscle waste metabolites, carbon dioxide and lactate. This response is largely driven by increased, unopposed actions of the sympathetic nervous system (SNS) mediating β-adrenergic stimulation and vagal inhibition, allowing increased cardiac contractility by positive ionotropic effect and invoking the "fight or flight" response (Hellsten and Nyberg, 2016; Schultz, La Gerche and Sharman, 2017). This sympathetic innervation also leads to the most important functional adaptation to exercise which is an increase in the maximal cardiac output, (CO which is denoted by the letter Q in the equation below) the volume of blood being pumped by the left ventricle per unit time, usually expressed as litres per minute (L/min) and in the resting state is usually 5 L/min in adults (Hellsten and Nyberg, 2016). CO is the product of the heart rate (HR) or the number of beats per minute (BPM) and the stroke volume (SV), the volume of blood pumped by the left ventricle per heartbeat in millilitres (mL) (Rivera-Brown and Frontera, 2012) and calculated using the following equation:

Q (L/min) = HR (BPM) x SV (mL)

During maximal exercise, CO may increase from 5 L/min at rest to 15 L/min and 20 L/min in young females and males respectively and up to approximately 25 to 30 and 35 to 40 L/min in elite female and male athletes, respectively (Hellsten and Nyberg, 2016) to allow for delivery of oxygen, nutrients and removal of waste products via the bloodstream. The majority of CO augmentation during exercise is primarily provided by the previously mentioned sympathetic driven increase in HR with increasing intensity, with a smaller contribution from SV, being able to increase from 50 mL at rest to 120 mL which accounts for the first 40 - 60% increase in CO (Rivera-Brown and Frontera, 2012). In this respect, there is essentially a linear relationship between relative workload and HR during exercise which is independent of training status (Hellsten and Nyberg, 2016).

As with HR, systolic blood pressure (SBP) will normally increase with incremental exercise in a curvilinear fashion, for example during a graded exercise test, plateauing towards peak/maximal effort (Schultz, La Gerche and Sharman, 2017). Diastolic blood pressure (DBP) remains largely unchanged and may even drop marginally due to sympathetic mediated decline in systemic vascular resistance (Schultz, La Gerche and Sharman, 2017) but this effect is not so significant as to allow a drop in SBP and thus affect CO (Crisafulli, Marongiu and Ogoh, 2015) as seen in illness such as infection (Leone *et al.*, 2015). This

minimal change in DBP results in only a small to moderate change (Crisafulli, Marongiu and Ogoh, 2015) in the average blood pressure during a single cardiac cycle termed the mean arterial pressure (MAP). MAP plays an important clinical role as it is considered to be the perfusion pressure seen by organs in the body, which is especially important when considering blood flow to the kidneys and brain (Leone *et al.*, 2015). The latest guidelines suggest a MAP of 65 to 70 mmHg is sufficient to maintain perfusion to these vital organs (Leone *et al.*, 2015).

1.7.5 Respiratory changes during exercise

Whilst the cardiovascular system ensures the delivery of oxygen and nutrients and the removal of muscle metabolites and carbon dioxide from tissues, the respiratory system is responsible for absorbing and excreting these essential gases and waste products. Gas exchange by diffusion between alveoli in the lungs and pulmonary capillaries is key for these processes to occur. During acute exercise, increases in ventilatory rate are proportional to the increased oxygen demand and increases in carbon dioxide production (hypercapnia) such that arterial blood gas and acid-base balance are maintained at or near resting levels. The depth and rhythm of ventilation show a similar pattern in response to both increases in the level of hypercapnia and exercise intensity (Tsukada *et al.*, 2017).

There is an initial increase in the normal volume of air displaced between normal inhalation and exhalation termed the tidal volume (VT) and a rapid increase in ventilatory rate when this reaches plateau (Tsukada *et al.*, 2017). In response to hypercapnia and rising oxygen demand, there is an increase in the volume of gas exhaled from the lungs per minute termed the minute ventilation (Lühker *et al.*, 2017). There is a linear uptake in oxygen uptake to match the demand of skeletal muscle, until VO_2max is reached (Rivera-Brown and Frontera, 2012) the point at which no higher concentration of oxygen can be exchanged across the alveoli despite increasing exercise intensity. At this point, as discussed earlier, anaerobic respiration becomes the main pathway for energy production.

1.7.6 Skeletal muscle changes during exercise

Skeletal muscle is heavily dependent on aerobic respiration and as such receives almost all (85 - 95%) of the cardiac output and thus maximum oxygen delivery during maximal exercise (Heinonen *et al.*, 2014). As discussed, when a lack of or maximum delivery of oxygen is delivered there is a shift to the anaerobic pathway and the production of lactate and lactic acidosis resulting in fatigue. Exercise intensities below the point of onset of blood

lactate accumulation can be maintained for long periods as long as glucose is still readily available. Intracellular muscle glycogen stores decrease slowly until the availability of glucose is exceeded by glucose consumption at which point the exercise must stop to maintain brain glucose concentrations (Ament and Verkerke, 2009).

1.7.7 Adaptation to exercise

There is clear and abundant evidence that participation in regular exercise and increased fitness is associated with a lower prevalence of chronic diseases such as CVD, high blood pressure, diabetes as well as improving mental health (Rivera-Brown and Frontera, 2012). These are achieved as exercise training is associated with significant adaptations in many physiological systems that are specific to the type of training. Traditionally, exercise has been categorised, for want of better descriptors, as being of either “endurance/aerobic” vs. “resistance” modalities. The main distinction is that repeated endurance exercise (i.e., repeated low-intensity contractions that can be performed for prolonged periods of time) results in a phenotypic shift toward a population of fibres with greater oxidative capacity, whereas repeated resistance exercise induces muscle fibre hypertrophy and hyperplasia involving satellite cell activation (Kumar, Atherton, *et al.*, 2009).

Endurance exercise therefore elicits many adaptations in the metabolic and cardiorespiratory systems, such as increased maximal stroke volume, cardiac output and blood volume as well as improved skeletal muscle mitochondrial content (MacInnis and Gibala, 2017). There are important clinical implications of the adaptation that occurs as a consequence of repeated hemodynamic stimulation associated with exercise training in humans, including impacts on atherosclerotic risk in conduit arteries, the control of blood pressure in resistance vessels, oxygen delivery and diffusion, and microvascular health thereby decreasing cardiovascular risk (Green *et al.*, 2017). Resistance training elicits adaptations in mainly muscle mass through hypertrophy, improving lean muscle mass and insulin sensitivity whilst reducing adiposity and losses in bone mineral density and has also been shown to improve blood pressure by reducing endothelial stiffness (B. E. Phillips *et al.*, 2017).

1.8 Cardiorespiratory fitness and cardiopulmonary exercise testing (CPET)

1.8.1 Cardiorespiratory fitness and general health

CRF reflects the integrated ability to transport oxygen from the atmosphere to the mitochondria to perform physical work, therefore quantifying the functional capacity of an individual (Ross *et al.*, 2016). CRF is directly related to the integrated function of numerous systems and it is thus considered a reflection of cardiovascular and total body health (Ross *et al.*, 2016). Maintaining a good level of CRF is associated with better cardiovascular and general health as well as a longer life expectancy and decreased risk of comorbidity (Kaminsky *et al.*, 2013).

1.8.2 Cardiopulmonary exercise testing (CPET)

Resting pulmonary and cardiac function testing cannot reliably predict exercise performance. Compared with other exercise tests, cardiopulmonary exercise testing (CPET) provides a thorough assessment of exercise integrative physiology involving the pulmonary, cardiovascular, muscular, and cellular oxidative systems, allowing prognostic assessment of these key variables (Guazzi *et al.*, 2017). Owing to this thorough organ system analysis, in the clinical context, CPET allows for assessment of overall CRF and functional reserve (Levett *et al.*, 2018). It also allows for the ability to scrutinize reasons for symptomatic and impaired physiological response to exercise, such as dyspnoea and distinguish between cardiac or pulmonary causes such as heart failure or COPD (Guazzi *et al.*, 2017). In recent years, CPET has become more important in clinical research and particularly the improvement of CRF in pre-surgical patients (Older, 2013) and the elderly with co-morbidity (Corrà *et al.*, 2018). This has become possible due to the improvement in the safety, with the risk of death between 2 and 5 per 100,000 tests, repeatability of a CPET and improved understanding of the parameters studied (Decato *et al.*, 2018). In the simplest of terms, CPET involves measurement of oxygen uptake (VO_2), carbon dioxide production (VCO_2) and ventilatory measures during an incremental exercise test which progresses to maximum exercise effort and eventual cessation due to lactate accumulation (Albouaini *et al.*, 2007).

1.8.3 Indications for CPET

Traditionally, the main clinical indication for CPET was in heart failure for screening of candidates for heart transplantation (Corrà *et al.*, 2018) and only those without co-morbidity were considered. Today CPET is applicable to a variety of conditions including evaluation of exercise intolerance, cardiorespiratory disease, pulmonary rehabilitation, preoperative evaluation and prognostication of life expectancy (Mezzani, 2017; Levett *et al.*, 2018). CPET is considered the gold standard investigation of exercise intolerance and CRF, especially when specific questions remain unanswered after consideration of basic clinical data (Weisman *et al.*, 2003).

1.8.4 Methodology

The conduct of a CPET is performed in accordance with the guidelines set by the American Thoracic Society and American College of Chest Physicians (ATS/ACCP) in 2003 (Weisman *et al.*, 2003). These were adapted by and reproduced by in the UK in 2018 by the Perioperative Exercise Testing and Training Society (POETTS) (Levett *et al.*, 2018).

CPET is commonly performed using two modes of exercise: treadmill or cycle ergometry. For the majority of clinical circumstances, cycle ergometry is preferred as it allows work rate measurement and easier implementation of ramp protocols, collection of blood tests and physiological measures such as blood pressure and echocardiography (ECG) (Mezzani, 2017). Cycle ergometers are cheaper than treadmills, have a lower cost of operation and have overall been proven to be safer, especially for less active and elderly subjects and the clinically obese (Mezzani, 2017). In practice however, the likelihood of achieving VO_2max during incremental exercise testing is higher in whole body exercise such as treadmill running (Weisman *et al.*, 2003).

To ensure safety, various physiological parameters are measured during the CPET, including non-invasive blood pressure, oxygen saturations, heart rate and ECG (Figure 1.9). Ventilatory parameters and gas exchange are collected via an oro-nasal face mask, with specially developed sensors and analysers which are sensitive to even the acutest changes in oxygen and carbon dioxide. Incremental work rate protocols (also called the Bruce protocol after its inventor Robert Bruce) allow for rapid acquisition of data and are aimed at ensuring VO_2max is achieved within 8 - 12 minutes and the “ramp”, the rate at which the increments occur in minutes, is selected on a subject basis (Mezzani, 2017).



Figure 1.9: CPET using a cycle ergometer with gas collection mask, 12 lead ECG, cyclic BP and oxygen saturations being monitored constantly

The test itself consists of four main phases (Levett *et al.*, 2018):

1) Rest phase (2-3 minutes): During this phase, the ECG should be monitored for ischaemia or arrhythmia. Hyperventilation during this phase should be allowed to settle, or it may give a false AT.

2) Unloaded cycling or warm up phase (2-3 minutes): This phase allows the subject to acclimatise to controlled pedalling and encouraged to maintain a cadence of between 55 to 65 for the entire test.

3) Ramp phase (8-12 minutes): The subject is told when resistance is to start and encouraged to pedal for as long as possible, but not told how long they have actually exercised so as not to cause premature termination. See below for endpoints for a successful test.

4) Recovery phase (5 minutes): During this phase the load is removed and subjects are encouraged to pedal at a reduced cadence to prevent venous pooling in the legs and subsequent syncope. Monitoring should continue until ECG and BP have returned to baseline and HR has returned to within 10 BPM of resting value.

There is currently no gold standard to suggest a successful maximal CPET, however the metabolic endpoints to suggest a successful maximal test include (Weisman *et al.*, 2003):

1) Predicted peak oxygen uptake and/or plateau is observed
2) Predicted maximal heart rate achieved
3) Evidence of ventilatory limitation
4) Respiratory exchange ratio (RER) greater than 1.1 (see section 1.8.6)
5) Patient exhaustion or Borg Scale of perceived exertion rating of 9-10 on 0-10 scale.

1.8.5 VO_2max as a measurement of cardiorespiratory fitness

VO_2max is defined as the metabolic rate at the highest oxygen uptake (VO_2) attained on an incremental exercise test at maximal effort, or the point where VO_2 during an exercise intensity test reaches a maximum beyond which no increase in effort can raise it (Levett *et al.*, 2018) and is therefore a repeatable physiological end point. VO_2 can increase from a resting value of around 3.5 ml/kg/min to values of 30-50 ml/kg/min (Figure 1.10), i.e. 15 times the resting value during maximal exercise (Albouaini *et al.*, 2007). In practice, clinical testing requires a clear plateau in VO_2, which in healthy people occurs at near maximal exercise and this may not be physically achieved before psychological symptom limitation of exercise (Albouaini *et al.*, 2007). VO_2max therefore may be affected by patient volition.

VO_2peak, the highest obtainable VO_2 during a particular maximal exercise test, is therefore often used interchangeably with VO_2max but may not reflect what was potentially achievable for that patient (Weisman *et al.*, 2003; Levett *et al.*, 2018). VO_2peak has still however been shown to predict postoperative morbidity and mortality in surgical populations (Moran *et al.*, 2016) and as an independent factor of all-cause mortality (Kodama *et al.*, 2009) and therefore maintains good prognostic clinical value.

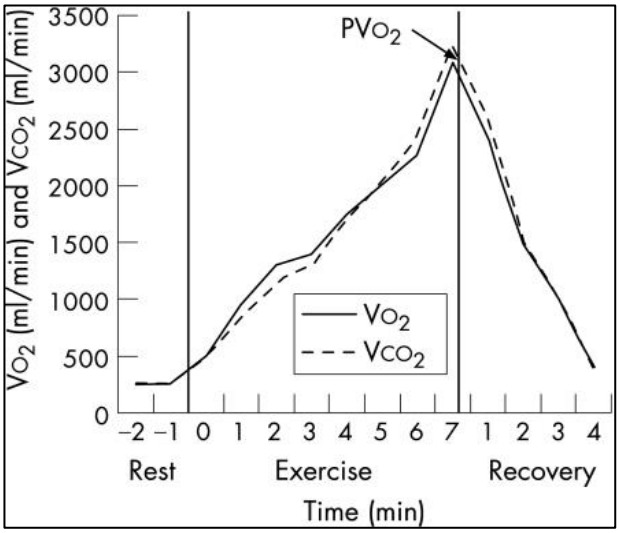


Figure 1.10: CPET in a healthy 49-year-old man using the Bruce protocol showing gas exchange and VO_2peak (PVO_2) (Albouaini et al., 2007)

1.8.6 Calculation of the anaerobic threshold

Using breath-by-breath measurements attained by the CPET analysis during the aerobic phase, expired ventilation increases linearly with VO_2 and is a reflection of aerobically produced CO_2 by skeletal muscle with blood lactate levels not substantially changing as muscle lactic acid production is minimal (Albouaini *et al.*, 2007). Lactate begins to accumulate as anaerobic glycolysis takes over for energy production later on due to the inability to provide sufficient oxygen for respiration, resulting in a metabolic acidosis (as discussed in section 1.7.3). The anaerobic threshold (AT) is a metabolic rate defined as the oxygen consumption above which aerobic energy production is supplemented by anaerobic mechanisms and seen as the point at which the lactate first begins to increase rapidly during incremental exercise (Beaver, Wasserman and Whipp, 1986). It is a good marker of the combined efficiency of the cardiac, pulmonary and circulatory systems (Agnew, 2010). Unlike the VO_2max, the AT cannot be volitionally influenced by the subject and therefore provides a reliable, repeatable index of submaximal exercise capacity and response to exercise therapy (Agnew, 2010).

Traditionally, the AT was calculated after the CPET using breath-by-breath data and the “V-slope” method (Figure 1.11). The two gradients for aerobic and anaerobic respiration are plotted and a linear regression line applied to each. A line is simply drawn where the two meet and is taken as the AT (Albouaini *et al.*, 2007; Levett *et al.*, 2018). This was previously validated if the ratio of CO2 output to O2 uptake per unit of time, also known as the

respiratory exchange ratio (RER) was greater than 1.1, indicating lactic acidosis from anaerobic respiration was taking place (Weisman *et al.*, 2003).

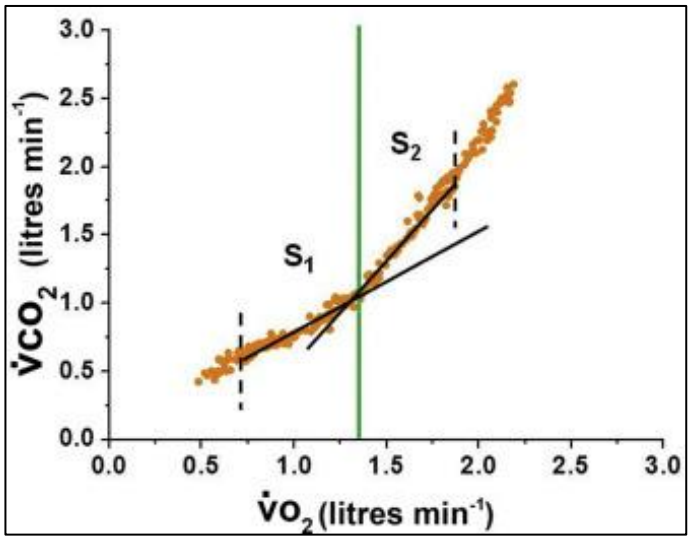


Figure 1.11: Estimation of the AT using the V-slope method. The VCO_2-VO_2 relationship is seen as two gradients S_1 (aerobic) and S_2 (anaerobic) and their point of intersection (green line) represents the AT (Levett et al., 2018)

Whilst previously accepted as a validated method for calculating the AT, the V-slope method has several inaccuracies when applied to a clinical context, such as not accounting for hyperventilation, resulting in an abnormally high RER, or patients with COPD who have impaired chemosensitivity and therefore sometimes an indeterminate AT (Weisman *et al.*, 2003). It is also less reliable when there is a curvilinear response to the CPET and the VCO_2-VO_2 relationship cannot be determined.

Current guidelines therefore suggest a dual methods approach or the “modified V-slope method” as described by the ATS/ACCP and POETTS (Weisman *et al.*, 2003; Levett *et al.*, 2018). Here, the V-slope method is compared to the ventilatory equivalents (VE, a measure of the efficiency of ventilation) graph for VO_2 and VCO_2 and the end tidal partial pressure (P_{ET} detected at the end of exhalation) graph of PCO_2 and PO_2.
Figure 1.12, taken from Levett et. al (2018) shows this relationship graphically. Graph A demonstrates the V-slope method as previously described. Graph B demonstrates the responses of the VE as a function of VO_2. Having been flat, the VE/VO_2 relationship begins to rise at AT whilst VE/VCO_2 decreases as O_2 is no longer efficiently used with hyperventilation as the anaerobic pathway is favoured (Levett *et al.*, 2018). Graph C demonstrates the responses of the P_{ET} expressed as a function of VO_2. $P_{ET}O_2$ increases with

no reciprocal decrease in $P_{ET}C_{O2}$ as the respiratory compensation for metabolic acidosis will not occur until several minutes after exercise and is not captured on a rapid incremental test such as a CPET (Levett *et al.*, 2018).

In summary, coincidental with the V-slope break point, the VO_2 at which VE/VO_2 and $P_{ET}O_2$ reach a minimum and thereafter begin to rise (hyperventilation relative to O_2), with no reciprocal change in VE/VCO_2 and $P_{ET}CO_2$ is demonstrative of the AT. This ensures that the three principles "agree" on a common AT as seen as the vertical green line in Figure 1.12. This method therefore calculates the AT based on an enhanced number of gas exchange measurements rather than the V-slope alone, making determination of the AT more reliable (Weisman *et al.*, 2003; Levett *et al.*, 2018). In order to ensure validity of this method, it is recommended that at least two experienced assessors should undertake the analysis and a consensus reached (Kaczmarek *et al.*, 2019).

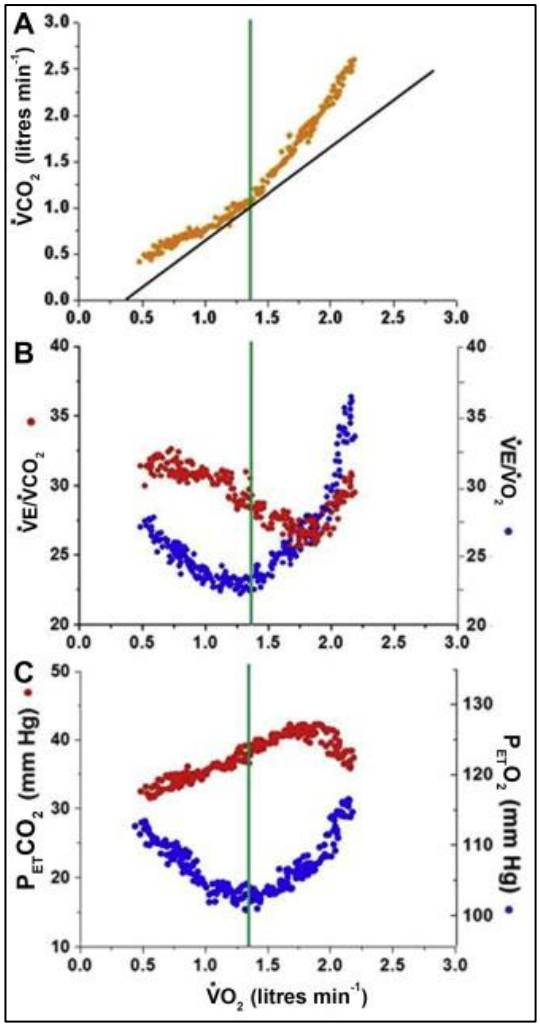


Figure 1.12: Example of AT estimation for a healthy individual during CPET using the modified V-slope method. Graph A is the V-slope method. Graph B shows the responses of the ventilatory equivalents. Graph C shows the end tidal volumes. The estimated AT is marked as the vertical green line where all three graphs "agree" on a common point (Levett et al. 2018)

The AT, like VO_2peak, is another important marker of CRF and low values are associated with increased morbidity and mortality. This is especially relevant in a clinical context when considering major surgery where an AT of least 11ml/kg/min is a predictor of good post-operative recovery required to safely perform major procedures without the need for a higher level of care such as the high-dependency unit (Agnew, 2010; Older, 2013).

1.8.7 Maximum wattage (Wmax)

Wmax is defined as the peak power output or work rate at termination of the ramp phase of the test expressed in Watts (Levett *et al.*, 2018) i.e. the highest recorded wattage during the CPET. In the clinical context, this represents a level of exercise tolerance with improvements in Wmax demonstrating an overall effectiveness of an intervention during a study (Whipp and Ward, 2009). As for VO_2 however, this outcome is entirely volitional and tolerance for exercise when using a cycle ergometer may be influenced by leg fatigue and the true Wmax may not be achieved as a result (Whipp and Ward, 2009).

Exercise capacity as for CRF is an independent predictor of all-cause mortality in older men based on a study of 5314 males aged 65 to 92 years old (Kokkinos *et al.*, 2010). Survival again improved significantly when unfit individuals in the study became fit through exercise.

1.8.8 Consequences of low cardiorespiratory fitness

Cardiovascular disease (CVD) remains a major cause worldwide of premature death and chronic disability, with IHD and stroke accounting for the majority of health lost to CVD (Roth *et al.*, 2017). Reduced CRF is a major risk factor for all-cause mortality (Kodama *et al.*, 2009) and in particular a stronger risk factor for CVD mortality than hypertension, smoking and diabetes (Booth, Roberts and Laye, 2012). Despite this, the acquisition of data regarding worldwide CRF has been limited and it is currently the only major risk factor not routinely or regularly assessed in the general clinical setting (Kaminsky *et al.*, 2013).

1.8.9 Ageing and reduced cardiorespiratory fitness

VO_2peak and therefore CRF reduces with increasing age after 57 years of age which amounted to 1.6% per year when expressed in mL/kg/min (Hakola *et al.*, 2011) (Figure 1.13). Men maintain a higher VO_2peak with increasing age and this gender difference is likely explained by lower SV and skeletal MM in women when compared to men (Hakola *et*

al., 2011) (Figure 1.13). Regulation of skeletal muscle blood flow and therefore oxygen delivery to skeletal muscle is reduced in the elderly as a result of decreased contractility (and therefore reduced SV) and inability to raise HR, leading to a decreased CO (Betik and Hepple, 2008) as discussed in section 1.7.4. Increases in systemic vascular resistance and lower vascular conductance as a result of arterial stiffening in the elderly also reduce skeletal muscle blood flow and therefore VO_2peak (Hearon and Dinenno, 2016). The association of ageing with decreased CRF and cardiac remodelling is a risk factor for CVD and increased risk of morbidity in the elderly (Hwang *et al.*, 2016).

Older individuals who maintain good CRF have a significantly lower risk of functional loss, morbidity and mortality (Blair and Wei, 2000). Conversely, having low levels of CRF is a major risk factor for premature mortality (Kodama *et al.*, 2009; Lee *et al.*, 2012). As a measure of functional capacity, lower levels of CRF have been demonstrated to be an important marker of functional limitations and directly associated with frailty in older adults (Kaminsky *et al.*, 2013). There are no guidelines for managing low CRF in older adults and strategies for improving low CRF in this age group need to be developed.

As discussed in section 1.7.2, mitochondria are essential to the production of ATP and therefore CRF. Mitochondrial dysfunction associated with ageing results in reduced ATP production, reducing skeletal muscle oxidative capacity (Betik and Hepple, 2008). Ageing is also associated with deterioration in the structure and function of the pulmonary circulation and alveolar beds, reducing gas exchange therefore reducing both the amount of available oxygen for ATP production and excretion of carbon dioxide during increasing exercise intensity (Coffman *et al.*, 2017). This again results in reduced functional capacity and an increased risk of morbidity and frailty in the elderly.

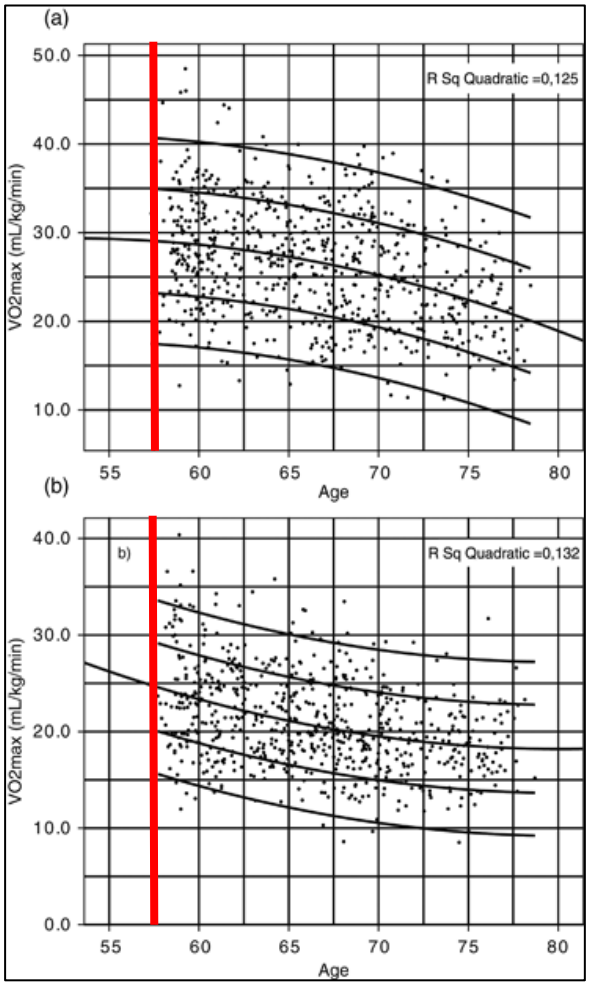


Figure 1.13: Decreasing VO_2max with increasing age in healthy and diseased subjects, (a) men and (b) women (Hakola et al., 2011). The red lines indicate 57 years of age

1.8.10 Cardiorespiratory fitness and postoperative outcomes in the elderly

CRF (as determined by aerobic performance) has been shown to be an independent predictor of postoperative mortality, which provides more accurate prognostic information than age alone (Snowden *et al.*, 2013). The stress of surgery causes a great demand for energy and hence oxygen in the immediate postoperative period (Older, 2013). AT is the most significant independent predictor for postoperative mortality and morbidity which increase with age as CRF declines (Older, 2013; Snowden *et al.*, 2013; Dunne *et al.*, 2016). Developing strategies to improve CRF in those scheduled for major surgery is therefore a key principal for improving post-operative recovery.

1.9 Exercise for improving cardiorespiratory fitness and muscle mass in the elderly

Although no amount of physical activity can stop the ageing process, it is likely that a combination of both aerobic and resistance type exercise will be most effective in ensuring "healthy ageing" and both should be prescribed for the elderly when considering exercise programmes (Chodzko-Zajko, Schwingel and Chae Hee Park, 2009). Physical inactivity in the elderly is a global health concern with huge socioeconomic consequences.

1.9.1 Exercise guidelines for the elderly

For those aged over 65 in the UK, the following guidelines are currently recommended by the World Health Organisation (World Health Organisation (WHO), 2018c):

- At least 150 minutes of moderate-intensity physical activity throughout the week
- *Or* at least 75 minutes of vigorous-intensity physical activity throughout the week
- *Or* an equivalent combination of both modalities.
- For added health benefits, moderate-intensity physical activity should be increased to 300 minutes per week.
- Those with poor mobility should perform physical activity to enhance balance and prevent falls 3 or more days per week.
- Muscle-strengthening activities should be done involving major muscle groups, 2 or more days a week.

These guidelines are extensive and poorly understood or followed. In fact, up to two thirds of adults across Europe do not follow these guidelines (Gomes *et al.*, 2016) contributing to the global reduction in overall physical activity which has major health implications. Exercise strategies should be individualised and easy to follow. Given that "lack of time" and a dislike to previously performed exercise regimes are reasons for poor adherence to exercise, (Trost *et al.*, 2002) newer strategies which are less time consuming need to be considered and implemented.

1.9.2 The benefits of exercise for the elderly

There is overwhelming evidence showing the capacity for exercise to prevent and treat many diseases (Warburton, Nicol and Bredin, 2006). Routine physical activity is associated with at least a 20 to 30% reduced risk for premature mortality and at least 25 chronic medical conditions, including coronary heart disease, type 2 diabetes, stroke, mental health and musculoskeletal conditions as well as some cancers (Warburton, Nicol and Bredin, 2006). One systematic review looking at improvements in CRF (as measured by VO_2max) from randomised control trials comparing exercise to control found a weighted mean difference of 2.56 ml/kg/min (95% CI 3.06 to 10.16; p<0.001), showing that exercise significantly improves CRF (Lin *et al.*, 2015).

As skeletal muscle is the main target of exercise training (Vina *et al.*, 2012), exercise is an important strategy in the battle against frailty and falls prevention. As discussed, RET has been shown to not only increase MM but strength and functional capacity as well, increasing self-independence thereby reducing the risk of falls (Lopez *et al.*, 2018). AE has been shown to improve mitochondrial protein synthesis, improving aerobic capacity and CRF whilst diminishing the effects of disease such as obesity and T2DM by reducing abdominal adiposity and improving insulin sensitivity and CVD by improving systemic vascular resistance and endothelial function by decreasing cholesterol (Vina *et al.*, 2012). In particular, the association between habitual exercise and reduced CVD and mortality is well documented (Wei, Liu and Rosenzweig, 2015). Beyond CRF, regular exercise is proven to enhance mood and be effective against anxiety and depression (Vina *et al.*, 2012) as well as promoting a healthy gut microbiota, protecting the function and permeability of the gut barrier (Fiuza-Luces *et al.*, 2018).

1.9.3 Resistance exercise

Resistance exercise training (RET) has been well established as an effective interventional strategy for healthy ageing and maintaining MM (Snijders, Verdijk and van Loon, 2009). In the clinical context, RET has been shown to reduce adiposity, lower BP, improve insulin sensitivity and enhance bone mineral density (B. E. Phillips *et al.*, 2017). RET is a powerful stimulus to promote net muscle protein anabolism, resulting in specific metabolic and morphological adaptations in skeletal muscle tissue (Koopman and van Loon, 2009). RET can effectively increase muscle strength, MM and as such improve physical performance and overall functional capacity (Koopman and van Loon, 2009).

Resistance training appears to increase basal muscle protein turnover however when compared to younger muscle, aging reduces the responses of myofibrillar protein and anabolic signalling to resistance exercise (Kumar, Atherton, *et al.*, 2009; Kumar, Selby, *et al.*, 2009). Whilst resistance exercise improves muscle protein balance in younger subjects, in the absence of nutritional intake, the balance remains negative in the elderly (Børsheim *et al.*, 2002). One study found that this was because despite increasing MPS, in the fasted state, MPB in the elderly still exceeded the rate of MPS (Wolfe, 2006a), whilst a variety of studies have shown that RET alone does not entirely eliminate net MPB in the fasting state in the elderly (B. E. Phillips *et al.*, 2017). Whilst strength adaptations to exercised muscle groups do occur with RET, whole-body MPS appears to change little with RET in the elderly (Brioche *et al.*, 2016). Equally, high protein diet alone does not have a beneficial effect on MPS or mitochondrial function (Walrand *et al.*, 2008). To reverse the effects of sarcopenia for example, RET in combination with good nutrition in the elderly is essential to account for this imbalance of MPS (Phillips, 2009; Churchward-Venne, Burd and Phillips, 2012).

The majority of RET studies usually require more than 10 weeks for changes to occur (Brioche *et al.*, 2016) even as high-velocity “short-term” therapy (Reid *et al.*, 2008). A recent systematic review concluded that existing evidence, whilst promising in improving strength, was inconsistent, using differing supplements, duration and methods of RET (Denison *et al.*, 2015). The benefits of RET are well documented and should form part of strategies to aid healthy ageing. This thesis will however primarily focus on improvements in CRF, which in the elderly is not directly influenced by RET alone and importantly short-term (4 weeks) duration, therefore RET will not be discussed further.

1.9.4 Aerobic exercise

Aerobic or endurance exercise (AE), allows for adaptations of increased oxygen extraction and utilisation. This is principally governed by the improvement in the availability, capacity and function of mitochondria and therefore increased ATP production (Brook *et al.*, 2016) as discussed in section 1.7.2. This manifests as an improvement in VO_2peak, angiogenesis and an increase in the number and size of functioning mitochondria (Brook *et al.*, 2016). AE has also been shown to increase the clearance of plasma cholesterol and triglycerides (B. E. Phillips *et al.*, 2017), potentially as a result of up-regulation of liver enzymes and increased inhibition of HMG-CoA reductase which plays a central role in the production of cholesterol and therefore reduce the risks associated with cardiovascular disease.

Whilst known to improve CRF and cited as being important for the elderly to maintain CRF, AE was previously thought not to vastly improve strength or increase myofibrillar MPS and hence MM (Marzolini, Oh and Brooks, 2012). In the fed state, Wilkinson *et al.* found that after 10 weeks of RET, myofibrillar protein synthesis was greatly increased and to a lesser extent mitochondrial protein synthesis (Wilkinson *et al.*, 2008). Moderate-intensity continuous training (MICT) AE (i.e. AE at a maximum of 75% of predetermined HR) was shown to only increase mitochondrial protein synthesis as hypothesized, confirming the phenotype-specific responses to each individual exercise (Wilkinson *et al.*, 2008).

Bell *et al.* conducted a study to look at MPS when comparing RET to traditional MICT AE, to a relatively new form of AE, high-intensity interval training (HIT), which will be discussed at length in section 1.10. Although the protocol was based on current exercise recommendations, they did not see an improvement in MPS when performing MICT AE, whereas they showed HIT to significantly improve MPS, albeit not as greatly as RET (Bell *et al.*, 2015). After 12 weeks of cycle ergometry training at over 80% maximal HR, Harber *et al.* found AE induced significant muscle hypertrophy in both young and elderly males (Harber *et al.*, 2012). There is therefore increasing evidence for the ability of higher-intensity AE regimes to improve both CRF and muscle mass.

1.9.5 Consequences of sedentary lifestyles for older adults

Several physiological changes associated with ageing cause progressive decline in function. Despite exercise training being a proven method to improve both CRF and MM, 39% of UK adults (almost 20 million people), a strikingly low proportion, meet the current government guidelines for exercise as described above (BHF, 2017). Up to one third of adults worldwide do not reach this target and this is associated with a poorer quality of life in middle age and disability and frailty in the elderly (Gomes *et al.*, 2016). Low physical activity is the fourth most important risk factor in the UK for premature death from any cause with an annual healthcare cost of around £1.2 billion (BHF, 2017), with those who are inactive having a 20 to 30% increased risk of death compared to those who adhere to guidelines (World Health Organisation (WHO), 2018c).

Ageing is associated with a progressive decline in the capacity for exercise (Betik and Hepple, 2008). As we age, changes in cardiovascular, pulmonary and skeletal muscle structure and function lead to reduced CRF and functional ability. The primary factor leading to reduced CRF with age is the reduction in cardiac output due to reduced stroke volume and increased peripheral vascular resistance (Rivera-Brown and Frontera, 2012). Our ability

to use the aerobic pathway thus becomes less efficient as we age, with increasing reliance on anaerobic respiration which is also less efficient with increasing age. Reduced CRF is a major risk factor for increased morbidity and all-cause mortality (Kodama *et al.*, 2009) and in particular a stronger risk factor for CVD mortality than hypertension, smoking and diabetes (Booth, Roberts and Laye, 2012). It is worth remembering and in particular reminding our patients that even modest levels of exercise are beneficial for health (Wei, Liu and Rosenzweig, 2015). However, not everyone is capable of performing regular exercise and non-exercise interventions should be researched for the future to ensure preservation of CRF and lean MM in these elderly individuals as well.

1.10 High-intensity interval training

High-intensity interval training (HIT) is a form of interval exercise which describes physical exercise that is characterized by brief, intermittent bursts of vigorous activity at or below maximum oxygen uptake, interspersed by periods of rest or low-intensity exercise (Gibala *et al.*, 2012; Weston, Wisløff and Coombes, 2014). Interval training is a potent stimulus for physiological remodelling in humans (MacInnis and Gibala, 2017). It has emerged as a time-efficient exercise strategy for primarily improving CRF (Little *et al.*, 2011), but also a number of other health related facets, such as lowering BP, reducing total body adiposity, increasing lean MM and improving insulin sensitivity (Laursen and Jenkins, 2002; Gibala and McGee, 2008). It has gained increasing popularity in the past few years for rapidly increasing CRF (Levinger *et al.*, 2015), inducing similar or even superior changes in VO_2max and mitochondrial adaptation when compared to RET and MICT AE (MacInnis *et al.*, 2017) with a reduced time commitment (Gibala and Little, 2010). HIT has also been shown to improve insulin sensitivity in younger adults in as little as 2 weeks (Babraj *et al.*, 2009).

1.10.1 Theoretical mechanisms for adaptations to HIT

HIT has been proven to improve CRF by increasing both central (SV, CO, blood volume) and peripheral (skeletal muscle mitochondrial content) adaptations (Laursen and Jenkins, 2002). Inactivity and ageing are associated with the loss of mitochondrial dynamics and proteins which are critical for the turnover and maintenance of healthy mitochondria (Wyckelsma *et al.*, 2017). HIT is a potent stimulus to increase skeletal muscle mitochondrial oxidative capacity and many of the biochemical and physiological adaptations that accompany HIT occur in response to increased muscle cell energy demands and cellular hypoxia (Laursen and Jenkins, 2002). This hypoxia increases blood flow, oxygen delivery

and fat metabolism (Laursen and Jenkins, 2002), improving CO and reducing adiposity. Adaptations to HIT mainly occur during the recovery time between efforts where aerobic metabolism increases in response to the energy deficit created during efforts (Laursen and Jenkins, 2002).

Another underlying principle of HIT is that the increased intensity leads to greater stimulus and recruitment of, in particular, type II muscle fibres (Gibala *et al.*, 2012). The effect on skeletal muscle phenotype can be dramatic and this change in phenotype is accompanied by enhanced mitochondrial enzyme expression and an increase in time to fatigue when electrically stimulated (Gibala *et al.*, 2012). HIT has been shown to induce significant increases in myofibrillar protein fractional synthetic rate and therefore whole-body MPS contributing to increased MM, which is only slightly less when compared to RET (Bell *et al.*, 2015).

On a cellular level, researchers have focused on the activity of mitochondrial enzymes, such as citrate synthase, a rate limiting enzyme in the regulation of the TCA cycle and mitochondrial protein synthesis rates which are both vastly increased following HIT (MacInnis and Gibala, 2017). Another key regulator of mitochondrial biogenesis is peroxisome-proliferator activated receptor γ coactivator (PGC)-1α, which is regarded as the “master regulator” of mitochondrial biogenesis in muscle (Gibala *et al.*, 2012) (Figure 1.14). HIT increases PGC-1α mRNA by several fold when measured three hours post exercise (Gibala *et al.*, 2009). This results in increased activation of transcription factors which increase mRNA expression of several mitochondrial genes, ultimately resulting in increased mitochondrial protein synthesis to drive mitochondrial biogenesis and capacity (Gibala *et al.*, 2012). PGC-1α is influenced by exercise intensity and the robust changes in the intramuscular ATP:ADP/AMP ratio after exercise (Gibala *et al.*, 2012).

HIT further stimulates 5'-AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) which are exercise-responsive signalling kinases which directly phosphorylate and therefore activate PGC-1α. Muscle PGC-1α is therefore a potent factor in increasing oxidative capacity and glucose uptake, being a possible explanation for the rapid responses seen in improving CRF and insulin sensitivity seen following HIT, further highlighting the potential benefits of this exercise modality (Gibala *et al.*, 2009). HIT is therefore a potent stimulator of aerobic fitness, muscle oxidative capacity and insulin sensitivity and would be beneficial to incorporate into exercise recommendations for older adults (Bell *et al.*, 2015).

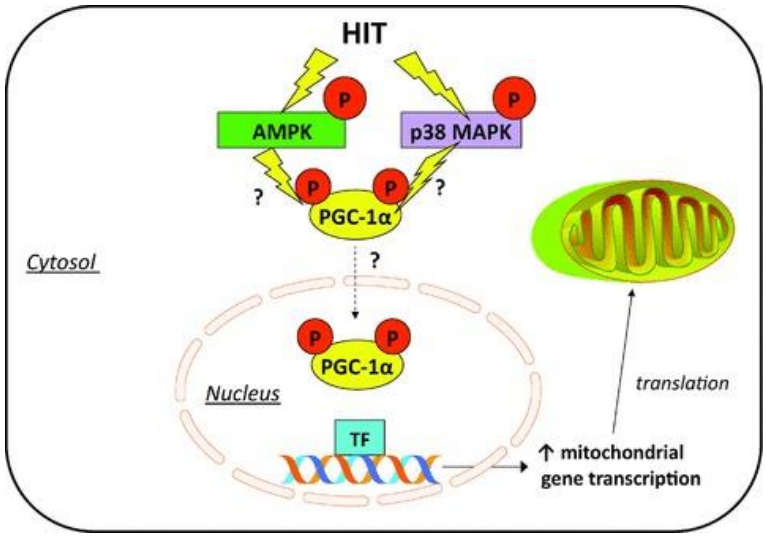


Figure 1.14: Potential intracellular signalling mechanisms involved in HIT-induced mitochondrial biogenesis (Gibala et al 2012)

1.10.2 Establishing HIT protocols

Traditionally, HIT was applied using the "Wingate technique", which consists of a 30-second "all-out" cycling effort against a supra-maximal workload and was found to be a potent stimulus for improving aerobic capacity and mitochondrial adaptation (MacInnis and Gibala, 2017). In a typical training session, subjects complete four to six Wingate tests interspersed with 4 min of rest, for a total of only 2 to 3 min of maximal exercise spread over a 15–30 min period (Gibala and Little, 2010). This requires a specialist cycle ergometer and an extremely high level of subject motivation, however this type of training may not be safe, tolerable or practical for many individuals (Gillen and Gibala, 2014) such as the elderly or those with co-morbidity.

Sprint interval training (SIT), is characterised by all out or supramaximal efforts (Figure 1.15), as with the Wingate test described above, to provoke intensities to produce VO_2peak (MacInnis and Gibala, 2017) which again may not be suitable for the majority of subjects aged over 65. In a review by MacInnis and Gibala, they found that VO_2max and mitochondrial adaptations were far superior after HIT compared to MICT, whereas improvements were similar for SIT and MICT (MacInnis and Gibala, 2017).

Recent studies have highlighted the potential for low-volume HIT modalities which may be more feasible but still time-efficient and resulting in similar adaptations to high-volume modalities (Gibala and Little, 2010; Gillen and Gibala, 2014). As little as three HIT sessions per week involving less than 10 minutes of intense exercise has been shown to improve aerobic capacity, skeletal muscle oxidative capacity, exercise tolerance and markers of disease risk after only a few weeks in both healthy individuals and people with cardiometabolic disorders (Gillen and Gibala, 2014). As it is still a relatively new modality, the optimal duration, training intensity and frequency of interval exercise protocols is subject to further study (MacInnis and Gibala, 2017). To achieve effective HIT, "near maximal" efforts are performed at intensities that aim to provoke > 85% of the maximal predicted HR (Ciolac *et al.*, 2011; MacInnis and Gibala, 2017). For the purposes of this thesis, owing to its more practical application to an elderly population (Boereboom *et al.*, 2016) we shall concentrate on HIT as the form of interval training to be used (Figure 1.15).

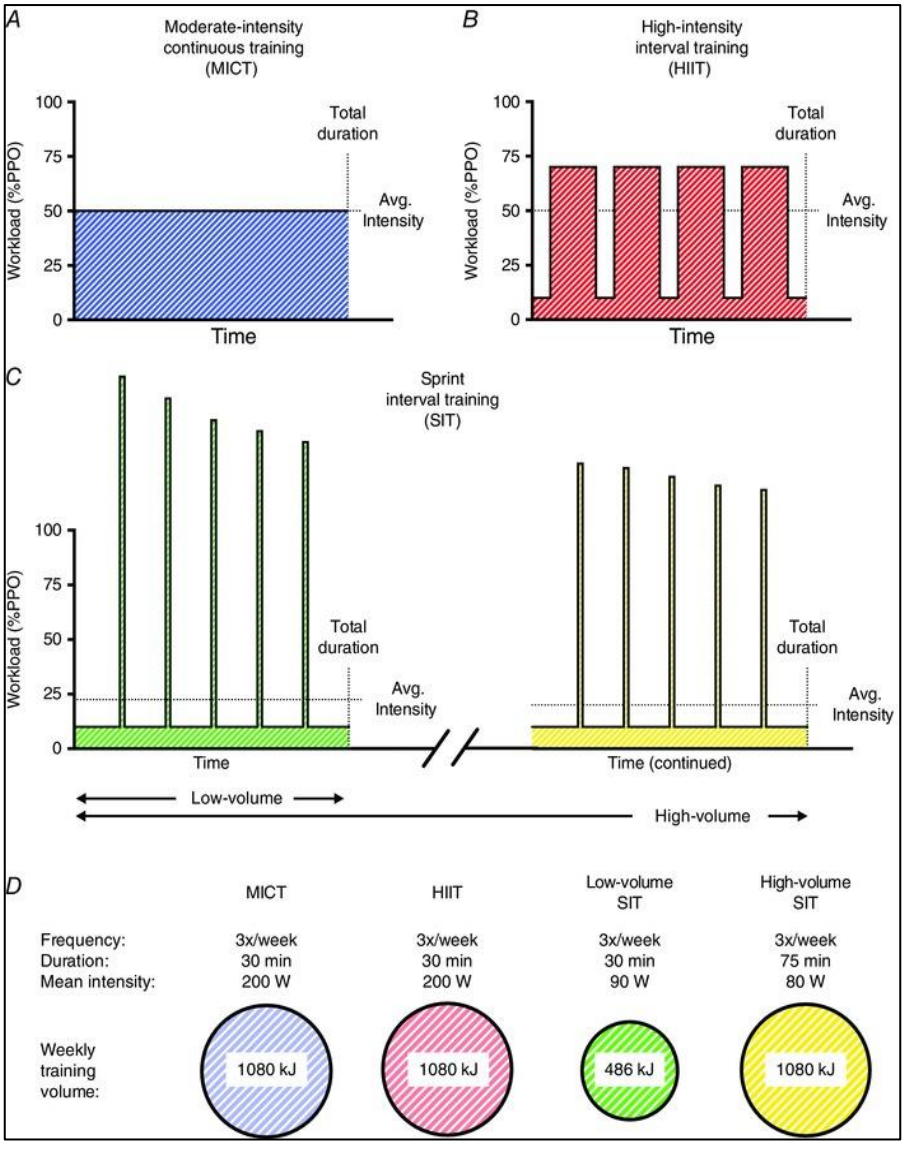


Figure 1.15: Depiction of the main types of aerobic exercise (MacInnis and Gibala, 2017)

1.10.3 Validation of the HIT protocol to be used

In a large study recently published, Phillips *et al.* adapted a proven interval training regime used previously by Ready *et al.* (Ready, Eynon and Cunningham, 1981) with the aim to show improvements in CRF, MAP and fasting insulin resistance in 189 adult subjects with impaired metabolic function (Phillips *et al.*, 2017). They began with a 7-by-1 interval at 100% VO_2max intervention, but at interim analysis also investigated the effects of a 5-by-1 protocol at 125% (starting at 85%) in comparison, both fully supervised, 3 times per week over 6 weeks using a cycle ergometer. Not only were they able to demonstrate significant improvements in all of the hypothesised variables, they also showed that the 5-by-1 protocol was able to significantly improve VO_2max greater than the 7-by-1 protocol (Phillips *et al.*, 2017).

This protocol was then adapted further by our research group to improve fitness in a clinically compliant 31-day (four week) progression to colorectal cancer surgery from diagnosis to see if relevant gains in CRF could be achieved in an age-matched to colorectal cancer, healthy population study (Boereboom *et al.*, 2016). In order to investigate this 5-by-1 intervention in a pilot, equipment-free, home-based study in middle-aged adults, our research group adapted the 5-by-1 protocol and used bodyweight exercises aiming for greater than 85% of the maximum predicted HR (MacInnis and Gibala, 2017) within a four week period (Blackwell *et al.*, 2017). Supervised as well as unsupervised HIT used in this manner produced significant improvements in CRF (Blackwell *et al.*, 2017) and this protocol was therefore chosen for this thesis. The full intervention can be seen in section 4.3.13.

1.10.4 Clinical applications of HIT

Given that “lack of time” remains one of the most commonly cited barriers to regular exercise participation (Trost *et al.*, 2002), HIT is a time-efficient exercise strategy that warrants consideration by health practitioners and fitness professionals. Boereboom *et al.* recently showed the benefits of laboratory based low-volume HIT in improving indices of CRF in elderly volunteers (mean age 67) within 31 days using a 5-by-1 protocol and also showed that it was feasible and enjoyable for participants (Boereboom *et al.*, 2016). A recent systematic review by Milanovic *et al.* showed that endurance training and HIT both elicit large improvements in the VO_2max of healthy, young to middle-aged adults, with the gains in VO_2max being greater following HIT when compared with traditional AE (Milanović, Sporiš and Weston, 2015). Dronkers *et al*. showed a significant improvement in CRF for pre-operative patients using laboratory based HIT compared to traditional home exercises

(Dronkers *et al.*, 2010). A meta-analysis by Weston *et al.* found that preoperative HIT at 90% VO_2max increased CRF by almost 20% compared to less than 10% with MICT at 50% VO_2max (Weston *et al.*, 2016). Finally, Tjønna *et al.* found that the increased intensity with HIT compared to MICT was a key factor for improving aerobic capacity and reversing the risk factors of the metabolic syndrome (Tjønna *et al.*, 2008). There is also evidence to suggest that despite higher ratings of perceived exertion, there is greater enjoyment associated with HIT than traditional MICT (Bartlett *et al.*, 2011; Stork *et al.*, 2017).

Whilst there are a wealth of positive results for improving CRF, recent studies certainly hold promise for the ability of AE and in particular HIT to improve lean MM as well unlike previously hypothesised (Konopka and Harber, 2014). The potential mechanisms behind lean MM improvements after AE are still not fully understood and further studies are warranted. Whilst these studies have shown excellent results with HIT in the laboratory setting using cycle ergometers, little research has been undertaken to show any potential improvements using a bodyweight only, equipment-free, home-based, HIT regime.

1.10.5 HIT and safety considerations for the elderly

The physical effort required to ensure 'proper' HIT on a cycle ergometer has previously been shown to be safe and effective in improving CRF in the elderly (Boereboom *et al.*, 2016) and superior when compared to traditional AE regimes for improving VO_2peak in the over 80 age group (Binder *et al.*, 2002). It has also been proven safe in elderly cohorts with comorbidity like coronary artery disease, a group of patients where HIT was previously thought not to be safe or practical (Warburton *et al.*, 2005). HIT is therefore well documented for being safe and practical for the elderly and this will be expanded on in Chapter 4.

1.11 Nutraceuticals as an alternative to exercise for maintaining muscle health in the elderly

Obtaining adequate nutrition from dietary food substances plays a vital role in maintaining normal functions of the human body. As previously discussed, regular exercise remains the most effective strategy to combat the changes in physiology as we age. However, given the previously discussed barriers to exercise in the elderly and infirm, more importance is being given to dietary strategies to improve health and these need further investigation. Nutrition in this respect may be an aid in improving health and reducing inflammation by immune

modulation (Boros, 2017). It is particularly important to research and develop nutritional compounds in the areas typically impaired in the elderly i.e. bone, skeletal muscle and cognitive function, as it is this age group which are more likely to encounter barriers to exercise (Iolascon *et al.*, 2017).

Natural products and health-promoting foods known as “nutraceuticals” have in the last decade received extensive attention from both health professionals and the common population (Zhao, 2007). Whereas dietary supplements act to increase the daily total intake of a nutrient but are not intended to treat disease, a nutraceutical is defined as a substance derived from natural or bioactive phytochemicals which not only supplements the diet for nutrition but has medicinal properties for promoting health and even preventing disease (Kalra, 2003; Zhao, 2007; Nasri *et al.*, 2014).

Whilst nutraceuticals have been used for thousands of years in traditional Eastern medicine for their antioxidant and anti-inflammatory properties, Western medicine has not yet used them therapeutically, even though their safety record is exceptional (Middleton, Kandaswami and Theoharides, 2000). A number of recent studies have shown promising results using nutraceuticals in combating various pathological conditions such as; obesity and type 2 diabetes (Bahadoran, Mirmiran and Azizi, 2013), cardiovascular and atherosclerotic disease (Goya *et al.*, 2016), hypertension (Ried, Fakler and Stocks, 2017), musculoskeletal disorders such as rheumatoid and osteoarthritis (Ellinger and Stehle, 2016), cancer (Manach *et al.*, 2004) and neurodegenerative disorders such as Alzheimer’s and Parkinson’s Disease (Nasri *et al.*, 2014). Whilst unlikely to have the same effects on CFR as exercise, these compounds have been shown to maintain and improve muscle mass and function by improving muscle micro and macro-vascular flow (Phillips *et al.*, 2016), thereby alleviating the negative effects of sarcopenia.

1.11.1 Polyphenols as phytonutrients

Phytonutrients refer to plant-derived nutrients with particular biological activities in supporting human health (or also defined as plant-derived nutraceuticals) (Zhao, 2007). Polyphenols are the active compounds derived from these natural plant metabolites (polyphenolics) and thousands of these are found in edible plants, beverages and plant products such as fruits, vegetables and legumes, cocoa products, wine, oils and teas (Myburgh, 2014).

Nutrition comprises macronutrients (carbohydrate, fat and protein) which are essential for producing energy and micronutrients (vitamins and minerals) which in turn are essential for facilitating basic biochemical processes (Williamson, 2017). Polyphenols belong to the 'other' group of compounds which are not preferentially stored in the body but which protect cells against oxidative stress and inflammatory processes, helping to improve long-term health (Williamson, 2017).

Polyphenols first became of interest to botanists around the 1930s due to role they played in protecting plants against stresses such as ultraviolet light damage and the colours they produced to attract and deter certain insects (Williamson, 2017). It was not until the 1990s that the antioxidant effects of polyphenols in humans were investigated more scientifically (Williamson, 2017).

1.11.2 Polyphenol families and classification

Polyphenolic compounds have at least one aromatic ring with one or more hydroxyl groups attached, the differing number of rings and hydroxyl groups distinguishing between the thousands of polyphenolics known (Manach *et al.*, 2004; Croft, 2016). These distinctions allow for classification of polyphenols into four major families; Flavonoids, stilbenes, phenolic acids and lignans, with each having hundreds of derivatives (Manach *et al.*, 2004; Han, Shen and Lou, 2007; Myburgh, 2014).

The flavonoid family are the most abundant polyphenols in the human diet, making up two thirds of the known phenolic compounds (Croft, 2016). The other third is made up mainly of the phenolic acids, with the stilbenoids and especially lignans being less readily available from diet (Bahadoran, Mirmiran and Azizi, 2013).

1.11.3 The biochemical actions of polyphenols and their effect on health

The molecular and cellular mechanisms of organic polyphenols remain unclear despite extensive research conducted in recent years. However, they are one of the few bioactives known today for which causality between intake and an improvement in health has been formally demonstrated (Sansone *et al.*, 2015). Polyphenols were originally heralded for their antioxidant and anti-inflammatory properties which was thought to lower the total number of circulating free radicals in the body (Crozier, Jaganath and Clifford, 2009) and it was reasonable to assume that this activity was key to their disease preventing properties (Croft,

2016). There is now however emerging evidence from *in vitro* studies suggesting that phenolic metabolites exert immune-modulating effects on intracellular signalling cascades vital for cellular functions such as growth, proliferation and apoptosis (Crozier, Jaganath and Clifford, 2009).

1.11.3.1 Oxidative stress, chronic inflammation and cancer

Oxidative stress results in the alteration of cell structure and phenotype which is considered to play a pivotal role in the pathogenesis of ageing and the previously discussed chronic diseases by releasing free radicals (Han, Shen and Lou, 2007). Nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) are transcription factors that regulate numerous physiological functions (Crozier, Jaganath and Clifford, 2009) and are involved in the pathogenesis of many diseases by expressing pro-inflammatory cytokines, leucocytes and inhibitors of apoptosis (Ellinger and Stehle, 2016). This in turn stimulates the production of pro-inflammatory mediators such as interleukin-1beta (IL-1β), tumour necrotic factor alpha (TNFα) and inducible nitric oxide synthase (iNOS) (Han, Shen and Lou, 2007). Dysregulation of these factors is associated with inflammatory diseases such as asthma, Crohn's disease, rheumatoid arthritis and some cancers (Crozier, Jaganath and Clifford, 2009).

Resveratrol, am example of a stilbenoid, has been shown to be able to scavenge free radicals in human endothelial cells by increased antioxidant enzyme activity and inhibiting pro-inflammatory NFκB and AP-1 transactivation both *in vitro* and *in vivo* (Han, Shen and Lou, 2007).

1.11.3.2 Endothelial function, atherosclerosis and cardiovascular protection

Increased vascular stiffness, endothelial dysfunction and systolic hypertension are hallmarks of vascular ageing (Heiss *et al.*, 2015). Dyslipidaemia, platelet aggregation and resultant endothelial dysfunction are considered to be amongst the main reversible pro-atherogenic metabolic abnormalities leading to CVD (Bahadoran, Mirmiran and Azizi, 2013). There is growing evidence that polyphenols could have protective effects against the propagation of all three abnormalities. The reversal of endothelial dysfunction has been suggested to slow the rate of atherogenesis and improve the prognosis of patients with cardiovascular disease (Grassi *et al.*, 2012).

Dyslipidaemia and reduced lipoprotein metabolism are also hallmarks of cardiovascular disease. Polyphenols have been shown to inhibit the oxidation of low density lipoproteins (LDLs), responsible for endothelial dysfunction through plaque formation (Han, Shen and Lou, 2007). Not only this, but they also stimulate the liver to upregulate the expression of serum high density lipoproteins (HDLs) which both reduce the circulating number of and reverse the harmful effects of LDLs (Han, Shen and Lou, 2007).

Chronic inflammation has been observed in many chronic degenerative diseases and especially plays a role in the development of CVD by causing endothelial dysfunction which is highly responsible for the development of atherosclerosis (Ellinger and Stehle, 2016). The cardiovascular protective effects of polyphenols are thought to involve signalling pathways that stimulate nitric oxide (NO) production which is essential for maintaining vessel compliance and improving endothelial function (Figure 1.16), even in pre-existing atherosclerosis (Hodgson and Croft, 2010). The resulting vasodilation and microvascular recruitment, whilst inhibiting vasoconstriction and decreased arterial compliance, improves muscle nutrient and oxygen delivery (Keske *et al.*, 2015) and hence maintains muscle health.

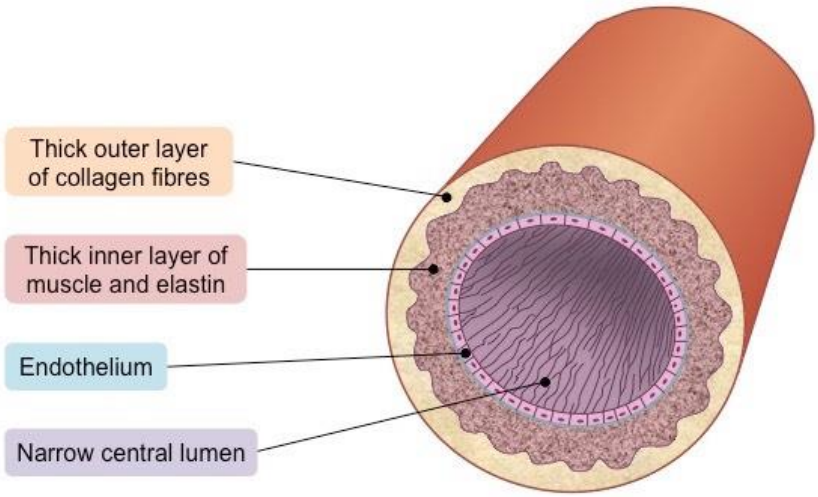


Figure 1.16: Structure of a typical artery

Platelet activation and aggregation also play a major role in the pathogenesis of CVD by promoting coagulation and plaque formation again contributing towards endothelial dysfunction (Hodgson and Croft, 2010). Antiplatelet effects were elicited following consumption of a cocoa based drink in a randomised control trial of 10 patients (Rein *et al.*, 2000) again providing evidence for the cardioprotective effects of polyphenols.

1.11.4 Examples of marketed nutraceuticals

Perhaps the best-known examples of nutraceuticals are the omega-3 fatty acids found in fish oils and marketed as capsules and bottled oils which have been shown to have excellent cardiovascular protective properties (Massaro *et al.*, 2010). Another popular nutraceutical are the plant sterols and stanols used in yoghurt-based drinks to lower cholesterol, particularly LDL cholesterol (Berger, Jones and Abumweis, 2004). They have been shown to inhibit HMGCoA reductase, discussed in section 1.5.3.3, albeit to a lesser but still significant effect compared to statins (Patti *et al.*, 2017).

1.12 Limb blood flow as a measurement of vascular health

Studies have consistently shown that ageing is associated with a 20-30% reduction in supine resting leg blood flow leading to an increase in vascular resistance (Donato *et al.*, 2006). This decrease in blood flow, and subsequent reduction in oxygen and nutrient delivery, is a key factor in the development of sarcopenia as discussed in section 1.3.8. As well as reduced delivery of oxygen and nutrients to tissues, there is a reduced capacity to clear waste metabolites, free radicals and atherogenic lipids which have all been shown to be involved in the development of cardiovascular and metabolic diseases (Dinenno *et al.*, 2001). Muscle blood flow is therefore a key link in the interplay and regulation of systemic and local muscle metabolism (Casey, Curry and Joyner, 2008).

As discussed in section 1.11.3, the vasodilation and vascular recruitment which has been seen following nutraceutical administration improves vascular compliance and endothelial function, reducing the risk of CVD (Hodgson and Croft, 2010) whilst also improving oxygen and nutrient delivery to tissues, improving muscle health (Keske *et al.*, 2015). Non-invasive methods of measuring these improvements have advanced greatly, allowing more accurate and reliable study of the effects of different stimuli such as exercise training and pharmacological agents on macrovascular changes (Salisbury *et al.*, 2018).

Limb blood flow (LBF) is a reliable, non-invasive and easily repeatable measure of macrovascular function which combines traditional 2D ultrasound with Doppler ultrasound or duplex, which calculates the shift in frequency that occurs as transmitted ultrasound waves reflected by the erythrocytes moving through the vessel (Hussain, 1997; Casey, Curry and Joyner, 2008). The velocity of flow through a large artery supplying blood to a specified region, such as the brachial or femoral, is first calculated using duplex (Casey, Curry and

Joyner, 2008). The arterial diameter is then measured using B-mode ultrasound and multiplied by the velocity of flow and by 60 to present values as millilitres per minute (Casey, Curry and Joyner, 2008). Reliability and repeatability of the measurement is achieved by simply measuring the distance to a fixed anatomical point, such as the femoral bifurcation, to the ultrasound probe at each measurement meaning that the measure can be repeated multiple times and reliably at multiple visits (Limberg *et al.*, 2014). Whilst technically not difficult to perform, to ensure accuracy of measurements, the isonation angle used is critical and must remain less than 60° as this will limit the error that may be induced in the velocity flow estimate (Casey, Curry and Joyner, 2008).

Improved LBF is not only an indicator of improved tissue oxygen and nutrient delivery but of improved vascular function in general, with studies showing that interventions which improve LBF reduce systemic markers of atherosclerotic risk and therefore reduce the risk of CVD (Limberg *et al.*, 2014).

1.13 Nutraceuticals to be studied

As described, there are a number of families of polyphenols with thousands of derivatives. For the purposes of this thesis, only the three compounds to be investigated will be expanded upon in more detail below.

1.13.1 Cocoa flavanols

There is considerable scientific evidence which suggests that the regular consumption of products containing cocoa flavanols (CF) may improve vascular health and reduce the risk of cardiovascular disease (Erdman Jr *et al.*, 2008). Consumed CF may reduce vascular inflammation by a reduced activation of monocytes and neutrophils which may reduce vascular inflammation and improve endothelial compliance (Ellinger and Stehle, 2016). Flavanol rich chocolate counteracted vascular impairment after sleep deprivation and restored working memory performance (Grassi *et al.*, 2016). The improvement in cognitive performance was thought to be because of the effects of CF on reducing blood pressure whilst improving central and cerebral blood flow (Grassi *et al.*, 2016).

West *el al.* also found that high-flavanol cocoa ingestion was associated with enhanced vasodilation in both conduit and resistance arteries and was accompanied by significant reductions in arterial stiffness in women with higher body mass index (BMI) (West *et al.*,

2014). Philips et al showed that acute cocoa flavanol (CF) supplementation improves muscle macro and microvascular responses to nutrition by improving vascular responsiveness, but ultimately did not improve MPS (Phillips *et al.*, 2016).

The FLAVIOLA study was a randomised control trial looking at the effects of CF supplementation in young and elderly adults. The primary outcome of flow mediated dilatation (FMD), a marker of endothelial function and secondary measure of SBP were significantly improved in both groups but particularly the elderly, providing clear evidence of the beneficial effects of CF in diminishing and perhaps reversing endothelial dysfunction (Heiss *et al.*, 2015).

A recent Cochrane review reported that CF caused a small (2mmHg) blood pressure lowering effect in healthy adults and further trials in hypertensive individuals were warranted (Ried, Fakler and Stocks, 2017). CF are well received generally by the public, however ensuring low sugar, quality supplements are produced is important to ensure they can be consumed in the context of a balanced diet (Goya *et al.*, 2016).

1.13.2 Green tea extracts

Many clinical and experimental studies have shown that green tea extract (GTE) consumption may be a potent intervention for improving cardiovascular health. As major polyphenolic compounds in green tea, catechins, have been shown to inhibit oxidation, vascular inflammation, atherogenesis, thrombogenesis, favourably modulate plasma lipid profile and vascular reactivity, suggesting a wide spectrum of beneficial effects of catechins on vascular function (Babu, Liu and Liu, 2008).

Epigallocatechin 3-gallate (EGCg) is the most abundant and potent green tea catechin and has been extensively studied for its beneficial health effects as a nutraceutical agent (Kim, Quon and Kim, 2014). In particular, *in vivo* experiments reveal that green tea polyphenols may be able to reduce hyperglycaemia and insulin resistance and may be effective in decreasing blood cholesterol levels (Tenore *et al.*, 2015). Whilst inhibiting development of vascular endothelial damage, EGCg has also been shown to reduce progression of existing atherosclerotic lesions (Chyu *et al.*, 2004).

In a recent review, Keske *et al.* reported that EGCg stimulates the GLUT-4 glucose transporter *in vitro*, therefore increasing glucose uptake postprandially and reducing insulin sensitivity (Keske *et al.*, 2015). The same review found that EGCg was a potent stimulator of

NO, causing vasodilatation and therefore improved vascular resistance and decreased blood pressure (Keske *et al.*, 2015). These results were repeated in another *in vitro* study which found that both green and black tea extracts were potent stimulators of NO and therefore lead to endothelial vasodilatation (Lorenz *et al.*, 2009). The latest Cochrane review concluded that the exact mechanisms contributing to the CVD protection imparted by green tea polyphenols are unknown and more long-term studies *in vivo* were needed to fully evaluate the effects of EGCg (Hartley *et al.*, 2013).

1.13.3 Curcumin

Curcumin (CU) is a phenolic compound present in the rhizomes of turmeric. It has been used by Asian communities for over 300 years and has long been theorised to possesses cardiovascular protective, anti-inflammatory, antioxidant, wound healing and cancer protective properties (Srivastava and Mehta, 2009). Many recent scientific animal studies have shown impressive antihypertensive results from CU ingestion and improved NO bioavailability leading to vascular remodelling and reduced endothelial dysfunction (Rungseesantivanon *et al.*, 2010; Boonla *et al.*, 2014).

One study in humans found that CU ingestion improved FMD significantly and equally in postmenopausal women when compared to a moderate aerobic exercise group and no changes were observed in the control group, suggesting that both can potentially improve the age-related decline in endothelial function (Akazawa *et al.*, 2012). Nakayama et al found that a single meal containing CU can significantly improve FMD in healthy males therefore potentially ameliorating postprandial endothelial dysfunction (Nakayama *et al.*, 2014).

The antibacterial effects of CU have long been heralded in Asian communities for millennia. *In vitro* studies have found excellent antibacterial action of CU as well as antiviral and antifungal (Moghadamtousi *et al.*, 2014) although these are yet to be translated into *in vivo* studies. The healing properties of CU are currently used in over 100 formulations for the treatment of a number of diseases, such as HTN, inflammatory bowel disease, diabetes and cancer (Mohanty and Sahoo, 2017). The scientific basis of the use of CU in this respect is its properties of being able to reduce inflammation and oxidative stress by inhibiting inflammatory mediators such as TNFα and NFκB (Mohanty and Sahoo, 2017). In particular, CU has been shown to antagonise the action of NFκB in various catabolic conditions such as sepsis, and this could lay the foundation for the development of treatments for such catabolic conditions in critically ill patients (Alamdari, O'Neal and Hasselgren, 2009).

1.14 Chapter conclusions

An increasingly elderly population will have significant socioeconomic consequences when considering the treatment of chronic disease and comorbidity and their implications for hospitalisation and social care for the older adult. Novel methods to improve CRF and lean MM need to be developed to ensure the elderly maintain good health and lead fulfilled lives in their later years. HIT is a time-efficient, low commitment methodology to achieve this, however further study into equipment-free HIT which can be performed by anyone, anywhere needs to be performed.

Although numerous studies have proven the positive benefits of regular exercise on health, exercise prescription is not always appropriate for the very elderly and / or infirm and it is this demographic which needs to be protected from the negative effects of sarcopenia. Whilst there is a wealth of literature on the benefits of nutraceuticals for improving MM and therefore functional reserve, little of it is in the form of high-powered randomised control trials and their further study is required before they can be reliably and safely developed for the wider elderly population.

Chapter 2: Hypotheses and Aims

2.1 Hypotheses

Age-associated declines in CRF, skeletal muscle mass and vascular function are all associated with negative health outcomes, adversely affecting the health-span of older adults. Given the trajectory of population ageing, strategies to promote healthy life years must be afforded significant attention. As a research group, we have previously shown that equipment-free HIT can improve the CRF of middle-aged adults, shifting the focus of exercise regimes away from a laboratory environment (Blackwell *et al.*, 2017).

In relation to this, the *primary hypothesis* of this thesis is that it is possible to improve indices of CRF in healthy older adults using a bodyweight-based, equipment-free HIT programme in a short timeframe of clinical relevance (Boereboom *et al.*, 2016). This thesis will also investigate whether level of supervision is important in eliciting these and other health-related improvements and if age impacts adaptive capacity to this type of exercise training.

Although our proposed HIT regime removes a number of stated barriers to exercise uptake and adherence, it must be recognised that HIT will not be a viable health-improvement strategy for all older adults. As such, this thesis will also explore the effect of plant polyphenol-enriched extracts on feeding-induced changes in muscle macrovascular blood flow in older adults, with a rationale that improving fed-state blood flow to skeletal muscle may improve muscle health.

2.2 Aims

Based on the above, the specific aims of this thesis are to:

1) Perform a systematic review and meta-analysis of randomised control trials comparing the efficacy of supervised versus unsupervised exercise regimens for improving CRF in adults.

2) Conduct and report on a randomised control trial investigating the effects of 4-weeks supervised versus unsupervised equipment-free HIT for improving the CRF of healthy young and older adults.

3) Conduct and report on a randomised control trial investigating the effects of three plant polyphenol-enriched extracts for improving muscle nutrient delivery in older adults.

Chapter 3: Supervised versus unsupervised exercise for improving cardiorespiratory fitness: a systematic review and meta-analysis

3.1 Chapter overview

Low CRF is an independent risk factor for poor health and premature mortality, however exercise is a proven strategy to improve CRF in adults. Current exercise guidelines for adults are laborious and often misinterpreted, leading to poor uptake and adherence to regular exercise. Unsupervised and home-based exercise interventions may remove some of the barriers to exercise participation, however research into their efficacy to is lacking and often poorly represented. This chapter summarises the design and results of a systematic review and meta-analysis of randomised control trials comparing the efficacy of supervised versus unsupervised exercise regimes for improving cardiorespiratory fitness in adults.

3.2 Introduction

Habitual physical activity is associated with a 20 to 30% risk reduction of all-cause premature mortality and at least 25 chronic medical conditions, including coronary heart disease, type 2 diabetes, stroke, mental health, musculoskeletal conditions and even certain cancers (Warburton, Nicol and Bredin, 2006; National Institute for Health and Care Excellence (NICE), 2013; Warburton and Bredin, 2016). There is extensive evidence that regular exercise not only improves physical health and cardiorespiratory fitness (CRF) but also has a positive effect on aspects of mental health including wellbeing, mood and release from daily stress (Vina *et al.*, 2012; National Institute for Health and Care Excellence (NICE), 2013). Physical *in*activity and its direct correlation to lower CRF (Kodama *et al.*, 2009) is thought to be responsible for 9% of premature deaths worldwide (Lee *et al.*, 2012). This statistic equated to 5.3 million deaths at the time of one study in 2008 (Lee *et al.*, 2012), with levels of physical activity having declined further since that time (World Health Organisation (WHO), 2018b).

Current public-health guidelines suggest that adults should perform at least 150-minutes of moderate-intensity, or 75-minutes of vigorous-intensity aerobic physical activity (or an equivalent combination of both) each week, plus resistance exercise on two or more days (World Health Organisation (WHO), 2018c) to improve CRF. Despite the clear benefits of habitual exercise, currently less than one in four adults worldwide meet these guidelines (World Health Organisation (WHO), 2018a) and this is associated with lower CRF and a poorer quality of life in middle-age as well as disability and frailty in the elderly (Gomes *et al.*, 2016). Perhaps more worryingly for the future, nearly 80% of adolescents also fail to reach these targets (Hallal *et al.*, 2012), almost undoubtedly contributing to the growing obesity epidemic (Eaton and Eaton, 2017) and rise in 'lifestyle-related' diseases (e.g. type 2 diabetes) (GBD 2015, Eastern Mediterranean Region Diabetes and Chronic Kidney Disease, 2018) in this age group. Physical inactivity therefore remains and looks set to continue as a pressing public health issue (Vina *et al.*, 2012).

Although exercise is a proven strategy for improving CRF and other physiological processes inhibited by inactivity, the basis for and importance of the 150-minutes of recommended exercise are often poorly explained to the public, leading to poor uptake and adherence to 'traditional' exercise training regimes (Sparling *et al.*, 2015). Indeed, the benefits of 'lesser' amounts of exercise are often overlooked when exercise advice is given, despite recommendations from bodies such as the World Health Organisation (WHO) suggesting

that aerobic exercise targets can be achieved in blocks of just 10-minutes or more (World Health Organisation (WHO), 2018c), and research findings showing that most sedentary adults will achieve some degree of health benefit when increasing their physical activity, even if this increase falls below public health recommendations (Sparling *et al.*, 2015).

Barriers to exercise in adults are often classified into external (factors beyond an individual's control) and internal (factors determined by an individual's personal decisions) factors (Korkiakangas, Alahuhta and Laitinen, 2009; Justine *et al.*, 2013; Sparling *et al.*, 2015; Morgan *et al.*, 2016), with both having a role to play in lack of engagement with physical activity. Examples of external factors include high costs of gym membership and a lack of local facilities or equipment which contribute to up to a third of adults not participating in regular exercise (Justine *et al.*, 2013; Morgan *et al.*, 2016; Ashton *et al.*, 2017). Whilst external barriers can often be overcome by strategies such as facilities expansion and improved public knowledge of facilities, internal barriers can be more difficult to overcome (Korkiakangas, Alahuhta and Laitinen, 2009). For example, older adults are often intimidated by modern fitness facilities and have anxiety about slowing others down in group exercise (Schutzer and Graves, 2004; Costello *et al.*, 2011). One barrier to exercise which can be classed as both internal and external is "lack of time". Lack of time is often cited as a principal barrier to both starting (Trost *et al.*, 2002; Korkiakangas, Alahuhta and Laitinen, 2009; Justine *et al.*, 2013; Gillen and Gibala, 2014; Ashton *et al.*, 2017; Thum *et al.*, 2017) and continuing with regular exercise, with over 50% of individuals withdrawing from an exercise programme within the first 6-months citing this reason (Thum *et al.*, 2017). This highlights a common belief that non-exercisers find physical activity impractical and time-consuming (Thum *et al.*, 2017).

Whilst superior exercise uptake and retention may be better achieved with supervision, direct supervision is expensive (Hunter *et al.*, 2018) and does not address a number of the barriers outlined above (e.g., local facilities, anxiety, costs for the individual). As such, unsupervised or home-based exercise (HBE) programmes may provide promising strategies to overcome numerous (internal and external) barriers to exercise (Dalal *et al.*, 2010) and improve CRF. Although previous studies have reported on the feasibility and efficacy of HBE programmes for improving physical function, and on adherence rates for these regimes, the majority of these studies have been conducted in specific co-morbid populations such as heart failure, post-coronary artery bypass graft and intermittent claudication (Dalal *et al.*, 2010; Aamot *et al.*, 2014), with alternative measures of physical function (vs. CRF) as the outcome. Therefore, we conducted a systematic review and meta-analysis of studies comparing supervised versus unsupervised exercise for improving CRF in adults.

3.3 Methods

3.3.1 Study design and inclusion criteria

This systematic review was registered prospectively with PROSPERO (registration number CRD42018096901) and was carried out in accordance with the PRISMA statement (Moher *et al.*, 2009). Only randomised control trials (RCTs) directly comparing the same supervised to unsupervised exercise protocol in adults aged 18 and over were included. Studies were excluded if they did not report VO_2 peak as measured by graded cardiopulmonary exercise testing (CPET), the gold standard investigation of exercise tolerance and CRF (Weisman *et al.*, 2003).

3.3.1.1 *Study PICOS*

Population: Adults aged 18 and over
Intervention: Exercise of any form in a supervised environment
Comparison: The same exercise at home or unsupervised
Outcomes: Improvements in CRF reported as VO_2 peak as measured by CPET.

3.3.2 Literature search

Literature searches were carried out by a trained Clinical Research Librarian using MEDLINE, EMBASE and CINAHL databases, all searched from their inception to 20th April 2019. The search strategies are listed in Appendix 1. The Cochrane Library of Systematic Reviews was searched for relevant reviews and a 'grey literature' search of Clinicaltrials.gov for relevant unpublished studies was performed. The reference section 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.

Abstracts were screened by one author (TS) with the aid of Rayyan systematic review software (Qatar Computing Research Institute, Doha, Qatar) (Ouzzani *et al.*, 2016) and considered for full text review if deemed to be potentially relevant. Full text versions of all potentially relevant primary studies were then independently screened against the inclusion

and exclusion criteria by two authors (TS and PT) and agreement for inclusion reached by consensus.

3.3.3 Data extraction

Study characteristics were extracted by one author (TS) with outcome data independently extracted and verified by two authors (TS and PT). Risk of bias for included studies was assessed using the Cochrane Collaboration RoB2 tool (The Cochrane Collaboration, 2016) for assessing risk of bias for RCTs independently by two authors (TS and PW) with any disagreement resolved by consensus.

3.3.4 Statistical analysis

Effect estimates are reported as difference in means with 95% confidence intervals. Where standard deviations of change were not reported, these were calculated as described previously based on Cochrane methodology (The Cochrane Collaboration, 2011). A correlation coefficient of 0.8 between pre and post-exercise values was assumed for these calculations with a random effects model used for the analysis. Our aim to conduct tests for publication bias based on mean differences and sample size and to conduct meta-regression was precluded by the low number of studies meeting our eligibility criteria. Risk of bias was assessed in duplicate using the Cochrane risk of bias tool with the I^2 to quantify statistical heterogeneity. Evidence quality was assessed using GRADE (Guyatt *et al.*, 2008). In order to reduce type I and II errors in analysis (Doleman, Williams and Lund, 2019), trial sequential analysis was conducted using software from the Copenhagen Trial Unit (Wetterslev, Jakobsen and Gluud, 2017). O'Brien-Fleming monitoring boundaries were constructed to adjust for multiple comparisons at a type I error rate of 5%. The required information size to give a power of 80% using empirical estimates of effect sizes, variance and diversity was calculated. All other analyses were conducted on Stata Version 15.2 (StataCorp LLC, 2017). Meta-analysis and trial sequential analysis were carried out by an independent statistician.

3.4 Results

3.4.1 Search results

A total of 3121 unique studies were identified after removing duplicates, 3115 from the original literature search and 6 from other sources as described in the methods section of this manuscript. Twenty-eight of these studies were identified for full text review of which 24 did not meet the full inclusion criteria, leaving 4 studies (Gettman, Pollock and Ward, 1983; Hsieh *et al.*, 2009; Kraal *et al.*, 2014; Hunter *et al.*, 2018) included in both the qualitative and quantitative synthesis (Figure 3.1).

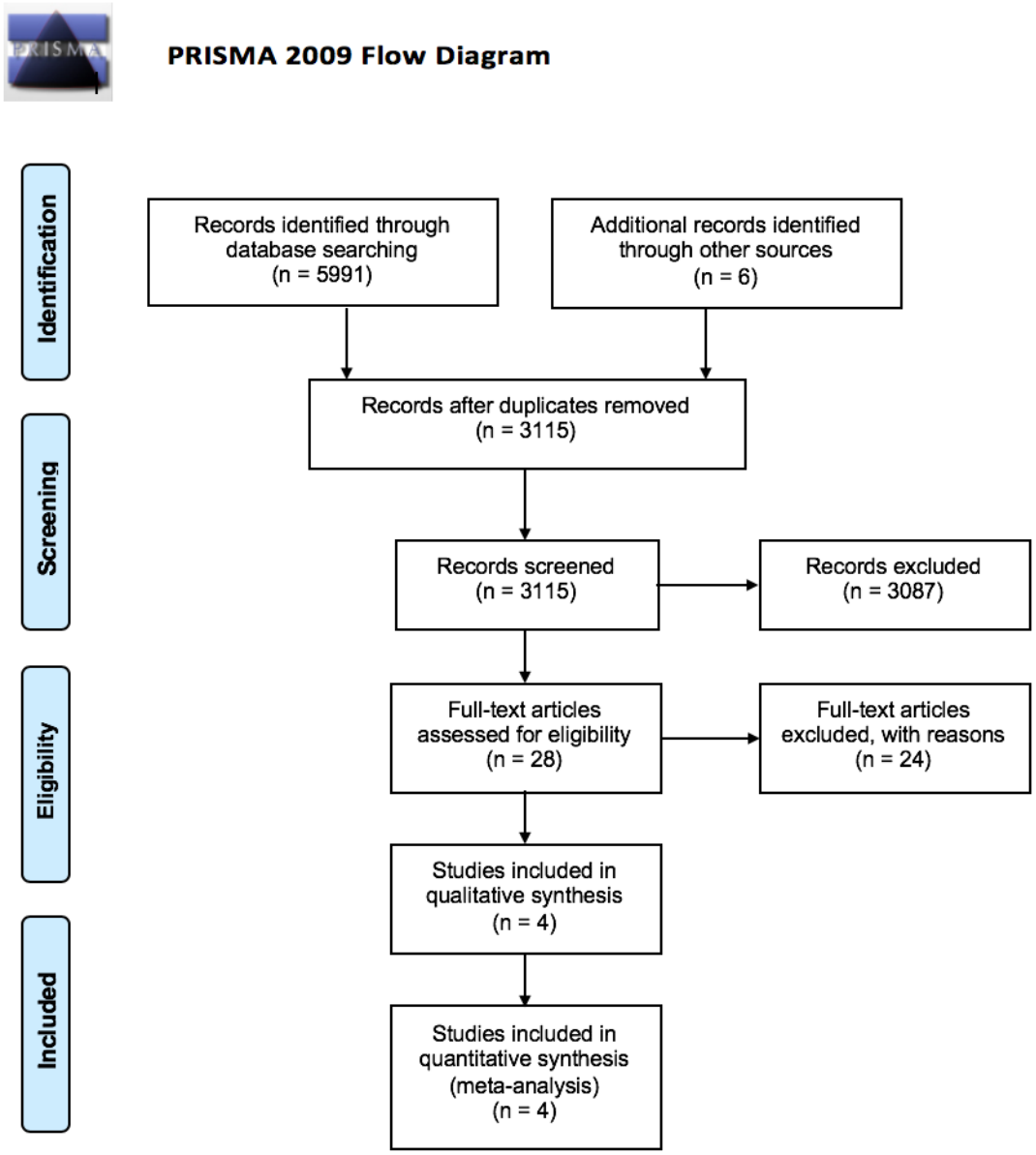


Figure 3.1: PRISMA flow diagram

3.4.2 Study characteristics

The study characteristics of the final included studies can be seen in Table 3.1. The earliest study meeting the inclusion criteria was published in 1983 (Gettman, Pollock and Ward, 1983) and the latest in 2017 (Hunter *et al.*, 2018). All studies were RCTs and all were full articles in peer reviewed journals. One study included a non-intervention control group (Gettman, Pollock and Ward, 1983) with the other three using the unsupervised group as the 'control' group (Hsieh *et al.*, 2009; Kraal *et al.*, 2014; Hunter *et al.*, 2018). All studies employed moderate to high-intensity aerobic exercise training (AET) at between 65-80% of predicted maximum heart rate with one study increasing this to 75-90% after 4-weeks exercise training (Hunter *et al.*, 2018). Intervention duration ranged from 8 to 20-weeks. One study combined aerobic exercise with resistance exercise training (RET) (Hunter *et al.*, 2018). Three out of the four studies used at least one piece of exercise equipment for AET (Hsieh *et al.*, 2009; Kraal *et al.*, 2014; Hunter *et al.*, 2018), with one study using an outdoor running track (Gettman, Pollock and Ward, 1983).

3.4.3 Risk of bias

All studies were assessed for bias using the Cochrane Collaboration RoB2 tool (The Cochrane Collaboration, 2016) (Figure 3.2*)*. All studies were found to have some concerns in the domain of measurement of the outcome. This arose due to the understandable and common difficulty in blinding assessors and participants during an exercise intervention.

3.4.4 Data synthesis

It was judged that there were sufficient studies to perform an independent meta-analysis for AET interventions. All studies were included in this meta-analysis.

Study	Country of Study	Mean age (years)	% Male	Sup N	Unsup N	Intervention	Sup mean change VO_2 peak	Unsup mean change VO_2 peak
Gettman 1983 (Gettman, Pollock and Ward, 1983)	USA	41.3	100	11	11	20-weeks mod intensity aerobic, 45-mins 3x per week	6.6	7.1
Hsieh 2009 (Hsieh *et al.*, 2009)	Taiwan	52.7	0	15	15	8-weeks mod intensity aerobic, 60-mins 3x per week	1.7	2.2
Hunter 2017 (Hunter *et al.*, 2018)	Australia	42.5	25%	25	25	8-weeks high intensity aerobic 30-mins + resistance 1-5x per week	2.4	3.2
Kraal 2014 (Kraal *et al.*, 2014)	Netherlands	58.4	86%	25	25	12-weeks mod intensity aerobic, 60-mins 3x per week	2.9	-0.9

Abbreviations: mod = moderate; N = number per group; Sup = supervised exercise; Unsup = unsupervised exercise

VO_2 peak values are presented as ml/kg/min

Table 3.1: Study characteristics of included RCTs

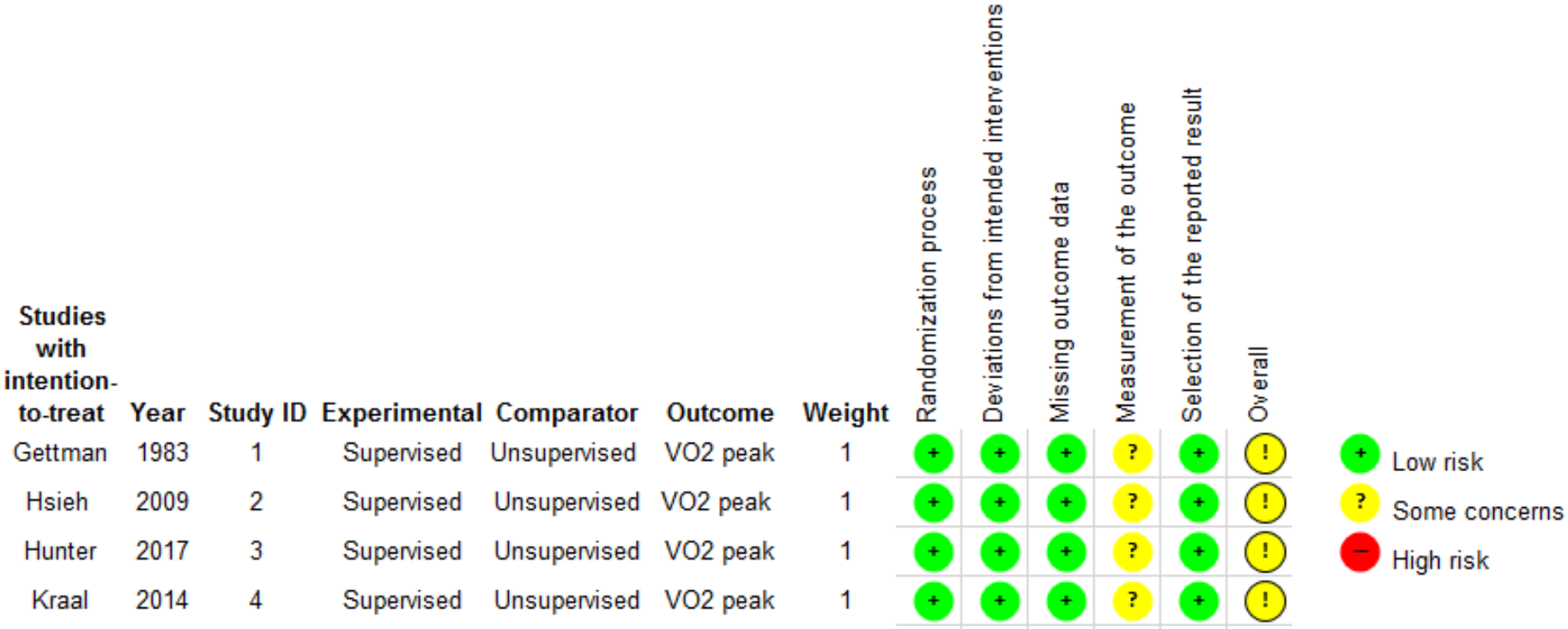


Figure 3.2: Risk of bias for included RCTs

3.4.5 Aerobic exercise outcomes

Collectively all studies reported CRF for a total of 152 participants before and after an exercise intervention (76 in a supervised intervention, 76 in an unsupervised intervention). There was no superior improvement in CRF in the supervised exercise group (mean difference 0.52, 95% confidence interval -1.62 to 2.66) (Figure 3.3). There was evidence of substantial statistical heterogeneity (I^2 = 87.2%) and trial sequential analysis showed an inadequate information size (power) to answer the clinical question (152/640 participants).

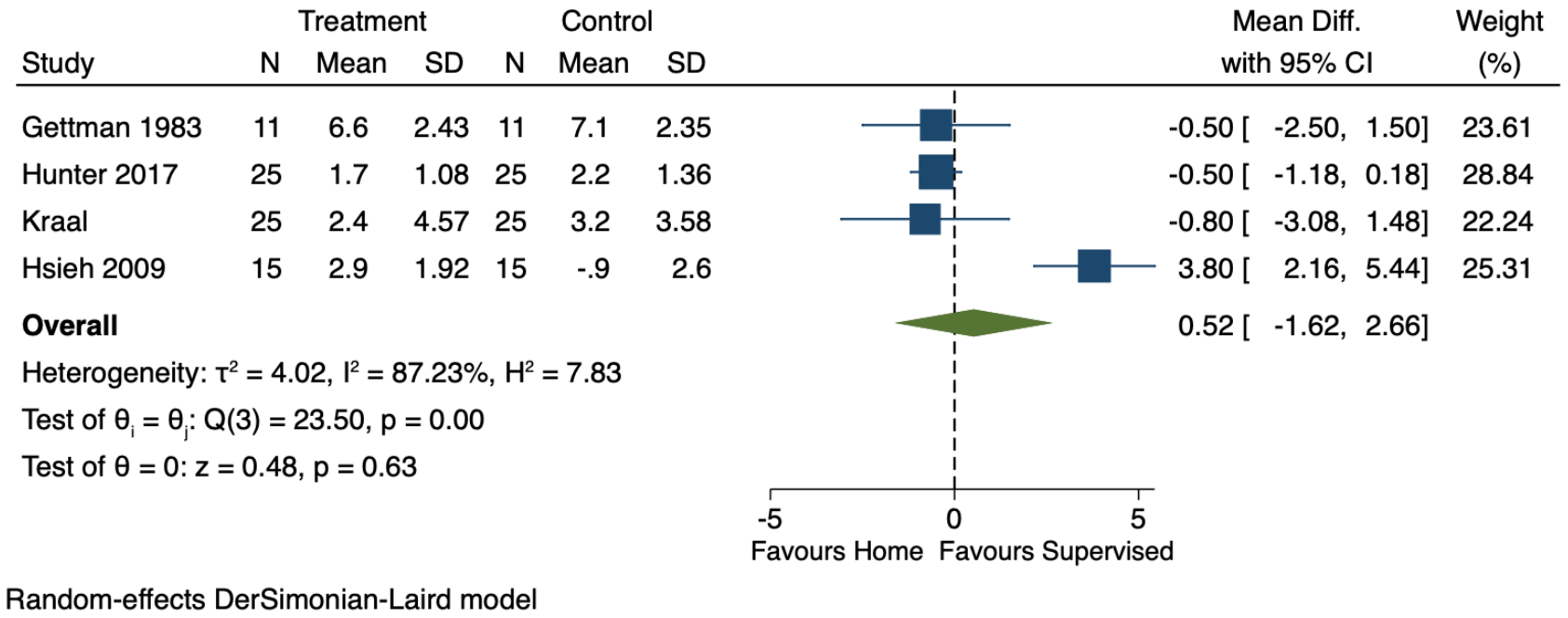


Figure 3.3: Forest plot of supervised versus unsupervised aerobic exercise training for eliciting improvements in VO2 peak

3.5 Discussion

This systematic review demonstrates that there is limited evidence for the superiority of supervised versus unsupervised exercise training for improving CRF in adults. Whilst one of the included studies suggests a superior improvement in CRF with supervised exercise this was not observed on meta-analysis (Figure 3.3*).* The meta-analysis of this review was not statistically significant as the low number of participants coupled with the substantial methodological weaknesses and statistical heterogeneity meant this outcome was underpowered (Doleman, Williams and Lund, 2019).

From the extensive literature search performed, only the four included studies directly compared the same exercise regime in a supervised and unsupervised setting. Several studies did not meet the inclusion criteria as, despite the time and number of exercise sessions being matched between supervised and unsupervised exercise, the supervised exercise often used equipment such as a treadmill or cycle ergometer, with the unsupervised exercise using walking or brisk jogging with consequent differences in intensity (Oncu, Durmaz and Karapolat, 2009). It would therefore appear that previous studies may have been heavily biased in favour of the supervised setting both in terms of the modality and intensity of the exercise. This bias may have led to greater improvements in CRF as often reported in studies where the exercise modality was not matched between the two groups (Hsieh *et al.*, 2009; Storer *et al.*, 2014).

Of the four studies included in this review, the shortest AET session was 30 minutes on a cycle ergometer, although this was followed by RET. The next shortest regime was 45 minutes on an outdoor running track, with the final 2 studies employing sessions of 60 minutes, with all these sessions taking place at least 3 times per week. Although these interventions would meet current physical activity guidelines for adults, the continuous nature of these sessions would not address one of the most commonly cited barriers to exercise, lack of time (Trost *et al.*, 2002; Korkiakangas, Alahuhta and Laitinen, 2009; Justine *et al.*, 2013; Gillen and Gibala, 2014; Ashton *et al.*, 2017; Thum *et al.*, 2017).

In addition, three of four studies included in this review used at least one piece of exercise equipment such as a treadmill or cycle ergometer, with only one promoting a potentially equipment-free exercise protocol, albeit using an outdoor running track (Gettman, Pollock and Ward, 1983). Equipment-free exercise is important in overcoming external barriers to exercise (equipment and local facilities), as well as a number of internal barriers related to

self-confidence and perceived ability (Schutzer and Graves, 2004; Costello *et al.*, 2011) as these exercise regimes can likely be performed in the home.

Although several studies have previously compared supervised versus unsupervised exercise in diseased cohorts, co-morbidities often prohibit participants in such studies from being able to perform a graded CPET for the assessment of fitness in these studies. They instead use an alternative measure of 'fitness' such as the six-minute walk test (6MWT) (American Thoracic Society, 2002; Kubo *et al.*, 2018) which, whilst validated for determining changes in functional capacity, does not determine peak oxygen uptake, or evaluate the causes of exercise limitation (American Thoracic Society, 2002). As such, these measures are not a reliable assessment of CRF which was our main outcome, and as such we did not include these studies in this review despite them directly evaluating exercise supervision.

Developing unsupervised HBE regimes may be a very useful tool to combat population inactivity and improve CRF. However, based on the evidence gap demonstrated by this review, further studies are required to fully determine the efficacy of equipment-free, unsupervised HBE for improving CRF and subsequently improving population health.

3.6 Limitations

As is common to all systematic reviews, a major limitation is the potential for missed studies from our literature search. In order to mitigate against this as far as possible, 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 search of all references of included studies was undertaken, together with another search of all articles citing included studies. The small number of studies eligible for inclusion in this review resulted in the data being underpowered for meta-analysis. In addition, we were unable to evaluate publication bias or investigate statistical heterogeneity due to the low number of studies.

Our primary outcome for this review was a change in CRF measured by a mean change in VO_2 peak during a graded CPET as this is considered the gold standard investigation of physical fitness (Weisman *et al.*, 2003). Although this design meant that only one variable was included in our final results for analysis, it is possible that other health and psychological outcomes may have shown a difference between supervision type not reported in this study.

3.7 Chapter conclusions

Physical inactivity and low CRF is a pressing, global public health issue and an independent risk factor for premature mortality. Although regular exercise is a proven strategy to improve CRF and overall health, uptake and adherence is poor. Unsupervised exercise regimes may remove a number of commonly cited barriers to exercise but to date they have been poorly studied with inconsistent comparisons to supervised regimes. Further studies are needed to qualify the fitness gains achievable by unsupervised, ideally equipment-free and time-efficient, exercise strategies to encourage exercise participation by physically inactive adults.

Chapter 4: The efficacy of short-term, home-based, equipment-free, high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness and cardiometabolic health in older adults

4.1 Chapter overview

There is a natural decline in cardiorespiratory fitness (CRF) and skeletal muscle mass (MM) with advancing age. Lower levels of CRF and MM are both independent risk factors for increased risk of premature mortality. Exercise is a proven strategy to improve CRF and other physiological processes inhibited by inactivity, however habitual exercise uptake and adherence in older adults is poor (as discussed in Chapter 3). As such, designing proven, repeatable, safe and most importantly enjoyable exercise regimens for improving CRF and MM in older adults remains an important challenge. This chapter describes the scientific basis for, methods and results of a randomised control trial assessing the efficacy of short-term, equipment-free, high-intensity interval training (HIT) for improving indices of CRF, body composition and cardiometabolic profile in older adults and the impact of supervision.

4.2 Introduction

Cardiovascular diseases (CVD) produce significant global health and economic challenges. CVD is the most common underlying cause of death worldwide, accounting for nearly one third of deaths: 17.3 million of 54 million total deaths per year (Ryan, Heath and Cook, 2018). There is a clear link between a sedentary lifestyle, or physical inactivity, and an increased risk of developing CVD with advancing age (LaMonte *et al.*, 2000). In some countries, physical inactivity is as high as 70% amongst adults, accounting for 1-3% of national health care costs, mainly due to changing patterns of transportation, increased use of technology and urbanization (World Health Organisation (WHO), 2018a).

There is robust evidence demonstrating that with advancing age there is a natural decline in CRF. This amounts to 1.6% of baseline VO_2peak per year from the age of 57 years (Hakola *et al.*, 2011). It has been suggested that this natural decline is linked to the decline in mitochondrial quality with age and a reduction in maximal ATP production and aerobic respiration rate (Nair, 2005; Seo *et al.*, 2016). An inverse association between physical fitness and the incidence of CVD and premature mortality in healthy or asymptomatic individuals has been previously demonstrated (Kodama *et al.*, 2009; Al-Mallah *et al.*, 2014) (Figure 4.1). Adults who spend increased time in sedentary behaviours and decreased time in physical activity are more likely to have reduced levels of fitness and more likely to have metabolic risks (Healy *et al.*, 2008) and muscle atrophy (Vainshelboim *et al.*, 2019). It is estimated that such sedentary adults with lower levels of CRF have a mortality risk that is 4.5 times higher than those that exercise regularly (Myers, 2003). To emphasize the problem, reduced CRF has also been shown to be a stronger risk factor for all-cause mortality than hypertension, smoking and diabetes (Booth, Roberts and Laye, 2012). Despite this, the acquisition of data regarding worldwide CRF has been limited and it is currently the only major risk factor not routinely or regularly assessed in the general clinical setting (Kaminsky *et al.*, 2013).

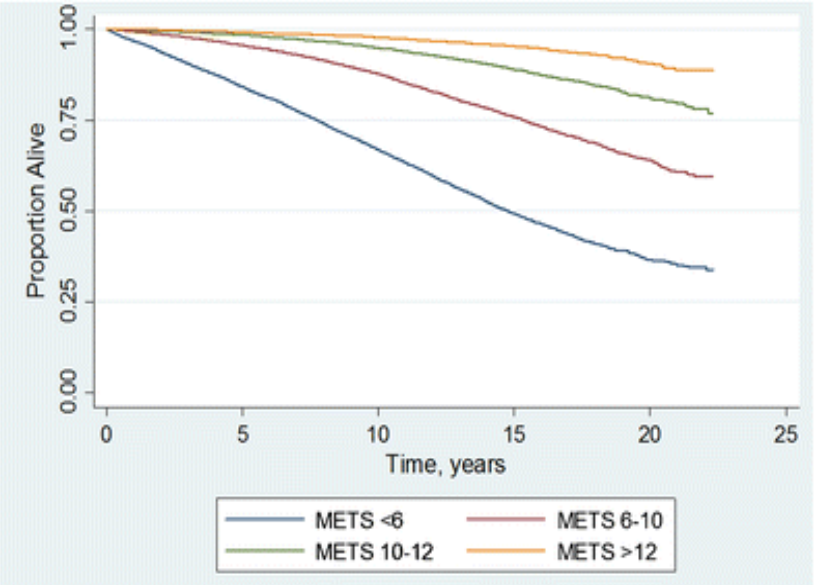


Figure 4.1: Kaplan-Meier survival graph showing graded decrease in survival with decreasing CRF with decreasing METS per week (Al-Mallah et. al, 2014)

Another important risk factor for increased rates of premature mortality in the elderly is the natural decline in lean muscle mass (LMM), which has been shown to be a more important predictor than raised body mass index (BMI) alone (Abramowitz *et al.*, 2018; Lee *et al.*, 2018). As discussed in section 1.9.1, a decrease in physical activity with advancing age can contribute to a great decrease in LMM of up to 30% (Evans, 2010) in the multifactorial process of sarcopaenia (Cruz-Jentoft *et al.*, 2019), which is a precursor to falls and frailty syndromes in older adults and is a source of considerable healthcare expenditure (de Labra *et al.*, 2015). Decreased LMM is directly correlated to lower VO_2peak and therefore lower levels of CRF in older adults (Boo *et al.*, 2019), again highlighting the negative health impact of lower levels of LMM.

As well as decreased LMM, excess accumulation of body fat is a significant and rapidly increasing global health issue. More than 39% of adults are considered to be overweight (BMI > 25kg/m^2) and 13% considered obese (BMI > 30kg/m^2), increasing the risk of atherosclerosis, CVD and in particular central and visceral adiposity which magnifies risk of chronic illness and mortality (Wewege *et al.*, 2017). The global rise of inactivity and obesity amongst adults has led to an increased prevalence of the Metabolic Syndrome (MetS) as discussed in section 1.6. Obesity is a precursor to dyslipidaemia which is a hallmark of MetS and is yet another CVD risk (Wood *et al.*, 2019).

Exercise is a proven strategy to improve CRF and LMM in older adults (Myers, 2003; Bruseghini *et al.*, 2015; Marzetti *et al.*, 2017) as well a number of other physical processes inhibited by inactivity and ageing (Warburton, Nicol and Bredin, 2006; de Labra *et al.*, 2015). A recent review concluded that regular physical activity by older adults increases aerobic capacity, muscle mass and strength and remains the best strategy for preventing and even reversing the effects of sarcopaenia, improving physical function and preventing disability (Marzetti *et al.*, 2017). The negative consequences of not engaging in regular physical activity are not however immediately apparent. For example, chronic diseases such as CVD and T2DM are strongly associated with sedentary behaviour (Castro *et al.*, 2018; Vainshelboim *et al.*, 2019) and may develop for decades before their more devastating effects are manifest as acute cardiac events or mortality (Chao, Foy and Farmer, 2000). Finally, there is also compelling evidence for the cognitive and psychological benefits attainable from regular exercise participation by older adults which is often overlooked (Chodzko-Zajko, Schwingel and Chae Hee Park, 2009).

For those over 65, in addition to the guidelines by the WHO discussed in Chapter 3, guidelines, it is recommended that they should perform muscle strengthening exercises on 2 or more days a week and if they have poor mobility, to perform physical activity to enhance balance and prevent falls on 3 or more days per week (World Health Organisation (WHO), 2018c). Currently less than one in four adults worldwide meet these guidelines (World Health Organisation (WHO), 2018a) with physical activity levels in older adults remaining the lowest (Langhammer, Bergland and Rydwik, 2018). As discussed in Chapter 3, “lack of time” is often cited as a principal barrier to both starting regular exercise (Trost *et al.*, 2002; Costello *et al.*, 2011) and adhering to an exercise programme (Korkiakangas, Alahuhta and Laitinen, 2009). Innovations in exercise prescription that show benefits despite a minimal time commitment therefore represent a valuable strategy to encourage physical activity participation (Gibala and Little, 2010). However, the optimal training regimen for improving CRF in older adults remains undefined (Hwang *et al.*, 2016).

Adherence is indisputably the measure of success of an exercise intervention and maximising long-term health benefits (Weston, Wisløff and Coombes, 2014). Exercise adherence in older adults is a multifactorial problem which encompasses many biopsychosocial factors (Rivera-Torres, Fahey and Rivera, 2019). It is estimated that up to half of older adults who begin a physical activity programme will drop out within six months (Chao, Foy and Farmer, 2000). There is also a preconceived notion amongst older adults, particular those with comorbidities and joint problems, that exercise may do more harm than actual good and may even worsen pre-existing conditions (Costello *et al.*, 2011). Whereas “lack of time” may be a barrier to

exercise in middle-aged adults, the elderly most frequently cite age and “poor health” as the leading barrier (Cohen-Mansfield, Marx and Guralnik, 2003), with many older adults being poorly advised as to the benefits of regular exercise on general health and improving co-morbidities in this age group (Schutzer and Graves, 2004). In a study of over 70,000 exercise logs in older adults, it was concluded that contrary to previous beliefs, older adults are able to perform MICT and HIT without strict supervision (Reitlo *et al.*, 2018). When designing unsupervised or home-based exercise regimens, they should be easy to follow with minimal numbers of exercises to complete, have good initial guidance on how to perform them, not worsen existing pain and be quick to complete, thereby bypassing “lack of time” to complete (Bachmann, Oesch and Bachmann, 2018). As such, unsupervised or home-based exercise (HBE) programmes may provide promising strategies to overcome numerous (internal and external) barriers to exercise (Dalal *et al.*, 2010), especially where programmes comprise an initial supervised exercise course (Stefanov *et al.*, 2013).

Another great challenge in convincing older adults to take up exercise for cardiometabolic modification is the vast improvement in pharmacological treatments available for a myriad of conditions. Whereas improvements in health may take a long time to manifest physically with exercise, pharmacological treatment is often much more rapid in effect and when compared to exercise, easier to be compliant with (Chao, Foy and Farmer, 2000). Designing effective, proven and importantly repeatable exercise regimes may be key to providing alternative methods other than pharmacological to improve health.

High-intensity interval training (HIT) has emerged as a time-efficient, feasible modality for improving CRF in the elderly (Boereboom *et al.*, 2016; Søgaard *et al.*, 2018) as discussed in length in section 1.10. HIT is also a proven method to reduce total body fat (Wewege *et al.*, 2017; Maillard, Pereira and Boisseau, 2018; Silveira-Coswig *et al.*, 2020), although evidence for the ability of aerobic based forms of HIT for improving LMM is less available (Blue *et al.*, 2018). Not only just being a potent stimulus for cardiorespiratory adaptations, studies have also shown an improvement in cardiometabolic disorders such as insulin resistance in as little as two weeks (Whyte, Gill and Cathcart, 2010), established T2DM (Little *et al.*, 2011; Francois and Little, 2015) and hypertension (Laursen and Jenkins, 2002) when using HIT. Furthermore, the treatment of established diseases and the geriatric syndromes discussed in section 1.2.3 is more effective following higher-intensity exercise interventions (Chodzko-Zajko, Schwingel and Chae Hee Park, 2009).

The main attraction of HIT is the significantly reduced time commitment needed to achieve these improvements when compared to MICT (Gibala and Little, 2010; Gillen and Gibala, 2014). Owing to its relatively new arrival on the exercise scene, the majority of studies investigating HIT have thus far been performed in the safety of the laboratory setting (Karlsen *et al.*, 2017) with supervision (Shepherd *et al.*, 2015; Gray *et al.*, 2016). The majority of these studies have also utilised specialised equipment such as treadmills and cycle ergometers and such conditions are unrealistic to create for a real-world population without expensive gym memberships. There are very few studies which have investigated the ability of HIT when performed unsupervised, such as at home (Blackwell *et al.*, 2017; Scott *et al.*, 2019) and at the time of writing this thesis, none in older cohorts. Our research group was the first to show that a home-based, unsupervised, bodyweight-based HIT programme can improve indices of CRF in as little as 4 weeks in middle-aged adults when compared to a supervised, cycle ergometer based programme (Blackwell *et al.*, 2017). However, with so many different HIT interventions having been studied, the optimal duration, training intensity and frequency of interval exercise protocols is subject to further study (MacInnis and Gibala, 2017). Furthermore, there is no universal criteria or framework for prescription and monitoring of HIT interventions to the wider public and safety concerns remain a common barrier for implementing HIT as standard care in clinical populations (Taylor *et al.*, 2019).

Maintaining health and postponing the onset of debilitating disease for as long as possible is necessary to avoid morbidity in later life (Schutzer and Graves, 2004). Considering the rapid rise of inactivity amongst older adults and the associated health care burden, more easily affordable, “real world” exercise regimes that maintain adherence and remove the barriers discussed are needed. Owing to the multiple benefits of HIT discussed, translating HIT away from the laboratory environment is critical for delivering these health improvements to clinical populations. At the time of writing this thesis, there were no comparative studies of supervised versus unsupervised or home-based, equipment free HIT studies investigating improvements in CRF in older adults. In order to assess the efficacy of HIT for improving the CRF, cardiometabolic profile and LMM of older adults, with two known barriers to exercise, namely equipment and supervision, removed, we investigated the efficacy of 4-weeks supervised and unsupervised, equipment-free HIT.

4.3 Experimental methods

4.3.1 Ethical approval

Ethical approval was applied for from the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee prior to any subjects being recruited into the study. This included ethical approval for the use of ionising radiation in the form of Dual-energy x-ray absorptiometry (DXA). The study was given the approval reference number of C16122016. The study was also prospectively registered with clinicaltrials.gov, identifier NCT number: NCT03473990. Full details of the ethical approval and amendments can be seen in Appendix 2.

4.3.2 Recruitment

Participants for the studies in this thesis were recruited through a variety of methods. Firstly, the study was advertised via a poster in local health clubs as well as the local newspaper. Two volunteer banks were used: the first being the Royal Derby Hospital Trust Voluntary Services Team; the second was the University of Nottingham registry of previous healthy volunteer subjects, who had consented to having their personal details stored on a secure database for the purpose of being contacted for future studies. Local societies and charity groups were contacted and oral presentations given at their respective monthly meetings.

4.3.3 Inclusion criteria

The inclusion criteria for this study were:

- Male or female
- Age 55 and over
- Full capacity to consent

4.3.4 Exclusion criteria

The exclusion criteria for this study were:

- Current participation in a formal exercise regimen (more than three times per week vigorous exercise)

- BMI < 18 or > 35 kg/m^2
- Active cardiovascular disease:
  - Uncontrolled hypertension (BP > 160/100)
  - Angina
  - Heart failure (class III/IV)
  - Significant cardiac 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
  - Diabetic patients needing medication
  - Uncontrolled thyroid disease
- Malignancy
- Clotting dysfunction
- Significant musculoskeletal or neurological disorders
- Family history of early (<55y) death from cardiovascular disease
- Participation in a research experiment within the last 3 months

4.3.5 Screening visit and consent

People replying to the advertisements were emailed the study participant information sheet as well as documents relating to the storage of their personal data and bodily samples on a secure database. Having been able to fully evaluate this information, they were then invited for a screening visit at the University of Nottingham Medical School at the Royal Derby Hospital. Participants were asked to fast from midnight the preceding night to ensure fasting blood tests could be taken as described later in this section. They were also asked to refrain from drinking alcohol 24 hours before this visit. Where possible, to ensure participants were not involved in more than 3 vigorous sessions of exercise per week (an absolute exclusion

criterion), the International Physical Activity Questionnaire Short Form (IPAQ-SF; Appendix 4) was completed as a screening tool (P. H. Lee *et al.*, 2011) via telephone. If that was not possible, it was completed at the screening visit.

During this visit, the participant was first given a full explanation of the study by an investigator. Having had the opportunity to ask any questions and still wishing to take part, they signed the study consent form (Appendix 5). The investigator then took a full, detailed medical history from the participant followed by a full medical examination of the cardiorespiratory system, abdomen, and upper and lower limbs. Anthropometric measures of height and weight were taken to calculate the participant's BMI. Having sat for five minutes, BP was measured from the left arm (where possible) (Beaney *et al.*, 2018) using an automated sphygmomanometer. Three readings were taken one minute apart (Beaney *et al.*, 2018) and the mean systolic and diastolic BP recorded. A resting 12-lead electrocardiogram (ECG) (CardioDirect 12, Spacelabs Healthcare, Washington, USA) was then performed to rule out existing cardiac rhythm abnormalities. Participants with abnormal BP readings, ECG changes, BMI outside the exclusion criteria or severe joint restrictions meaning they could not complete the exercises were excluded at this stage. Finally, baseline blood tests were performed for measurement of full blood count (FBC), urea and electrolytes (UEs), liver function tests (LFTs), lipid profile, thyroid function tests (TFTs), fasting glucose, glycated haemoglobin (HbA1c) and clotting screen to rule out any contraindications for inclusion in the study as per the exclusion criteria outlined later. Any abnormal findings and blood results were discussed with the participant and with their consent, a letter to their GP was sent for further investigation and follow up.

4.3.6 Acute study day

Having fulfilled all the necessary criteria for inclusion, participants were asked to attend for an acute study day. The same fast as for the screening day was observed the night preceding. This day allowed for pre-intervention baseline physiological assessment. Each intervention is listed below and then described in more detail in following sections.

1) Oral glucose tolerance test
2) Dual-energy x-ray absorptiometry (DXA)
3) Standardised meal
4) Muscle architecture scan
5) CPET

4.3.7 Oral glucose tolerance test (OGTT)

The two-hour fasted OGTT as described by the World Health Organisation (World Health Organisation (WHO) and International Diabetets Federation, 2006) was performed prior to and after the 4-week intervention period by all participants.

4.3.7.1 *Test procedure*

Participants were asked to starve from midnight the night preceding to ensure a minimum of 8 hours fasting prior to the test (World Health Organisation (WHO) and International Diabetets Federation, 2006) but were allowed water freely. Following a full explanation of the test, participants were asked to lie or sit on an examination bed. A 22-gauge intravenous cannula (BD Venflon™ Pro, Becton Dickson, New Jersey, USA) was inserted in a retrograde position into a vein on the dorsum of the non-dominant hand or wrist, where possible. The cannula was connected to a 3-way tap to facilitate repeated blood sampling. A slow continuous infusion of 0.9% sodium chloride (Baxter International Inc., Illinois, USA) was then attached to maintain line patency and to allow periodic flushing of the cannula after blood sampling to prevent the line from clotting. The cannulated hand was then placed into a hand warming box (Figure 4.2) and heated to 55°C to allow arterialisation of the venous blood (Liu *et al.*, 1992).



Figure 4.2: Retrograde cannula inserted into the dorsum of the hand and connected to a continuous saline infusion. The hand was then placed into the pictured hand warmer for the duration of the OGTT.

A baseline sample of 4 mL of blood was taken and the blood glucose concentration measured with a near-patient glucose analyser (YSI Life Sciences, Ohio, USA). The remaining sample was placed in ice and after centrifugation, the separated plasma was frozen at -80°C for future analysis of insulin concentrations. Participants were then asked to ingest a glucose drink made with 75g of oral dextrose powder (Myprotein, Cheshire, UK) dissolved in 200mL of plain water. Following ingestion, blood samples were drawn at regular 15 minute intervals for a duration of 120 minutes (World Health Organisation (WHO) and International Diabetets Federation, 2006). 3ml of blood was drawn from the cannula to expel blood which had been diluted by the continuous 0.9% sodium chloride infusion and discarded, and then a 4ml sample and analysed above, after which the line was again flushed with 5ml of 0.9% sodium chloride and the slow continuous infusion restarted.

4.3.7.2 *Measurement of plasma insulin*

Plasma insulin concentrations were measured using an ultrasensitive enzyme linked immunosorbent assay (ELISA) (Mercodia AB, Uppsala, Sweden), according to manufacturer's instructions. Samples were thawed at room temperature and then vortexed to ensure thorough mixing of defrosted samples. All postprandial samples were diluted 1:12 using the ELISA kit "Calibrator 0" (Mercodia AB, Uppsala, Sweden) to ensure that the measured concentration fell within the reference range of the assay.

25μL of plasma and calibrator solutions were then pipetted into the plate wells before enzyme conjugate solution was added to each well and incubated on a plate shaker for 1 hour at room temperature. The plate was then manually washed six times with pre-prepared wash buffer solution. Substrate TMB was added to each well and incubated for 30 minutes at room temperature. Stop solution was then added and the plates mixed on a shaker for approximately 5 seconds to ensure mixing of the substrate complex and stop solution. Finally, the fully prepared plates were read at 450nm using an optical plate reader (Thermo Scientific Multiskan Ascent, ThermoFisher Scientific, USA) to allow calculation of the insulin concentrations against the calibrator concentration curve (Figure 4.3).

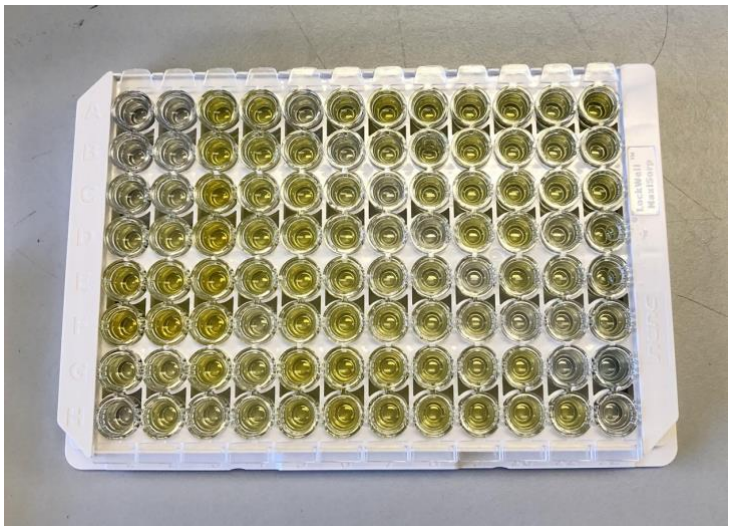


Figure 4.3: Mercodia Insulin ELISA sample plate after addition of stop solution before reading on optical plate reader

4.3.7.3 *Insulin resistance*

Plasma samples were also used to calculate Insulin resistance using HOMA-IR as described in section 1.5.6.3.

4.3.7.4 *Data analysis*

Calculated glucose and insulin levels were plotted against the 15-minute time intervals and concentrations for the area under the curve (AUC) were subsequently calculated.

4.3.8 Body composition and Dual-energy x-ray absorptiometry scan

Following the OGTT, participants had their body composition measured using a whole-body DXA scan (Lunar Prodigy 2, GE Medical Systems, Buckinghamshire, UK). This allowed for pre and post-intervention measurement of whole-body mass, total fat mass (TF), body fat percentage (FM%) and lean muscle mass (LMM) (Figure 4.4). In line with University of Nottingham policy, all DXA scans were performed by an Ionising Radiation Medical Exposure Regulations (IR(ME)-R) trained operator. Participants were asked to first void their bladder immediately prior to scanning to prevent distortion of the images from excess urine. Immediately prior to scanning, participants were again fully informed of and consented to the minimal radiation exposure during the scan. Measurements for specific regions of interest were calculated using the integrated DXA software (Figure 4.5).

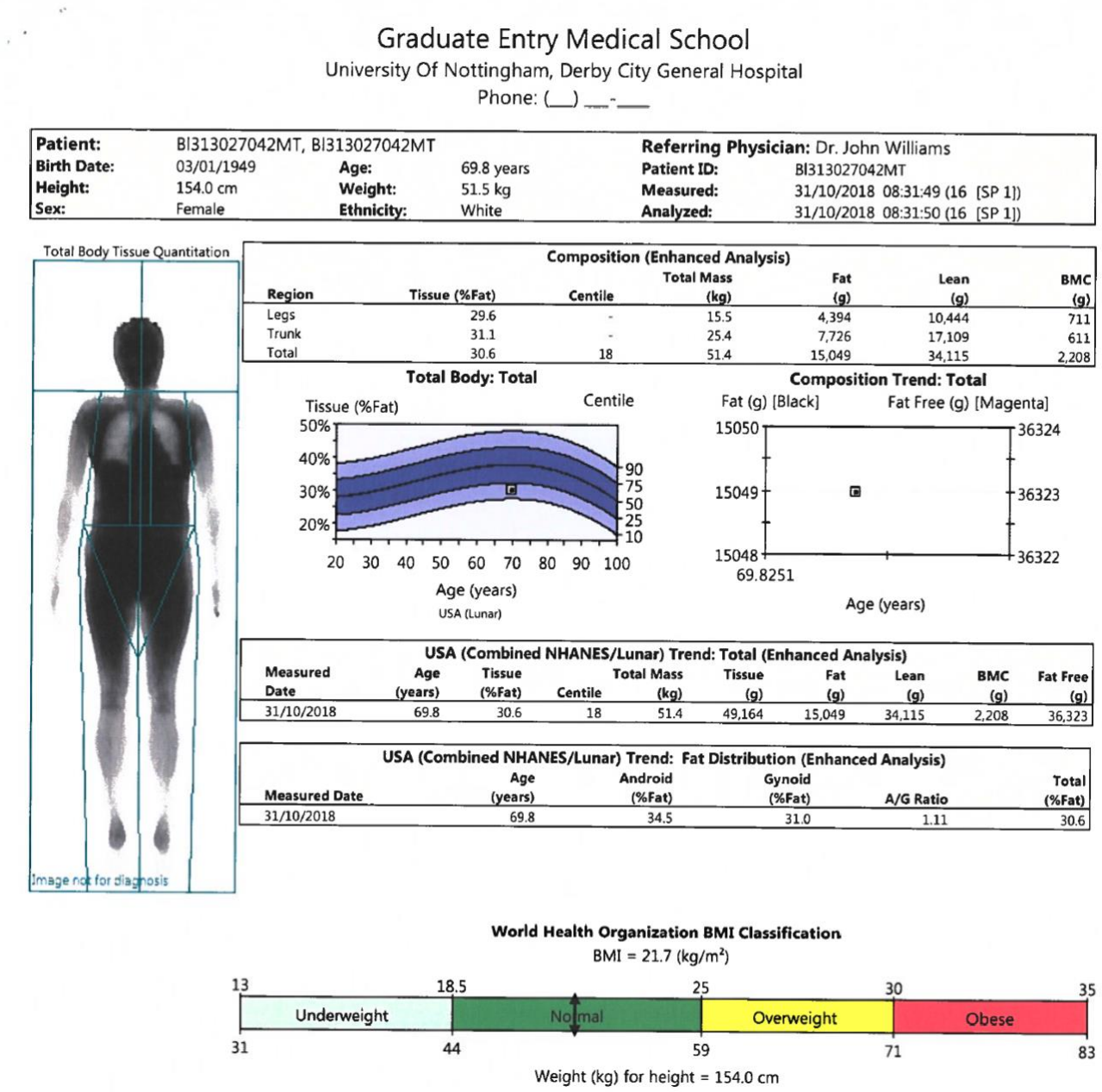


Figure 4.4: Measures of total mass including total fat, lean and leg tissue as calculated by the Dual-energy x-ray absorptiometry (DXA) scanner integrated software

Graduate Entry Medical School
University Of Nottingham, Derby City General Hospital
Phone: (__) __-____

Patient:	BI313027042MT, BI313027042MT			**Referring Physician:**	Dr. John Williams
Birth Date:	03/01/1949	**Age:**	69.8 years	**Patient ID:**	BI313027042MT
Height:	154.0 cm	**Weight:**	51.5 kg	**Measured:**	31/10/2018 08:31:49 (16 [SP 1])
Sex:	Female	**Ethnicity:**	White	**Analyzed:**	31/10/2018 08:31:50 (16 [SP 1])

BODY COMPOSITION: Total Body (Enhanced Analysis)

Region	Tissue (%Fat)	Region (%Fat)	Tissue (g)	Fat (g)	Lean (g)	BMC (g)	Total Mass (kg)
Arms	37.2	35.5	5,713	2,125	3,589	264	6.0
Arm Right	37.2	35.5	2,920	1,085	1,835	135	3.1
Arm Left	37.2	35.6	2,793	1,040	1,754	129	2.9
Arms Diff.	-0.1	-0.1	127	45	81	7	0.1
Legs	29.6	28.3	14,838	4,394	10,444	711	15.5
Leg Right	30.2	28.8	7,423	2,238	5,185	359	7.8
Leg Left	29.1	27.8	7,415	2,156	5,259	351	7.8
Legs Diff.	1.1	1.0	9	82	-74	8	0.0
Trunk	31.1	30.4	24,835	7,726	17,109	611	25.4
Trunk Right	31.5	30.7	12,227	3,853	8,375	302	12.5
Trunk Left	30.7	30.0	12,607	3,873	8,734	309	12.9
Trunk Diff.	0.8	0.8	-380	-20	-359	-7	-0.4
Android	34.5	34.1	3,484	1,201	2,282	36	3.5
Gynoid	31.0	30.2	7,443	2,308	5,135	192	7.6
Total	30.6	29.3	49,164	15,049	34,115	2,208	51.4
Total Right	30.9	29.5	24,629	7,614	17,015	1,137	25.8
Total Left	30.3	29.0	24,535	7,435	17,100	1,070	25.6
Total Diff.	0.6	0.5	94	178	-85	67	0.2

Figure 4.5: Measures of regions of interest as calculated by the Dual-energy x-ray absorptiometry (DXA) scanner integrated software

4.3.9 Standardised meal

Owing to the prolonged fast from the preceding night for the OGTT, one hour prior to CPET all participants were given a standardised meal of two slices of toast with margarine spread and a preserve of their choice (jam, marmalade etc). This was given along with a hot drink of their choice and as much water as required. Their choices were replicated on the repeat assessment day.

4.3.10 Muscle architecture

Changes in muscle architecture were assessed using ultrasound (MyLab™50; Esaote, Genoa, Italy). Participants were asked to lie semi-supine on a couch with the knees fully extended. The midpoint of the vastus lateralis muscle (VL) of the dominant leg was marked out using the midpoint of the greater trochanter of the femur and the mid-patella point (Figure 4.6). Calculation of fibre pennation angle (PA), muscle thickness (MT) and fascicle length (FL), as discussed in section 1.4.3, were made using Image J software version 1.44

(Rueden *et al.*, 2017). For all three parameters, three measurements were made per recorded US image, resulting in a total of 9 measurements per parameter per visit and the average value for each parameter was calculated for each visit (Franchi *et al.*, 2018).



Figure 4.6: Demonstration of measurement of the midpoint of the vastus lateralis muscle

4.3.11 Cardiopulmonary exercise testing (CPET)

To establish baseline CRF, the basis of the primary outcomes VO_2peak and AT, all participants performed a CPET pre and post intervention. CPET is considered the gold standard of investigation of exercise tolerance and CRF (Weisman *et al.*, 2003). All CPET assessments were conducted in the Clinical Physiology Exercise Suite at the University of Nottingham Royal Derby Hospital Centre. All tests were carried out in accordance with the ATS/ACCP (Weisman *et al.*, 2003) and POETTS (Levett *et al.*, 2018) guidelines. Participants were given a full safety briefing, including the criteria for termination (Table 1) (Weisman *et al.*, 2003) before each test. All CPETs were supervised by two investigators, at least one of whom had a valid Advanced Life Support Provider certificate and the other a minimum of a Basic Life Support Provider certificate (Resuscitation Council UK, London, UK).

4.3.11.1 *CPET equipment and calibration*

CPETs were performed on a cycle ergometer (Lode Corival, Lode BV, Groningen, Netherlands) (Figure 5). Breath-by-breath analysis was measured using an inline gas analysis system (ZAN 680, nSpire Health, Colorado, USA). Routine calibration of the gas analysis system was performed prior to every CPET as per manufacturer guidelines taking into account barometric pressure, ambient humidity and temperature. The flow sensor was calibrated for volume using a precision 3 litre syringe and was then fitted to a silicone oro-nasal mask (7450 Series V2™ mask, Hans Rudolph Inc., Kansas, USA).

The participant's weight was taken prior to each CPET to allow for calculation of gas exchange variables in ml/kg/min. The mask was tightened onto the participants face with Velcro straps to ensure an adequate seal was formed. To test the seal and to ensure prevention of gas leaks, the flow sensor was occluded against forced expiration. Before each test the participant was positioned on the cycle ergometer, the foot straps tightened, and the seat adjusted to their preference to ensure comfort. These alterations were replicated for each test.

4.3.11.2 *Participant monitoring during CPET*

Heart rate was continuously monitored using a 12-lead ECG. BP was measured at 2-minute intervals using an automated BP cuff (Orbit-K™, SunTech Medical®, North Carolina, USA) which was placed over the brachial artery around the participant's right arm. Finally, continuous pulse oximetry was monitored using a pulse oximeter attached to a freestanding monitor (iMEC 8, Mindray Medical, Shenzhen, China) placed on the index or middle finger of the participant's left hand (Figure 4.7).



Figure 4.7: Participant positioned on cycle ergometer with oro-nasal gas collection mask, 12 lead ECG, cyclic BP and oxygen saturations being monitored constantly. The gas analyser is seen beneath the printer.

4.3.11.3 *CPET protocol*

Participants were asked if they had any final questions then were asked not to speak when the test started unless there was an urgent concern (Table 4.1) so as not to distort respiratory gas analysis. An incremental ramp was estimated for each participant based on their weight and self-reported level of physical activity taken from the IPAQ questionnaire. The aim was to ensure incremental effort lasting between eight to twelve minutes and for VO_2peak to be achieved in this time for each CPET (Weisman *et al.*, 2003).

Indications for exercise termination
Chest pain suggestive of ischemia
Ischemic ECG changes
Complex ectopy
Second or third degree heart block
Fall in systolic pressure > 20 mm Hg from the highest value during the test
Hypertension (> 250 mm Hg systolic; > 120 mm Hg diastolic)
Severe desaturation: $Sp_{O2} \leqslant 80\%$ when accompanied by symptoms and signs of severe hypoxemia
Sudden pallor
Loss of coordination
Mental confusion
Dizziness or faintness
Signs of respiratory failure

Table 4.1: ATS/ACCP Criteria for CPET termination taken from Weisman et al. 2003

The test was then performed according to ATS/ACCP guidelines (Weisman *et al.*, 2003) as discussed in section 1.8.2. In brief, each test began with a 3-minute period of observed rest on the cycle ergometer to allow for initial hyperventilation to settle and to monitor for arrythmia not previously seen. This was followed by a 2-minute warm up of unloaded cycling at a sustained cadence of 55-60 rpm, to allow participant acclimatisation to the ergometer. The ramp protocol was then initiated and participants encouraged to volitional exhaustion by the two investigators, with the termination criteria as discussed in section 1.8.2.

On termination of the ramp protocol, participants continued to cycle against a reduced resistance of 15 watts for three minutes and then no resistance for two minutes. They were then supervised whilst dismounting the ergometer, asked to sit in a chair and the face mask removed. Ongoing continuous ECG and pulse oximetry was performed as well as

intermittent BP measurement until the participant felt they had recovered and the HR was below 100 bpm.

4.3.11.4 *Analysis of gas exchange*

Breath-by-breath data was analysed and then measures of VO_2Peak and AT calculated relative to weight in ml/kg/min. VO_2Peak was defined as the highest VO_2 attained during the test (Levett *et al.*, 2018) and was calculated by the gas analysis machine. AT was later defined using the modified V-slope method as described in section 1.8.6 (Levett *et al.*, 2018) by two experienced assessors (Kaczmarek *et al.*, 2019). Both were blinded to the participant ID, their group allocation and whether the CPET was pre or post intervention.

4.3.11.5 *Watt max analysis*

Wmax or peak exercise tolerance was defined as the highest recorded power out in Watts during the ramp phase (i.e. at termination of the ramp phase). As described, the same ramp protocol was used for CPET pre and post intervention so Wmax could be reliably compared.

4.3.12 Randomisation and experimental groups

After CPET, participants were randomised into one of three experimental groups using sealedenvelope.com (Sealed Envelope Ltd. 2019, simple randomisation service, London UK). This service was entirely independent of the trial investigators and uses random permuted blocks of 4 and 6, thereby ensuring allocation concealment.

The three experimental groups were as follows:

1) Lab HIT (LHIT): Fully supervised, laboratory-based with encouragement

2) Home HIT (HHIT): Entirely unsupervised, home-based with no influence

3) Control (CON): Non-intervention control group

4.3.13 HIT intervention

The full HIT protocol can be seen in Figure 4.8 and is based on previous work undertaken by our research group looking at unsupervised, home-based exercise regimens for improving CRF in middle-aged adults (Blackwell *et al.*, 2017). All sessions began with a warm-up period of two minutes of walking on the spot. Five sets of one minute, high-intensity efforts were performed, interspersed by 90 seconds of recovery again in the form of walking on the spot. Following the final effort, two minutes of a cool down period were completed, again by walking on the spot. This meant each session in total would last no longer than 17 minutes if completed correctly.

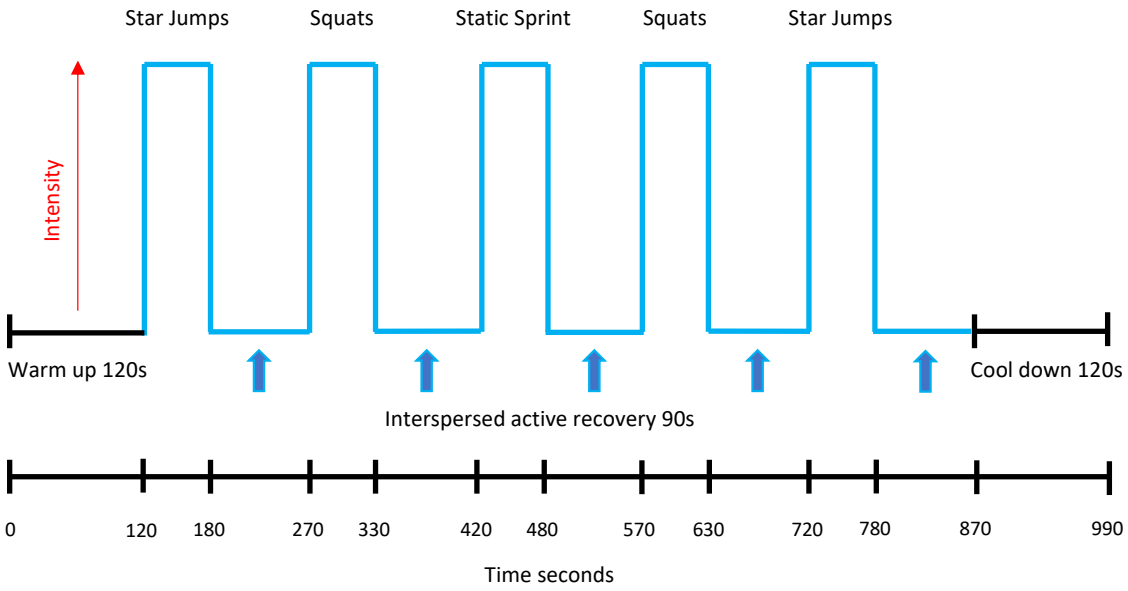


Figure 4.8: HIT protocol for exercise groups

After allowing a minimum of 48 hours rest post CPET, participants assigned to an exercise group were asked to attend the laboratory for an introductory session to the HIT protocol. This was to ensure they were comfortable with the exercise and rest periods, ensure proper form during exercises and importantly to show them how to record each progress each session. To ensure a heart rate sufficient enough to achieve HIT was elicited, participants were asked to exercise to above 85% (MacInnis and Gibala, 2017) of the calculated predicted maximum heart rate using the equation 220 - age (years) (Fox, Naughton and Haskell, 1971).

Pre and post-session blood pressure was measured. All participants were fully monitored with a 3-lead ECG to capture changes in heart rate during the exercises and cool down periods. This was to ensure that no arrhythmias were encountered during this first session which would have resulted in exclusion from further sessions due to safety. The same termination criteria were observed as for the CPET, with participants being told to stop any efforts immediately if any such events occurred.

To maintain exercise intensity, as a method of showing improvement and to some extent a means of motivation to complete sessions, the maximum number of repetitions achieved with correct form for a single effort, which successfully raised the participants' heart rates to the required level to achieve HIT, was recorded. This number was subsequently matched or bettered during each future session. Efforts one (star jumps) and two (squats) should then be matched by or improved on when repeated in efforts four (squats) and five (star jumps). This was required for all efforts except for the static sprint which would have been technically difficult to record. In rare circumstances where a participant struggled with a particular effort e.g. due to joint restriction, this was noted and one of the other efforts simply repeated in its place.

LHIT participants continued to receive one-to-one supervised training sessions with an investigator who held at least a Basic Life Support Provider certificate (Resuscitation Council UK, London, UK) and were given verbal encouragement throughout each session to evoke a sufficient HR response. HHIT participants were asked to maintain the appropriate intensity during sessions at home and given a full safety briefing on what to do if they developed any of the adverse symptoms in Table 1.

4.3.14 Retest day

All participants were asked to attend for a repeat acute study day after four weeks. All measures taken during the acute study day were repeated. The exercise groups were asked to observe a minimum 48-hour rest period after their final HIT sessions to ensure CPET performance was not restricted by fatigue, or that cardiometabolic measures were not affected by prior acute exercise. The same ramp was used for the CPET as for the pre-test.

4.3.15 HIT acceptability

Participants in the exercise groups were asked to complete a questionnaire about their experience of the HIT programme after the retest (Boereboom *et al.*, 2016). This used a 5 (strongly agree) to 1 (strongly disagree) scoring system. The questionnaire can be seen in section 4.4.1.0.

4.3.16 Statistical Analysis

4.3.16.1 *Power Calculation*

Based on AT and VO_2peak data from a previous study by our research group (Blackwell *et al.*, 2017), an *a priori* power calculation based on an effect size F of 0.6 for three groups and a within group standard deviation of 5 (6 ml/kg/min), determined that 30 participants (10 per group) would be required to achieve an α of 0.05 and a 1-β of 0.80. Whilst this value of 6 ml/kg/min is slightly high, including baseline values as a covariate improves accuracy and therefore power (see section 4.3.16.3), making this assumption much more reliable.

4.3.16.2 *Within group analysis*

As this was a RCT, significance testing of baseline differences was not performed as per the CONSORT 2010 statement (de Boer *et al.*, 2015). Effects are reported as number (%), mean (SD) or +/- mean difference (MD) (SD). Within-group analysis for pre and post intervention change was performed using repeated measures t-tests. The level of significance was set at $p<0.05$. All analyses were performed using GraphPad Prism for Mac OS X Version 7.0d (GraphPad Software, La Jolla, San Diego, California USA).

4.3.16.3 *Between group analysis*

To analyse pre and post intervention change between groups, ANCOVA with baseline values as the continuous covariates were performed. Effects are reported as number (%) or mean differences (MD) with 95% confidence intervals (CI) and p values. The assumptions of normality in groups (Shapiro-Wilk test), normality of residuals (histograms) and equality of variance were tested with robust tests for equality of variances, linearity, homoscedasticity (Breusch-Pagan/Cook-Weisberg test) and homogeneity of regression slopes. Differences

between groups and 95% CI were adjusted for multiple comparisons using Sidak's *posthoc* test. All analyses were conducted using Stata Version 15 (StataCorp LLC, TX, USA).

4.4 Results

4.4.1 Participant demographics

Thirty participants of mean age 71(5) years, of which 14(47%) were male, were recruited to this study. Demographics can be seen in Table 4.2.

	L-HIT (n=10)	H-HIT (n=10)	CON (n=10)
Age (years)	70 (5)	71 (4)	71 (7)
Male / Female	5/5	3/7	6/4
BMI	26 (3)	25 (3)	26 (1)
AT	13.40 (4)	14.90 (6)	14.27 (3)
VO_2	26.37 (8)	25.47 (8)	28.05 (4)
SBP	127 (14)	126 (10)	128 (11)

Table 4.2: Participant demographics

4.4.2 Study adherence

The study Consort diagram can be seen in Figure 4.9. Two participants declined to progress to the acute study day for personal reasons. One participant developed asymptomatic ECG changes during the CPET and was excluded before randomisation, having been referred for further clinical investigations. All participants who entered the intervention phase remained in the study with none lost to follow up.

All randomised participants completed a pre and post intervention CPET for the primary outcomes of VO_2peak and AT. Two participants could not have a post intervention DXA scan owing to a CT scan being performed for both during the 4-week intervention period, one randomised to the home group, the other randomised to the control group. Training compliance was reported as 100% for both exercise groups, with all participants completing at least 12 sessions in four weeks (Figure 4.9).

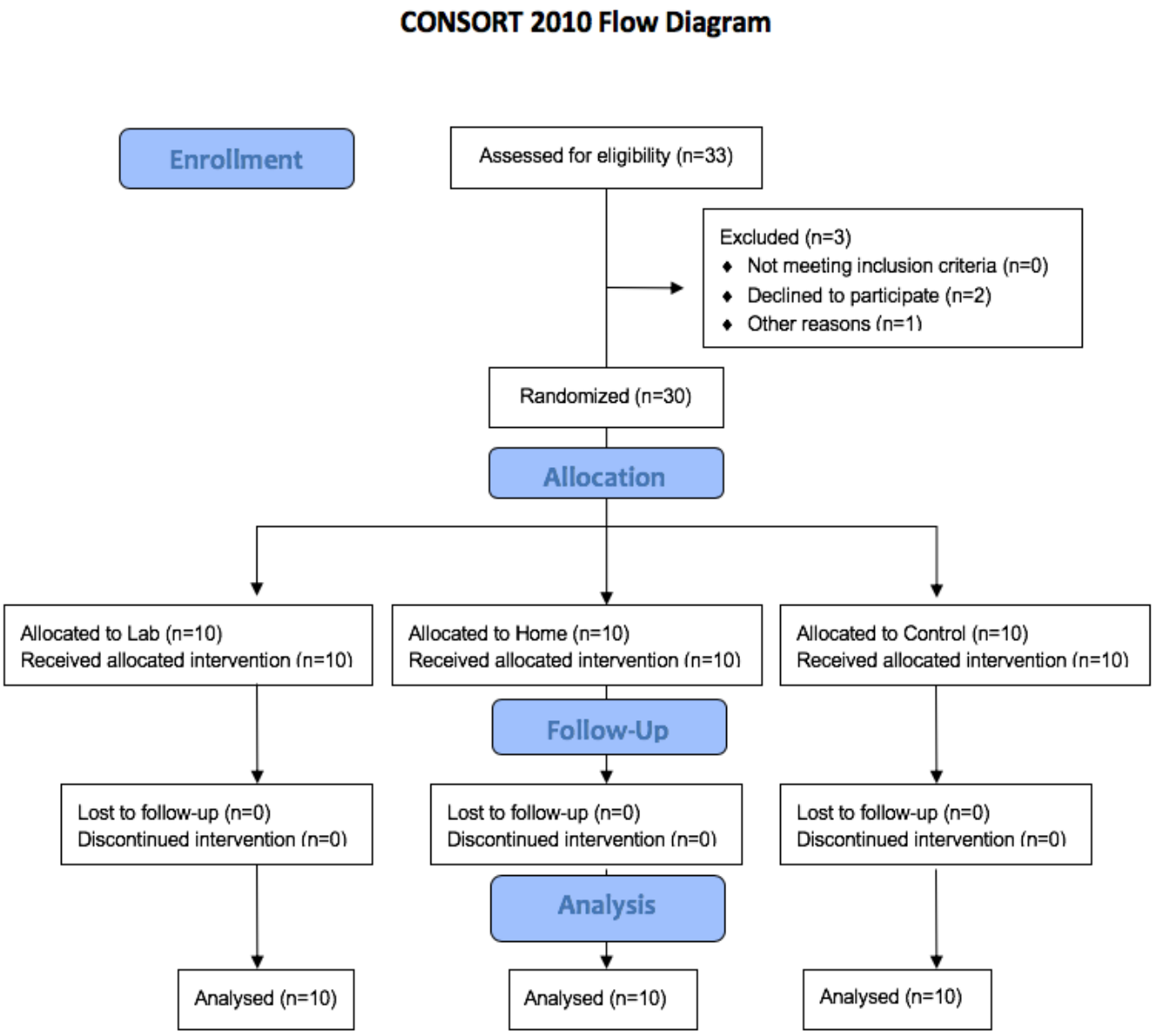


Figure 4.9: Study Consort diagram

4.4.3 Within group differences

Within group changes following a 4-week period of LHIT, HHIT or a CON intervention period are shown in Table 4.3.

	LHIT		HHIT		CON	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
AT (ml/kg/min)	13.40 (4)	15.24 (5)**	14.90 (6)	16.94 (7)**	14.27 (3)	13.94 (3)
VO_2 (ml/kg/min)	26.37 (8)	28.98 (8)**	25.47 (8)	28.42 (9)**	28.05 (4)	27.7 (4)
SBP (mmHg)	127 (14)	121 (10)	126 (10)	122 (12)	128 (11)	129 (10)
DBP (mmHg)	73 (7)	69 (7)*	68 (12)	64 (9)*	77 (7)	73 (12)
BMI (kg/m^2)	25.95 (3)	25.77 (3)*	24.54 (3)	24.37 (3)*	25.57 (1)	25.48 (1)
Total Fat (g)	23808.0 (8774)	22905.5 (8751)**	21762.0 (6182)	21064.8 (6015)**	21596.7 (3981)	21421.6 (3845)
% Fat	32.85 (10)	31.82 (10)*	33.63 (8)	32.54 (8)*	32.53 (6)	31.98 (7)
Lean Muscle (g)	47353.9 (9652)	48036.0 (9972)**	38664.1 (6407)	39496.6 (5972)*	45615.4 (7163)	46070.0 (7268)
Lean Leg (g)	15623.4 (3642)	15856.0 (4020)	13698.9 (3227)	13862.0 (3205)	14217.3 (2226)	14172.4 (2466)
Cholesterol	5.1 (1.5)	4.5 (1.2)**	5.7 (0.9)	5.2 (0.8)*	5.1 (0.7)	5.1 (0.8)
HDL	1.6 (0.5)	1.6 (0.4)	1.9 (0.4)	1.9 (0.4)	1.5 (0.2)	1.5 (0.2)
Non-HDL	3.5 (1.7)	2.9 (1.4)**	3.8 (0.9)	3.3 (0.7)**	3.7 (0.7)	3.6 (0.7)
PA (degrees)	11.90 (2)	14.03 (1)**	11.69 (2)	13.76 (2)**	11.04 (1)	11.26 (1)
MT (mm)	1.94 (0.4)	2 (0.3)	1.85 (0.5)	1.84 (0.3)	1.89 (0.3)	1.96 (0.3)
FL (mm)	7.21 (2)	7.40 (2)	6.66 (2)	6.89 (2)	6.97 (2)	6.90 (2)
Glucose AUC	894.84 (103)	895.03 (125)	920.69 (129)	915.01 (118)	956.18 (234)	950.12 (213)
Insulin AUC	5284.8 (4176)	5431.8 (4644)	6645.5 (2374)	8409.6 (5410)	5411.1 (2263)	6576.7 (3341)
HOMA-IR	0.98 (0.5)	0.97 (0.4)	2.2 (1.9)	1.3 (1.0)	1.7 (1.5)	2.5 (2.4)

Table 4.3: Assessment parameters pre and post intervention.
*Data are presented as mean (SD). Analysis via repeated measures t-tests. *=p<0.05, **=p<0.01 versus pre-intervention in the same group*

4.4.4 Cardiorespiratory fitness

4.4.4.1 *Anaerobic threshold*

There was a significant increase in AT within the LHIT (MD +1.84 (1.6) ml/kg/min; *p*=0.006) and HHIT (MD +2.04 (1.7) ml/kg/min; *p*=0.004) groups after the intervention period, with no significant change in the control group (*p*=0.44) (Table 4.3, Figure 4.10).

When compared to the control group, there was a significant increase in AT in the LHIT (MD +2.27 (0.57 to 3.98) ml/kg/min; *p*=0.007) and HIT (MD +2.29 (0.59 to 4) ml/kg/min; *p*=0.006) groups after the intervention period. There was no significant difference between the LHIT and HIT groups (MD -0.02; 95% CI -1.74 to 1.7; *p*=1) (Figure 4.10).

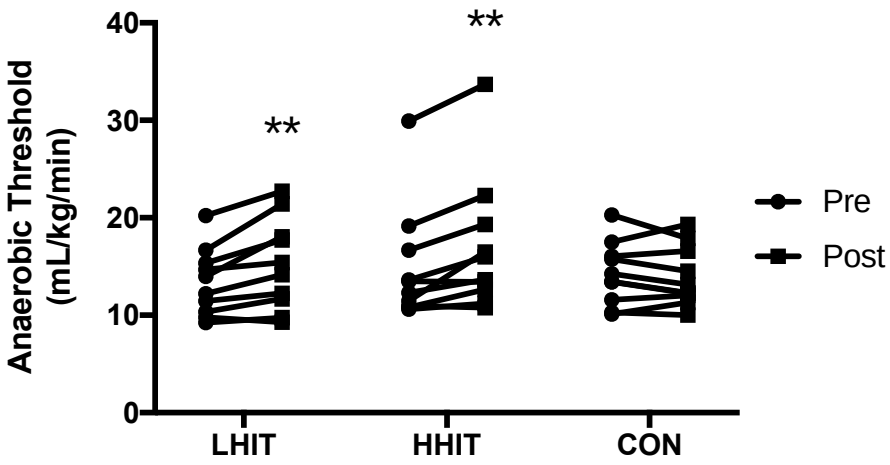


*Figure 4.10: AT (ml/kg/min) pre and post intervention. ** = p<0.01 versus pre-intervention when compared to control group*

4.4.4.2 *VO_2peak*

There was a significant increase in VO_2peak within the LHIT (MD +2.60 (2.0) ml/kg/min; *p*=0.003) and HHIT (MD +2.95 (2.2) ml/kg/min; *p*=0.002) groups after the intervention period with no significant change in the control group (*p*=0.48) (Table 4.3, Figure 4.11).

When compared to the control group, there was a significant improvement in VO_2peak in the LHIT (MD +3.05 (0.81 to 5.29) ml/kg/min; *p*=0.005) and HHIT (MD +3.45 (1.19 to 5.7)

ml/kg/min; *p*=0.002) groups after the intervention period. There was no significant difference between the LHIT and HHIT (MD -0.4 (-2.63 to 1.84) ml/kg/min; *p*=0.96) (Figure 4.11).

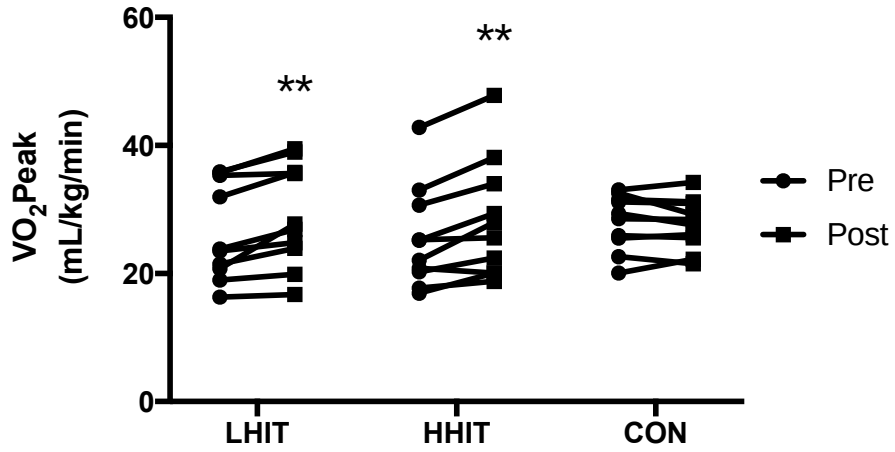


*Figure 4.11: VO_2peak (ml/kg/min) pre and post intervention. ** = p<0.01 versus pre-intervention when compared to control group*

4.4.4.3 *Maximum wattage*

There was a significant increase in WMax within the LHIT (MD +8.4 (6.1) W; *p*=0.002) and HHIT (MD +8.2 (5.5) W; *p*=0.001) groups after the intervention period with no significant change in the control group (*p*=0.46) (Table 4.3, Figure 4.12).

When compared to the control group, there was a significant increase in Wmax in the LHIT (MD +10.64 (2.9 to 18.38) W; *p*=0.005) and HHIT (MD +10.57 (2.98 to 18.16) W; *p*=0.005) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD +0.07 (-7.68 to 7.82) W; *p*=1) (Figure 4.12).

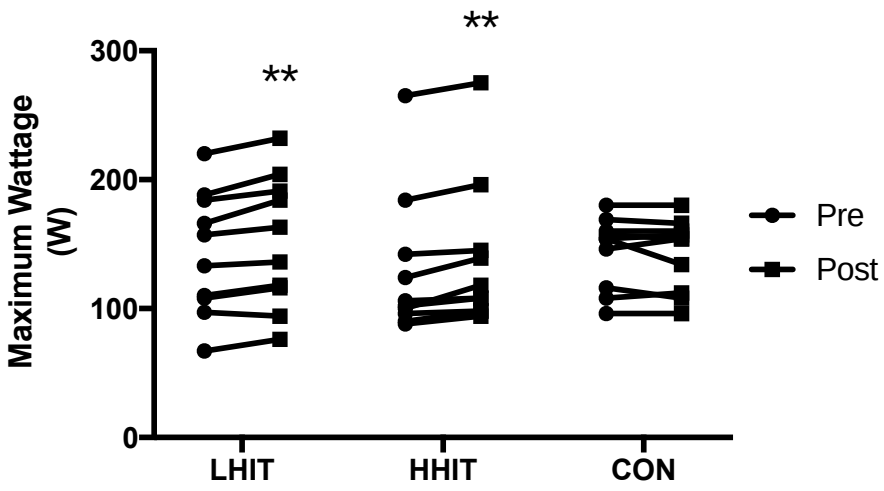


*Figure 4.12: WMax (W) pre and post intervention. ** = p<0.01 versus pre-intervention when compared to control group*

4.4.5 Resting blood pressure

4.4.5.1 *Systolic blood pressure*

There was a significant decrease in resting systolic BP within the LHIT (MD -6 (6) mmHg; *p*=0.002) and HHIT (MD -4 (4) mmHg; *p*=0.007) groups after the intervention period with no significant change in the control group (*p*=0.7) (Table 4.3, Figure 4.13).

When compared to the control group, there was a significant improvement in resting systolic BP in the LHIT (MD -6.25 (-11.6 to -0.91) mmHg; *p*=0.02) and HHIT (MD -5.37 (-10.72 to -0.02) mmHg; *p*=0.05) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD -0.88 (-6.21 to 4.45) mmHg; *p*=0.97) (Figure 4.13).

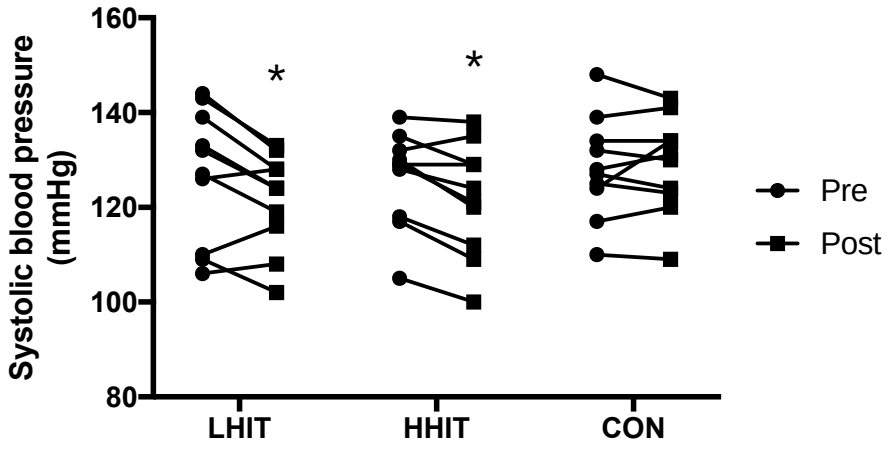


*Figure 4.13: Resting systolic BP (mmHg) pre and post intervention. * = p<0.05 versus pre-intervention when compared to control group*

4.4.5.2 *Diastolic blood pressure*

There was a significant decrease in resting diastolic BP within the LHIT (MD -4 (10) mmHg; *p*=0.02) and HHIT (MD -4 (15) mmHg; *p*=0.04) groups after the intervention period with no significant change in the control group (*p*=0.2) (Table 4.3).

When compared to the control group however, there were no significant differences in resting diastolic BP in the LHIT (MD +5.74 (-6.52 to 18) mmHg; *p*=0.57) or HHIT (MD +1.69 (-9.83 to 13.21) mmHg; *p*=0.98) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -4.05 (-15.76 to 7.66) mmHg; *p*=0.77).

4.4.6 Glucose concentration

There was no significant change in blood glucose AUC in response to a 75g oral glucose load within any of the groups after the intervention period (Table 4.3).

When compared to the control group, there were no significant differences in glucose AUC in the LHIT (MD -4.29 (-97.55 to 88.97) mmol/120 mins; *p*=1) or HHIT (MD -5.72 (-98.2 to 86.77) mmol/120 mins; *p*=1) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD 1.43 (-90.87 to 93.73) mmol/120 mins; *p*=1).

4.4.7 Insulin concentration

There were no significant changes in insulin AUC in response to a 75g oral glucose load within any of the groups after the intervention period (Table 4.3).

When compared to the control group, there were no significant differences in glucose AUC in the LHIT (MD -1003.95 (-4498.08 to 2490.19) uU/ml/120 mins; *p*=0.85) or HHIT (MD +455.6 (-3090.34 to 4001.53) uU/ml/120 mins; *p*=0.98) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -1459.54 (-5016.64 to 2097.55) uU/ml/120 mins; *p*=0.66).

4.4.7.1 *Insulin resistance*

There were no significant changes in HOMA-IR within any of the groups after the intervention period (Table 4.3).

When compared to the control group, there were no significant differences in HOMA-IR in the LHIT (MD -0.48 (-1.11 to 0.15); *p*=0.13) or HHIT (MD -0.47 (-1.08 to 0.14; *p*=0.12) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -0.01(-0.64 to 0.61); *p*=0.97).

4.4.8 Cholesterol profile

4.4.8.1 *Total cholesterol*

There was a significant decrease in total cholesterol within the LHIT (-0.6 (0.5) mmol/L; *p*=0.007) and HHIT (-0.5 (0.6) mmol/L; *p*=0.02) groups after the intervention period, with no significant change in the control group (*p*=0.2) (Table 4.3, Figure 4.14).

When compared to the control group, there was a significant decrease in total cholesterol in the LHIT (MD -0.51 (-1.01 to -0.01) mmol/L; *p*=0.04) and HHIT (MD -0.46 (-0.87 to -0.05) mmol/L; *p*=0.05) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD -0.15 (-0.66 to 0.37) mmol/L; *p*=0.85) (Figure 4.14).

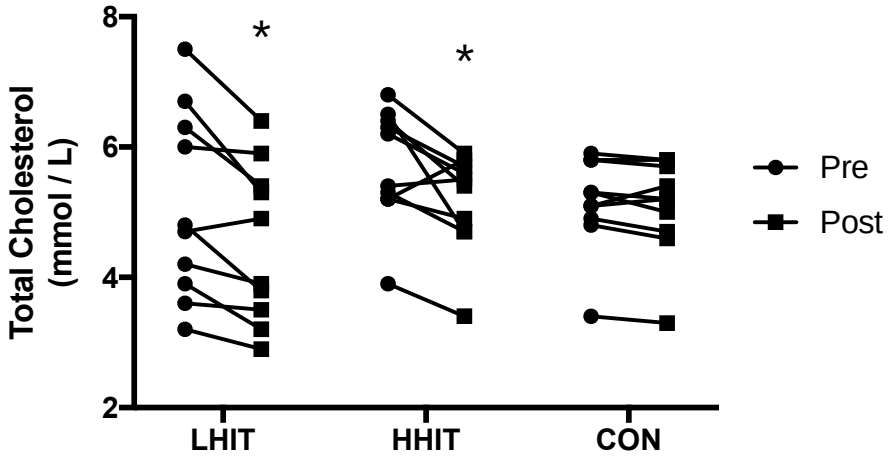


*Figure 4.14: Total cholesterol (mmol/L) pre and post intervention. * = p<0.05 versus pre-intervention when compared to control group*

4.4.8.2 *HDL cholesterol*

There was no significant change in HDL cholesterol within any of the groups after the intervention period (Table 4.3).

When compared to the control group, there were no significant differences in HDL cholesterol in the LHIT (MD -0.48 (-1.11 to 0.15) mmol/L; *p*=0.13) or HHIT (MD -0.47 (-1.08 to 0.14) mmol/L; *p*=0.12) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -0.01(-0.64 to 0.61) mmol/L; *p*=0.97).

4.4.8.3 *Non-HDL cholesterol*

There was a significant decrease in non-HDLc within the LHIT (-0.6 (0.5) mmol/L; *p*<0.01) and HHIT (-0.5 (0.5) mmol/L; *p*<0.01) groups after the intervention period, with no significant change in the control group (*p*=0.08) (Table 4.4, Figure 4.15).

This equated to a percentage change decrease in non-HDLc of -17.7% in the LHIT group and -16.1% in the HHIT group (Table 4.4).

	LHIT	HHIT	CON
Pre	3.5 (1.7)	3.8 (0.9)	3.7 (0.7)
Post	2.9 (1.4)	3.3 (0.7)	3.6 (0.7)
Change	-0.6 (0.5)	-0.5 (0.5)	-0.1 (0.2)
p	0.006	0.005	0.08
% change	-17.7	-16.1	-3.5

Table 4.4: Non-HDL cholesterol (mmol/L) pre and post intervention

When compared to the control group, there was a significant decrease in non-HDL cholesterol in the LHIT (MD -0.46 (-0.81 to -0.11) mmol/L; *p*=0.008) and HHIT (MD -0.38 (-0.73 to -0.03) mmol/L; *p*=0.03) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD -0.08 (-0.43 to 0.28) mmol/L; *p*=0.93) (Figure 4.15).

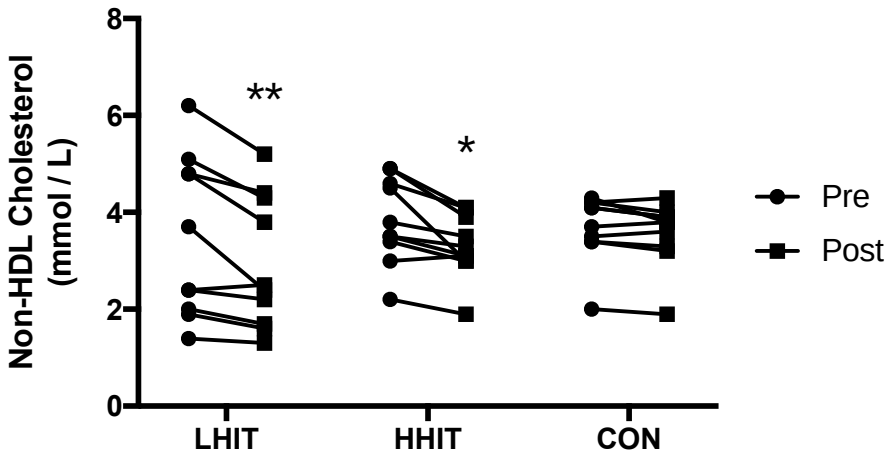


*Figure 4.15: Non-HDL cholesterol (mmol/L) pre and post intervention, ** = p<0.01, * = p<0.05 versus pre-intervention when compared to control group*

4.4.9 Body composition

4.4.9.1 *BMI*

There was a significant decrease in BMI within the LHIT (MD -0.18 (0.2) kg/m^2; *p*=0.04) and HHIT (MD -0.17 (0.2) kg/m^2; *p*=0.04) groups after the intervention period, with no significant change in the control group (*p*=0.4) (Table 4.3).

4.4.9.2 *Total fat mass*

There was a significant decrease in total fat mass within the LHIT (MD -902.5 (857.1) g; *p*=0.009) and HHIT (MD -697.2 (318.5) g; *p*=0.002) groups after the intervention period, with no significant change in the control group (*p*=0.49) (Table 4.3).

When compared to the control group, there was a significant decrease in total fat in the LHIT versus control group (MD -796.25 (-1581.86 to -10.64) g; *p*=0.05) after the intervention period. However, there was no significant difference in the HHIT versus control (MD -630.1 (-1428 to 167.83) g; *p*=0.15) or LHIT versus HHIT (MD -166.19 (-950.7 to 618.28) g; *p*=0.93) groups post after the intervention period.

4.4.9.3 *Percentage body fat*

There was a significant decrease in percentage body fat in the LHIT (MD -1.03 (1.1) %; *p*=0.02) and HHIT (MD -1.09 (0.8) %; *p*=0.03) groups after the intervention period, with no significant change in the control group (*p*=0.13) (Table 4.3).

When compared to the control group however, there were no significant changes in body fat percentage in the LHIT (MD -0.47 (-1.65 to 0.71) %; *p*=0.68) or HHIT (MD -0.52 (-1.73 to 0.69) %; *p*=0.63) groups after the intervention periods. There was also no significant difference between the LHIT and HHIT groups (MD 0.05 (-1.13 to 1.23) %; *p*=1).

4.4.9.4 *Lean muscle mass*

There was a significant increase in LMM in the LHIT (MD +683.1 (543.6) g; *p*=0.009) and HHIT (MD +832.4 (784.6) g; *p*=0.03) groups after the intervention period, with no significant change in the control group (*p*=0.12) (Table 4.3).

When compare to the control group however, there was no difference in the LHIT (MD +155.66 (-732.55 to 1043.88) g; *p*=0.96) or HHIT (MD +176.6 (-729.5 to 1082.7) g; *p*=0.95) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -20.94 (-922.54 to 880.67) g; *p*=1).

4.4.9.5 *Lean leg mass*

There were no significant changes in lean leg mass in any of the groups post intervention (Table 4.3).

When compared to the control group, there were no significant differences in lean leg mass in the LHIT (MD -253.43 (-1444.21 to 937.34) g; *p*=0.93) or HHIT (MD -204.56 (-1406.32 to 997.2) g; *p*=0.96) mmol/120 mins; *p*=1) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD -48.87 (-1258.84 to 1161.1) g; *p*=1).

4.4.10 Muscle architecture

4.4.10.1 *Pennation angle*

There was a significant increase in pennation angle in the LHIT (MD +2.13 (1.4)º; *p*=0.001) and HHIT (MD +2.07 (1.4)º; *p*=0.0005) groups after the intervention period, with no significant change in the control group (*p*=0.31) (Table 4.3, Figure 4.16).

When compared to the control group, there was a significant increase in pennation angle in the LHIT (MD +1.95 (0.72 to 3.17) º; p=0.001) and HHIT (MD +1.8 (0.59 to 3.01) º; p=0.002) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD +0.14 (-1.05 to 1.34) º; p=0.99) (Figure 4.16).

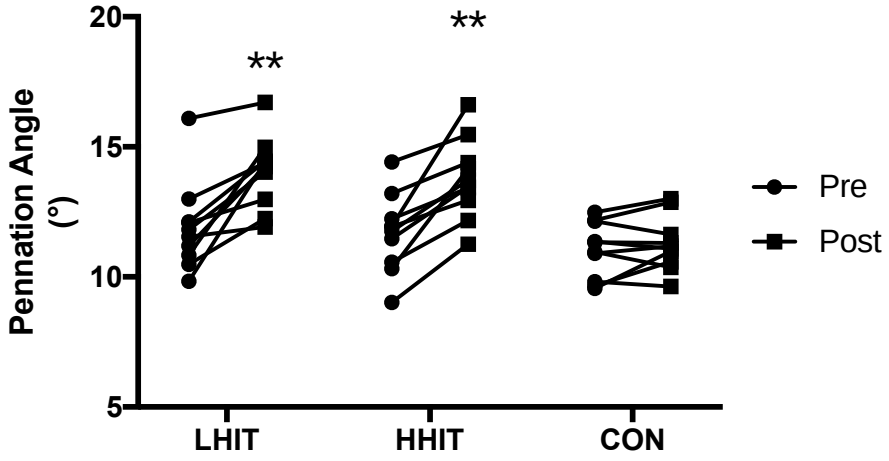


*Figure 4.16: Muscle pennation angle pre and post intervention. ** = p<0.01 versus pre-intervention when compared to control group*

4.4.10.2 *Muscle thickness*

There were no significant changes in muscle thickness within any of the groups after the intervention period (Table 4.3).

When compared to the control group, there were no significant differences in muscle thickness in the LHIT (MD +0.01 (-0.13 to 0.15) mm; *p*=1) or HHIT (MD -0.08 (-0.23 to 0.06) mm; *p*=0.41) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD +0.09 (-0.05 to 0.24) mm; *p*=0.32).

4.4.10.3 *Fascicle length*

There were no significant changes in fascicle length within any of the groups post intervention (Table 4.3).

When compared to the control group, there were no significant differences in fascicle length in the LHIT (MD +0.37 (-0.38 to 1.12) mm; *p*=0.53) or HHIT (MD +0.25 (-0.5 to 1) mm; *p*=0.79) groups after the intervention period. There was also no significant difference between the LHIT and HHIT groups (MD +0.12 (-0.64 to 0.89) mm; *p*=0.97).

4.4.11 HIT acceptability

All participants in the exercise groups completed a questionnaire at the end of the retest. The programme was well received by the participants, with 100% of exercising participants agreeing that they enjoyed it and felt physically fitter having performed it (Figure 4.27). 19/20 (95%) stated that they would continue with the programme, with one participant wanting to adapt the programme for use on an exercise bike at home. Only one participant would have preferred to have been in a group. Two participants in the laboratory group stated that they would have preferred to have exercised at home. All participants would recommend the programme to a friend.

Free-text responses to the question “what was the most challenging part” included:

- “the first few sessions”
- “pushing myself to complete the last effort”
- “trying to stay in a rhythm for the star jumps”
- and most commonly; “the CPET”

Free-text responses to the question “what was the best part” included:

- “getting fitter”
- “feeling better in myself”
- “I got to do it at home”
- “meeting and working with the team”

Question	**Median Score (Range)** **5 - Strongly agree** **1 - Strongly disagree**
The HIT study was adequately explained	5 (5)
HIT has been an enjoyable experience	5 (4-5)
HIT has been a significant time burden	1 (1-2)
I would recommend HIT to friends	5 (4-5)
HIT has been physically more demanding than I expected	4 (3-5)
I would perform the same HIT regimen again	5 (4-5)
This study has interfered with other aspects of my life due to a) the time commitment	

b) the travelling involved c) the physical strain	1(1-2)
I believe HIT has improved my fitness	5 (4-5)
I am pleased to have done something to improve my fitness	5 (5)
I would have preferred to exercise in a group setting	1 (1-4)
(If in the lab group) I would have preferred to exercise at home	1 (1-4)

Figure 4.17: Results of HIT acceptability questionnaire for the exercise groups

4.5 Discussion

This study has shown that just four weeks of a time efficient, equipment free, bodyweight-based HIT programme is able to elicit improvements in the cardiorespiratory fitness, body composition and cardiometabolic health of older adults. We report significant improvements in CRF, DXA-derived measures of body composition, muscle architecture, cholesterol profile and resting systolic blood pressure.

Whilst the positive effects of HIT on CRF in older adults is well established, at the time of writing this thesis and to the best of our knowledge, this is the first study to show that equipment-free HIT can result in these improvements in this age group within this short time frame. Furthermore, to the best of our knowledge, this is also the first study to show that these improvements are achievable in this age group independent of the supervision received. The programme was safe, easy to learn, well received by all exercising participants and maintained 100% training compliance. Furthermore, it was not associated with any serious medical complications in both supervised and unsupervised participants.

As previously discussed, “lack of time” is a commonly cited barrier to starting and maintaining regular physical activity in older adults (Trost *et al.*, 2002; Costello *et al.*, 2011). The improvements we report were achieved with a total time commitment of less than 50 minutes per week; less than one third of the time required to meet current guidelines for moderate continuous exercise (World Health Organisation (WHO), 2018c). This reduced time commitment would likely result in greater exercise adherence (Korkiakangas, Alahuhta and Laitinen, 2009).

In addition to “lack of time”, level of supervision during exercise has also been reported to impact adherence, although the notion of supervision being favourable is not unequivocal. For example, Hunter *et al.* found that direct exercise supervision afforded no greater benefit for improving CRF via a combined aerobic and resistance exercise programme in middle-aged, physically inactive adults (Hunter *et al.*, 2018). Further supporting this, they also found at 15-month follow-up that exercise supervision had no influence on exercise adherence and physical activity behaviour (Hunter *et al.*, 2018). In agreement with these findings we also saw no significant difference between the supervised and home-based groups for our primary outcomes of CRF (AT nor VO_2peak), suggesting that the level of supervision is not important for our particular HIT regimen. In addition, our HIT regimen does not require any exercise facilities or equipment other than a small amount of floor space and therefore can realistically be performed anywhere, with one participant even completing a week of the

protocol on a balcony whilst on holiday. Collectively our HIT protocol can remove three potential barriers to exercise in older adults, namely i) lack of time, ii) access to facilities/ cost of equipment and iii) embarrassment of exercising in public (Korkiakangas, Alahuhta and Laitinen, 2009; Costello *et al.*, 2011; Morgan *et al.*, 2016; Ashton *et al.*, 2017). Our results also alleviate previous concerns that the intensity required to achieve 'true HIT' requires expensive equipment, such as cycle ergometers and cannot be achieved outside of a laboratory environment (Hardcastle *et al.*, 2014). In addition, our protocol only included three exercises which were easily adaptable for those with, for example, joint restrictions, which commonly occur in older age, again potentially improving adherence. In reality, any choice of exercise which can raise the HR to above 85% of maximum predicted could be utilised, again improving the adherence.

Scott *et al.* successfully completed a non-randomised, 12 week, virtually supervised bodyweight-based study whereby the participants exercised at home and received feedback remotely via a heart rate monitor and mobile phone application (Scott *et al.*, 2019). They suggested that future studies should investigate more novel monitoring and direct feedback systems to increase adherence (Scott *et al.*, 2019). That our study had 100% reported adherence, with no direct monitoring, answers a question previously proposed as to whether adherence to a Home-HIT programme is maintained when participants were not aware that they were being constantly monitored (Ferreira, 2020). It is more than likely however that on completing the four-week retest, participants would be more likely to continue with the programme knowing that their CRF had improved. More studies showing the benefits of home-HIT entering the public domain are needed and can only serve to improve knowledge of the modality and its effect on health, again overcoming another barrier in lack of knowledge of exercise modalities in the elderly (Cohen-Mansfield, Marx and Guralnik, 2003; Schutzer and Graves, 2004).

Significant improvements in AT, VO_2peak and maximum wattage in our participants adds to previous studies reporting that HIT is safe and effective for improving measures of CRF in older adults in a reduced timeframe, again highlighting the potency of HIT for improving CRF (Knowles *et al.*, 2015; Boereboom *et al.*, 2016; Hwang *et al.*, 2016; Bruseghini *et al.*, 2020). Our results for CRF using a bodyweight-based protocol are similar to previous work by our research group using a 5-by-1 minute, four week protocol on a ramped cycle ergometer in older adults who were healthy (Boereboom *et al.*, 2016) and those awaiting surgery for urological cancer (Blackwell *et al.*, 2020), validating its effectiveness in improving CRF in this age group. As discussed in section 1.8.6, AT cannot be volitionally influenced and as such provides a reliable validation of changes in CRF (Agnew, 2010). Despite this, few other studies have included the AT as a marker of CRF when describing HIT studies in the elderly.

Improvements in AT observed in our participants were above the previously stated clinically significant increase of 1.5mL/kg/min following participation in a cycle-ergometer based, supervised HIT programme for subjects undergoing liver resection for metastatic colorectal cancer (Dunne *et al.*, 2016), further validating our equipment-free method of HIT for improving CRF in older adults.

The ~10% increase in VO_2peak reported for both exercise groups in this study is consistent with a an eight week, all-extremity (upper and lower limb) HIT programme in older adults using a non-weight-bearing ergometer (Hwang *et al.*, 2016), albeit in half the time-frame and using no equipment. Our results are also comparable with an eight week cycle ergometer based HIT programme in older adults, albeit with a more time efficient 5-by-1 minute as opposed to a 7-by-2 minute protocol (Bruseghini *et al.*, 2020). Our results are also consistent with two recent systematic review and meta-analyses of HIT in all forms and adult age groups using a 4-week or less time-frame (Wen *et al.*, 2019) and the effects of short-term (<12 weeks) HIT on cardiometabolic health (Batacan *et al.*, 2017). Exercise intensity and CRF is commonly quantified as metabolic equivalent tasks (METs), defined as the resting metabolic rate or the amount of oxygen consumed at rest, traditionally equivalent to a VO_2 of 3.5mL/kg/min (Jetté, Sidney and Blümchen, 1990). A study of 14,345 adult men over 11.4 years follow up reported that every one maximal MET improvement in VO_2peak is associated with a 15% and 19% lower risk of all-cause and CVD mortality respectively (D. Lee *et al.*, 2011). The significant increases in VO_2peak we report from this study for both exercise groups in just four weeks of exercise are very encouraging and close to this level of clinical significance.

In addition to the improvements in CRF outlined above, we have also seen significant improvements in aspects of body composition and muscle architecture. At the whole-body level we observed a modest reduction in BMI, a major contributor to development of the Metabolic Syndrome (Kahn *et al.*, 2005; Kassi *et al.*, 2011). Taking into account that our cohort was not obese to begin with, these small yet significant reductions suggest that obese individuals may be able to use this exercise programme to try and modify their body weight. In addition, lower levels of LMM are directly correlated to low VO_2peak in older adults (Boo *et al.*, 2019), hence improving LMM levels not only opposes the numerous detrimental effects of sarcopaenia, including improving balance and gait (Snijders *et al.*, 2019) but also contributes to improved CRF. In addition we have, for the first time, shown that body-weight HIT can modestly improve LMM and lower fat mass in the older adult in just four weeks. Whilst we observed an increase in LMM within our exercise groups following the intervention period, when compared to the control group, these changes were non-significant. Reasons for this include the possibility that those in exercise groups maintained stricter dietary control

(which was not an outcome for this study) or that the numbers within each group were not large enough to show a difference across the groups.

Older adults are at risk for developing insulin resistance due to reduced physical activity levels and gains in adiposity (Robinson *et al.*, 2017). We report a within group reduction in total body fat and percentage body fat, possibly improving insulin sensitivity and reducing the risk of developing MetS and T2DM (Kahn *et al.*, 2005; Kassi *et al.*, 2011). Coswig *et al.* also demonstrated significant decreases in total fat mass and fat percentage using an eight week treadmill based HIT programme in older females, albeit using a much longer protocol of 4-by-4 minute intervals (Silveira-Coswig *et al.*, 2020). An eight week all-extremity HIT intervention did not produce any changes in body composition (Hwang *et al.*, 2016). That significant improvements in body composition were not seen in two four week studies using the 5-by-1 minute protocol utilising a cycle ergometer (Boereboom *et al.*, 2016; Blackwell *et al.*, 2020) suggests that a body-weight protocol is superior for improvements in body composition in this short timeframe. Our relatively modest decrease of 1% fat is not however consistent with a systematic review of young, overweight or obese males where an approximately 6% reduction was seen in on average ten weeks of HIT (Wewege *et al.*, 2017). The mean BMI for our study was 25.7, only just entering our participants into the overweight category compared to 32.7 in the HIT group for the systematic review (Wewege *et al.*, 2017) and it may be for this reason as well as the reduced timeframe of just four weeks compared to ten that the level of fat loss was less pronounced compared to the systematic review.

In relation to muscle architecture, we have also shown significant improvements via an improvement in the pennation angle of *m. vastus lateralis* post-intervention. Fibre PA is an accepted marker of force and contractile capabilities (Lee *et al.*, 2015), with previous work demonstrating that an increase in PA corresponded to an increase in muscle cross-sectional area and therefore increased muscle strength (Aagaard *et al.*, 2001). Studies of muscle architecture in elderly individuals have also shown a correlation between decreased PA and sarcopaenia (Strasser *et al.*, 2013), and again our HIT programme may be a tool to prevent the development of sarcopaenia in later life. We did not however see further changes in muscle architecture (muscle thickness or fascicle length) or improvements in lean leg mass via DXA. This is not in keeping with findings of the two previous 5-by-1 minute HIT studies using a cycle ergometer, both of which found changes in both the pennation angle (Blackwell *et al.*, 2020) and muscle thickness (Boereboom *et al.*, 2016; Blackwell *et al.*, 2020) of *m. vastus lateralis.* It is possible that the recruitment of more muscle fibres using the cycle ergometer led to more significant changes to muscle hypertrophy in the lower limbs and therefore muscle thickness.

Despite a wealth of evidence suggesting that insulin sensitivity can be improved in older adults using a HIT intervention (Hwang *et al.*, 2016; Robinson *et al.*, 2017; Søgaard *et al.*, 2018) and that HIT is an excellent tool for combatting the severity and effects of T2DM (Little *et al.*, 2011; Francois and Little, 2015), we were not able to show improvements in markers of insulin sensitivity using our HIT intervention. Robinson *et al*. and Søgaard *et al*. both utilised the insulin clamp method (Robinson *et al.*, 2017; Søgaard *et al.*, 2018) which as discussed in section 1.4.6.1 is the gold standard method to assess insulin sensitivity (Tam *et al.*, 2012) and as such may have been more sensitive to detect changes in insulin sensitivity. We deemed this method for detecting insulin resistance as being too invasive for our participants. Hwang *et al.* reported a 26% decrease in insulin resistance in a cohort of similar BMI to ours using an eight week all-extremity HIT intervention but did not see any changes in other cardiometabolic parameters such as cholesterol and total body fat (Hwang *et al.*, 2016). A meta-analysis of 50 studies and 2,033 participants of which 1,383 performed a HIT intervention, suggested that ten weeks of HIT are needed to produce improvements in insulin resistance are more pronounced in subjects with existing insulin resistance or established T2DM (Jelleyman *et al.*, 2015). It may well be that our subject cohort who are considered to have average insulin sensitivity and not considered to be insulin resistant could not have improved further with our HIT intervention. It may also again simply be that four weeks of our intervention is not long enough.

Resting BP fell significantly in both exercise groups which has been consistently shown in older adults following HIT regimens (Bruseghini *et al.*, 2015; Blackwell *et al.*, 2020). The significant reductions in resting BP in just four weeks of our HIT protocol are superior to traditional aerobic exercise training protocols which are reported to be - 5/3 mmHg over a three month timeframe (Herrod *et al.*, 2018). Our findings for BP are similar to another study of older, sedentary males who reported a decrease of -7.7/4.6 mmHg, albeit using a cycle ergometer HIT protocol over six weeks with a preceding six week conditioning period (Grace *et al.*, 2018). Their protocol involved six, 30s sprints interspersed with three minutes of rest (Grace *et al.*, 2018), suggesting that our more time-efficient, five by one-minute HIT protocol can achieve similar reductions in resting BP in a mixed sex cohort. Our findings for resting BP did not however amount to a clinically significant decrease of 10mmHg systolic and 5mmHg diastolic BP which has been shown to reduce risk of myocardial infarction (22%) and stroke (41%) (Law, Morris and Wald, 2009). As our subjects were healthy older adults with relatively few co-morbidities, their mean resting BP was 127/70 placing them in the normotensive range (National Institute for Health and Care Excellence (NICE), 2019b). A possible reason for not reaching clinically relevant decreases in BP is that the ability of exercise regimens to reduce BP is not as effective for normotensive participants as it is for

hypertensive participants. It may also simply be that our four-week intervention was not long enough to elicit changes of this magnitude or that the study was not adequately powered to achieve the clinically significant changes. Our results are still promising however and the decrease in systolic BP will serve to decrease the cardiovascular risk profile in these participants, highlighting the potential for this method of HIT to provide an alternative exercise model for the improvement of vascular health in this population.

In a meta-analysis of 170,000 individuals, it was reported that a 1mmol/L reduction in LDL cholesterol is associated with a 10-20% reduction in the risk of cardiovascular events and all-cause mortality (Cholesterol Treatment Trialists' (CTT) Collaboration *et al.*, 2010). In just four weeks, we have seen reductions of 0.5mmol/L in non-HDLc for our exercise groups, again showing promise for our programme to be associated with favourable metabolic results in older adults and to be a clinically relevant tool in reducing dyslipidaemia and the risk of CVD. As discussed in section 1.5.3.3, NICE guidelines are to aim for a reduction in non-HDL cholesterol (non-HDLc) of 40% in three months with statin therapy (National Institute for Health and Care Excellence (NICE), 2019a). With this HIT programme, we have seen a promising decrease in mean non-HDLc of 17% in our exercise groups in only four weeks. These findings may be of interest to individuals who do not wish to start pharmacological intervention for improvement of dyslipidaemia as well as the previously discussed CRF benefits. As has been previously consistently described, a higher intensity of exercise is needed to lower non-HDLc effectively (Leon and Sanchez, 2001; Mann, Beedie and Jimenez, 2014; Hsu *et al.*, 2019). That our 5-by-1 minute protocol lowered non-HDLc so well again validates the intensity of the proposed exercises. Whilst these decreases in as little as four weeks are promising, more long-term studies are required to determine whether bodyweight-based HIT can produce a similar decrease in non-HDLc as statins in the same three-month time period.

Whilst we found significant decreases in non-HDLc, we did not see the predicted increases in HDLc following aerobic exercises which have been previously reported in a number of systematic reviews (Leon and Sanchez, 2001; Kodama *et al.*, 2007; Gordon, Chen and Durstine, 2014; Hsu *et al.*, 2019). However, the shortest study included in these reviews was eight weeks, double the timeframe of our study and the mean intervention period was 27 weeks in one review (Kodama *et al.*, 2007). The all-extremity HIT study did not find any significant changes in lipid profile after eight weeks of HIT or MICT (Hwang *et al.*, 2016) which is in contrast to the results we have observed with non-HDLc. Søgarrd *et al.* employed a six week cycle ergometer based HIT programme in older adults and unusually found a significant decrease in HDLc in females but did find also a significant decrease in LDLc (Søgaard *et al.*, 2018). Bruseghini *et al.* did not observe any changes in lipid profile after

eight weeks of cycle ergometer HIT in older adults either, but did see decreases in LDLc after resistance training (Bruseghini *et al.*, 2015). It may simply be that short-term HIT is not long enough to elicit changes in HDLc in non-comorbid individuals and longer-term studies involving HIT are again needed.

4.5.1 Limitations

This randomised control trial was adequately powered by a prior power calculation to draw valid conclusions of its primary outcome of changes in CRF. However, the small sample size may still have led to a possibility of type 2 errors.

Our participants were healthy subjects who mainly displayed an interest in the project due to the primary outcome of improving CRF. This may have created selection bias within our final cohort. Whilst the number of repetitions achieved was a good measure of exercise effort, data on heart rate variability were not recorded and this is scope for future work.

Another limitation of this study, as with many studies of HIT regimens, is that there is no evidence as to the long-term effects of these interventions as the study only looked at short-term improvements. Future studies will need to focus on whether the gains in CRF are sustainable with this form of HIT and indeed whether further improvement is possible beyond four weeks.

4.6 Chapter conclusions

Ageing leads to a decrease in CRF and loss of LMM, increasing the risk of disability, the Metabolic Syndrome and even premature mortality. Exercise is a proven strategy to combat these developments, but older adults are least likely to take up regular exercise due to various barriers. HIT has emerged as a time-efficient, feasible strategy to rapidly improve CRF in older adults. Until now its application has been mainly laboratory-based using specialised equipment, creating further barriers for older adults.

Owing to the multiple benefits of HIT discussed, translating HIT away from the laboratory environment is critical for delivering these health improvements to clinical populations. A four-week, equipment-free HIT intervention is able to elicit improvements in the CRF, body composition and cardiometabolic health of older adults with a reduced time commitment of less than 50 minutes per week. This study provides convincing evidence that the application

of HIT in this form is safe, feasible, easy to learn and equally successful when performed in an unsupervised setting such as at home, as it is in a supervised setting. The application of HIT at home may provide a novel approach to combating the rise of inactivity and the associated poor health outcomes in older adults, who are a growing section of society.

Chapter 5: Short-term, home-based, equipment-free high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness in young adults

5.1 Chapter overview

There has been a worrying rise in inactivity and obesity amongst adolescents and young adults. In this chapter we will discuss why younger adults are less physically active than last generation's and the health implications that being overweight and having low levels of CRF can have at that stage of life. Studies have shown that the exercise habits, levels of fitness, and that being overweight in young adulthood are carried forward into later adulthood. Therefore this age group should be targeted with effective and time efficient novel exercise interventions to combat the rise of inactivity and obesity. Whilst younger adults are generally more active than older ones, the number of younger adults who achieve the recommended weekly exercise targets is staggeringly low. Following on from our positive results in Chapter Four, we repeated the HIT exercise protocol in younger, recreationally active adults to see if it could have similar results in improving CRF in this age group and allow us to determine the efficacy of this intervention as a function of age.

5.2 Introduction

The health benefits of being physically active in adolescence and young adulthood are well documented. These include improved cardiorespiratory and muscular fitness, bone and cardiometabolic health and a positive effect on weight status (Guthold *et al.*, 2020), as well as improved cerebrovascular, and cognitive functioning abilities (Hwang *et al.*, 2018). There is also evidence that these health benefits are carried forward into later adult life (Guthold *et al.*, 2020). CRF in young adulthood is a modifiable, prognostic marker of long-term mortality and CVD risk, with higher fitness levels at an earlier age being associated with a lower future risk for all-cause mortality and adverse cardiovascular events (Valle *et al.*, 2015; Shah *et al.*, 2016) as well as being associated with a lower risk for developing insulin resistance and diabetes by middle age (Chow *et al.*, 2016). Equally, as discussed in section 4.2 for older adults, poor CRF in young adults is associated with the development of CVD risk factors and mortality (Carnethon *et al.*, 2003; Shah *et al.*, 2016). Low CRF in young adulthood has also been associated with abdominal obesity and increased cardiometabolic risk (Díez-Fernández *et al.*, 2018).

Sedentary behaviour, defined as any waking activity characterized with an energy expenditure < 1.5 METs whilst in a sitting, reclining or lying posture (Tremblay *et al.*, 2017), is becoming more prevalent in modern societies. The reasons for this include changes in the physical, social and economic environments for today's younger adults (Castro *et al.*, 2018). Television viewing, computer use, electronic games, prolonged mobile phone use and travelling on public transport or sitting in cars are all examples involving prolonged periods of these lower levels of metabolic energy expenditure (Owen *et al.*, 2010). Television viewing is the most commonly reported daily activity, occupying up to 40%, or four hours of daily free time in several European countries (Grøntved and Hu, 2011). The numerous adverse health outcomes associated with sedentary behaviours have been well established, including an increase in cardiovascular and all-cause mortality (Kodama *et al.*, 2009; Ekelund *et al.*, 2016; Young *et al.*, 2016) (Figure 5.1) as discussed in section 4.2. Although sedentary behaviour in younger adults, such as university students, is less well studied it is nevertheless important, as a significant proportion of their time is spent sitting in class or studying elsewhere (Castro *et al.*, 2018; Vainshelboim *et al.*, 2019). The change in residence, place and circumstance as well as relationship status for many such individuals is associated with a decrease in regular physical activity (Allender, Hutchinson and Foster, 2008; Howie *et al.*, 2018).

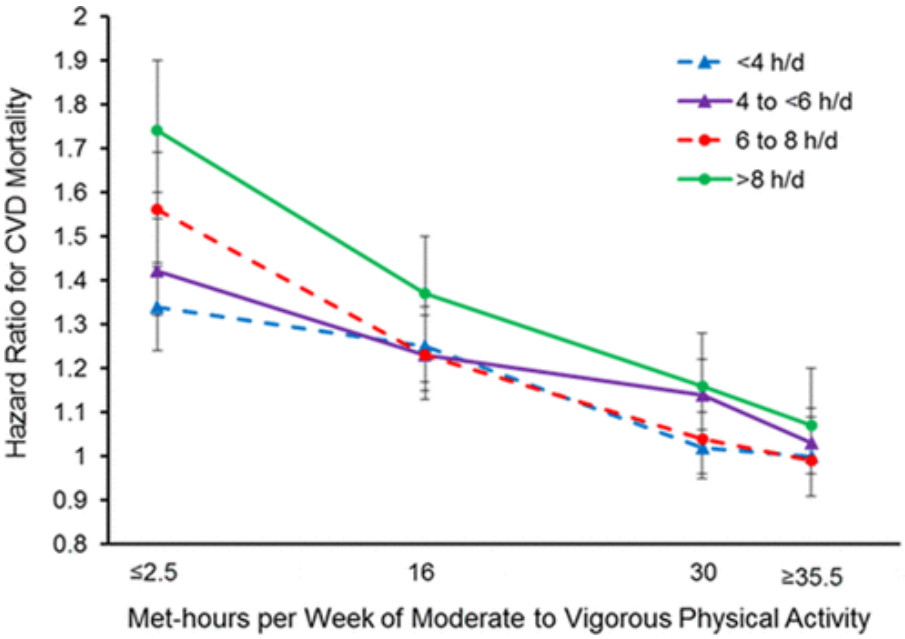


Figure 5.1: Hazard ratios for the joint association of sitting time and physical activity with cardiovascular disease (CVD) mortality (Eklund et. al 2016)

As it is for older adults, physical inactivity is also a growing concern in younger adults. The average time performing moderate or higher-intensity physical activity is half that at age 16-19 when compared to 12-15 years old (Troiano *et al.*, 2008), with a further marked decline in physical activity in young adulthood at 18-29 years old where assimilation into adult study or work and family roles begins (Caspersen, Pereira and Curran, 2000). Whilst younger adults are still more likely to be more physically active on a daily basis than older adults, the amount of time spent in sedentary behaviours is still high (Vainshelboim *et al.*, 2019) which has major implications for metabolic health (Owen *et al.*, 2010; Vainshelboim *et al.*, 2019). Within this group, inactivity amongst university students has been reported to be even lower than that of desk-based workers of a similar age (Castro *et al.*, 2018), with university attending females having the lowest levels of regular physical activity overall (Towne *et al.*, 2017).

Combined with the low levels of physical activity outlined above, increased energy intake is considered the main contributor to influence body weight and obesity (Zeppa *et al.*, 2020). In a survey of over one thousand 18-25 year olds, approximately 60% and more than four unhealthy snacks a day consisting of chocolates, crisps and fizzy drinks (Poobalan *et al.*, 2014) and this increase in unhealthy eating habits has undoubtedly led to the rise of obesity in this age group. Cardiometabolic risk factors are more prevalent amongst overweight or obese children and young adults than those of a healthy weight (Skinner *et al.*, 2015).

Despite massive public health interventions worldwide, at least 1.1 billion people are now classed as overweight (Tjønna *et al.*, 2008) and 300 million are obese (Misra and Khurana, 2008). Evidence has consistently shown that adolescent adiposity and fat distribution extends into early adulthood (Jia *et al.*, 2019), with 50-80% of obese adults having been obese as adolescents (Fisher *et al.*, 2015).

A reduction in skeletal muscle insulin sensitivity is seen in physical inactivity which is thought to be due to altered insulin signalling, contributing to redistribution of energy substrates into alternative tissues (Booth *et al.*, 2017). This redistribution increases central fat accumulation and ectopic storage within the liver and other organs, exacerbating insulin resistance (Booth *et al.*, 2017). The rise in obesity in adolescence and young adulthood is undoubtably the main cause of the rise in incidence of the Metabolic Syndrome (MetS) (Kassi *et al.*, 2011) as discussed in section 1.6. It is also well established that increased time in sedentary behaviours, with decreased participation in physical activity, is independently associated with increased risk of developing MetS (Healy *et al.*, 2008). This complex, multifactorial condition is associated with an increased risk for cardiovascular morbidity and mortality, with an odds ratio of 3 and 1.8 respectively (Isomaa *et al.*, 2001). Adolescents with high BMI have an increased risk of developing MetS in early adulthood and may already have established hallmarks of the disease such as elevated blood glucose levels, insulin resistance, increased carotid intima thickness and raised serum cholesterol levels (Magnussen *et al.*, 2010). Higher levels of sedentary behaviour are also significantly associated with an increased risk of T2DM (Wilmot *et al.*, 2012; Patterson *et al.*, 2018). In particular, one systematic review and meta-analysis concluded that prolonged television viewing is associated with an increased risk of T2DM as well as CVD and all-cause mortality (Grøntved and Hu, 2011). It is estimated that 8% of all mortality and 29% of T2DM in the English population are associated with certain sedentary behaviours (Patterson *et al.*, 2018).

There is an inverse association reported between sitting time and physical activity (Castro *et al.*, 2018) and manifestations of risk factors for CVD and reduced CRF can be seen from early adulthood. Imbalances between the production and destruction of reactive oxygen species by antioxidant defence systems associated with physical inactivity promote the uncoupling of endothelial nitric oxide synthase (Lavie *et al.*, 2019). These abnormalities result in reduced NO bioavailability and prolonged disruption of endothelial function and the associated reduction in vascular compliance because of physical inactivity is particularly damaging to cardiovascular health (Lavie *et al.*, 2019). As well as physical inactivity speeding the decline in VO_2max, biological ageing beginning in the third decade of life contributes to decreases in CRF (Booth *et al.*, 2017). Sitting time is also inversely correlated

to lean body mass in young adults whilst being directly correlated to increased fat percentage in young female adults and decreased upper body strength in young males (Vainshelboim *et al.*, 2019), again increasing the risk of insulin resistance and MetS. Promotion of health interventions to reduce the morbidity and mortality from inactivity, obesity and poor CRF therefore need to be developed for all age groups throughout healthcare system worldwide, especially in Western populations were the burden of cardiometabolic diseases remains extremely high (Lavie *et al.*, 2019).

Exercise is a proven strategy to combat the risk of mortality associated with sedentary behaviour (Ekelund *et al.*, 2016). Individuals who are physically fitter maintain a more favourable caloric balance and lower body weights, both of which protect against the development of CVD risk factors (Carnethon *et al.*, 2003). Appetite and hunger are also suppressed following exercise, in particular in the immediate post-exercise state, and this effect, known as “exercise-induced anorexia”, is linked to the suppression of orexigenic hormones and the increase of satiety hormones (Zeppa *et al.*, 2020). Regular physical activity can positively affect eating behaviour and food choices in a healthy direction, helping in correcting inappropriate long-term eating habits (Zeppa *et al.*, 2020). However, despite the clear benefits of regular exercise participation, up to 62% of young adults are not meeting these physical activity guidelines (Valle *et al.*, 2015). Furthermore, regardless of an individual’s baseline fitness, any improvements in CRF improve the overall CVD risk profile (Myers *et al.*, 2015).

When considering exercise strategies to improve CRF in younger adults, RET is a powerful stimulus to promote net muscle protein anabolism, resulting in specific metabolic and morphological adaptations in skeletal muscle tissue which effectively increase muscle strength (Koopman and van Loon, 2009). AE allows for adaptations of increased oxygen extraction and utilisation principally governed by the improvement in the availability, capacity and function of mitochondria and therefore increased ATP production and improved CRF (Brook *et al.*, 2016). However, as previously discussed in depth in Chapter 3, recommended exercises are often poorly explained to the public, leading to poor uptake and adherence to ‘traditional’ exercise training regimes (Sparling *et al.*, 2015). “Lack of time” remains a chief barrier to exercise participation in younger adults (Fisher *et al.*, 2015), especially for those with more physically demanding employment or more intense degree courses, such as medicine and nursing students (Blake, Stanulewicz and Mcgill, 2017). Other commonly faced barriers preventing exercise participation in younger adults include a lack of access to expensive gym equipment, high costs of gym memberships and negative body image when exercising in public places (Korkiakangas *et al.*, 2011; Scott *et al.*, 2020). Interest in

optimizing the magnitude of adaptation from exercise, whilst minimizing the time and effort, is therefore a topic of considerable interest within the exercise-for-health community (Fisher *et al.*, 2015; Foster *et al.*, 2015; Shepherd *et al.*, 2015).

HIT has emerged as a time-efficient strategy to rapidly improve CRF (Gillen *et al.*, 2014; Weston *et al.*, 2014) and indices of cardiometabolic function such as weight loss and insulin sensitivity in younger adults (Gibala and Little, 2010; Weston, Wisløff and Coombes, 2014). The physiological mechanisms for these changes have been discussed in length in Chapter 1. The NHS also recently suggested that 10 minutes of a HIT exercise intervention contributed towards the 150 minutes per week (National Health Service (NHS), 2018), building on the concept that 75 minutes of vigorous activity per week can be just as effective as 150 minutes per week (World Health Organisation (WHO), 2015). There is also evidence to suggest that HIT can promote healthier eating choices in younger adults (Zeppa *et al.*, 2020). As previously discussed, these rapid adaptations have, for the majority, been studied in supervised, laboratory conditions and employed specialist equipment such as cycle ergometers and treadmills (Karlsen *et al.*, 2017; Scott *et al.*, 2019). Studies in younger adults have also focused primarily on proving the superiority of HIT over MICT (Fisher *et al.*, 2015; Kong *et al.*, 2016). There is very little evidence in the form of randomised control trials as to the efficacy of unsupervised or home-based HIT interventions for improving CRF and cardiometabolic health in younger adults.

Many health behaviours such as healthy eating and regular exercise develop during adolescence and young adulthood (Towne *et al.*, 2017). Improving CRF and reducing sedentary behaviour amongst both active and inactive younger adults could be a public health strategy for health promotion and chronic disease promotion (Carnethon *et al.*, 2003; Vainshelboim *et al.*, 2019). To aid this initiative, designing equipment free exercise interventions with a reduced time commitment may be more appealing to younger adults (Fisher *et al.*, 2015). Following on from the success of our study in older adults on the efficacy of short-term, equipment-free, home-based HIT for improving CRF, we repeated the protocol in younger, recreationally active adults to see if we could achieve similar results. If positive, our home-based HIT regime could offer an attractive exercise intervention for younger adults, which may, across the adult life-course, mitigate age-associated declines in CRF and cardiometabolic status.

5.3 Experimental methods

5.3.1 Ethics approval

Ethics approval was sought for amendments to study C16122016 to allow us to recruit supervised exercise, unsupervised exercise and control groups of younger adults (aged 18-35 years). Full details of the ethical approval and amendments can be seen in Appendix 6.

5.3.2 Recruitment

Participants for this study were recruited from the staff and student cohort at the University of Nottingham studying at the Royal Derby Hospital by flyers, direct approach and the University of Nottingham registry of previous healthy volunteer subjects who had consented to having their personal details stored on a secure database for the purpose of being contacted for future studies.

5.3.3 Screening visit and consent

The screening visit and consent process were exactly as for the older adult study (section 4.3.3), involving a single screening session to determine study eligibility against the inclusion and exclusion criteria and the provision of written informed consent.

5.3.4 Inclusion criteria

The inclusion criteria for this study were:

- Male or female
- Age 18 to 35
- Full capacity to consent

5.3.5 Exclusion criteria

The exclusion criteria for this study were:

- Current participation in a formal exercise regimen (more than three times per week vigorous exercise)

- BMI < 18 or > 35 kg/m^2
- Active cardiovascular disease:
  - Uncontrolled hypertension (BP > 160/100)
  - Angina
  - Heart failure (class III/IV)
  - Significant cardiac 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
  - Diabetic patients needing medication
  - Uncontrolled thyroid disease
- Malignancy
- Clotting dysfunction
- Significant musculoskeletal or neurological disorders
- Family history of early (<55y) death from cardiovascular disease
- Participation in a research experiment within the last 3 months

5.3.6 Acute study day

Owing to time constraints, an approval amendment for additional DXA scans was not able to be actioned for the young participant groups. All other experimental tests were completed as for the older adult study (section 4.3.6). In brief, the assessments on this day included:

1) Oral glucose tolerance test
2) Standardised meal
3) Muscle architecture scan

4) CPET - carried out in accordance with the ATS/ACCP (Weisman *et al.*, 2003) and POETTS (Levett *et al.*, 2018) guidelines

5.3.7 Randomisation and experimental groups

Participants were randomised into one of three experimental groups using sealedenvelope.com (Sealed Envelope Ltd. 2019, simple randomisation service, London UK). This service was entirely independent of the trial investigators and uses random permuted blocks thereby ensuring allocation concealment. The three groups were as follows:

1) Lab HIT (LHIT): Fully supervised, laboratory-based HIT with encouragement
2) Home HIT (HHIT): Entirely unsupervised, home-based with no interaction
3) Control (CON): Non-intervention control group

5.3.8 HIT regime

Participants randomised to an exercise group performed exactly the same regime as for the older adult study (section 4.3.13). In brief, each HIT (laboratory and home-based) session comprised 5, 1-minute bursts of high-intensity bodyweight-based activity (star-jumps, squats, static sprints) interspersed with 90-seconds rest or active recovery.

5.3.9 Retest day

All participants were asked to attend for a repeat acute study day after four weeks. All measures taken during the acute study day were repeated. The exercise groups were asked to observe a minimum 48-hour rest period after their final HIT sessions to ensure CPET performance was not restricted by fatigue, or that cardiometabolic measures were not affected by prior acute exercise. The same ramp was used for the CPET as for the pre-test.

5.3.10 Statistical Analysis

5.3.10.1 Power calculation

As for section 4.3.15.1, based on AT and VO_2peak data from a previous study by our research group (Blackwell *et al.*, 2017), an *a priori* power calculation based on an effect size

F of 0.6 for three groups and a within group standard deviation of 5, determined that 30 participants (10 per group) would be required to achieve an α of 0.05 and a 1-β of 0.80.

5.3.10.2 Within group analysis

As this was a RCT, significance testing of baseline differences was not performed as per the CONSORT 2010 statement (de Boer *et al.*, 2015). Effects are reported as number (%), mean (SD) or +/- mean difference (MD) (SD). Within-group analysis for pre and post intervention change was performed using repeated measures t-tests. The level of significance was set at $p<0.05$. All analyses were performed using GraphPad Prism for Mac OS X Version 7.0d (GraphPad Software, La Jolla, San Diego, California USA).

5.3.10.3 Between group analysis

To analyse pre and post intervention change between groups, we performed ANCOVA with baseline values as the continuous covariate. Effects are reported as number (%) or mean differences with 95% confidence intervals (MD (CI)) and p values. We tested the assumptions of normality in groups (Shapiro-Wilk test), normality of residuals (histograms), equality of variance with robust tests for equality of variances, linearity, homoscedasticity (Breusch-Pagan/Cook-Weisberg test) and homogeneity of regression slopes. Differences between groups and 95% CI were adjusted for multiple comparisons using Sidak's *posthoc* test. All analyses were conducted using Stata Version 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

5.3.10.4 Comparison of CRF to older adult exercise groups

To analyse the results of CRF from the younger adult exercise groups when compared to the older adult exercise groups, we performed ANCOVA with baseline values as the continuous covariate. Effects are reported as number (%) or mean differences with 95% confidence intervals (MD (CI)) and p values. We tested the assumptions of normality in groups (Shapiro-Wilk test), normality of residuals (histograms), equality of variance with robust tests for equality of variances, linearity, homoscedasticity (Breusch-Pagan/Cook-Weisberg test) and homogeneity of regression slopes. Differences between groups and 95% CI were adjusted for multiple comparisons using Sidak's *posthoc* test. All analyses were conducted using Stata Version 15 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

5.4 Results

5.4.1 Participant demographics

Thirty participants of mean age 24(5) years of which 15 (50%) were male were recruited to this study. Baseline characteristics can be seen in Table 5.1.

	L-HIT (n=10)	H-HIT (n=10)	CON (n=10)
Age (years)	22 (4)	27 (4)	24 (6)
Male / Female	4 / 6	5 / 5	6 / 4
BMI	25 (4)	26 (4)	25 (4)
AT	14.89 (4.6)	20.27 (5.8)	18.22 (4.2)
VO_2	31.08 (7.9)	36.06 (4.8)	37.86 (8.8)
SBP	120 (11)	126 (6)	118 (14)

Table 5.1: Participant baseline characteristics.
Data depicts mean (SD). Abbreviations: L-HIIT, Laboratory (supervised) high intensity interval training; H-HIIT, Home-based (unsupervised) HIIT; CON, No-intervention control group; BMI, body mass index (kg/m^2); AT, anaerobic threshold (mg/kg/min); VO_2, VO_2peak (mg/kg/min); SBP, systolic blood pressure (mmHg). No significant differences between groups.

5.4.2 Study adherence

The study Consort diagram is shown in Figure 5.2. One participant declined to progress to the acute study day for personal reasons. One participant failed to complete 12 exercise sessions and was removed from the final analysis.

All randomised participants in the LHIT and CON groups completed a pre and post intervention CPET for the primary outcome of CRF (as measured by VO_2peak and AT). One participant in the HHIT group did not complete the number of required training sessions in the four weeks of intervention and was excluded from the study. Training compliance was otherwise 100% for both HIT groups, with all participants completing 12 sessions in four weeks.

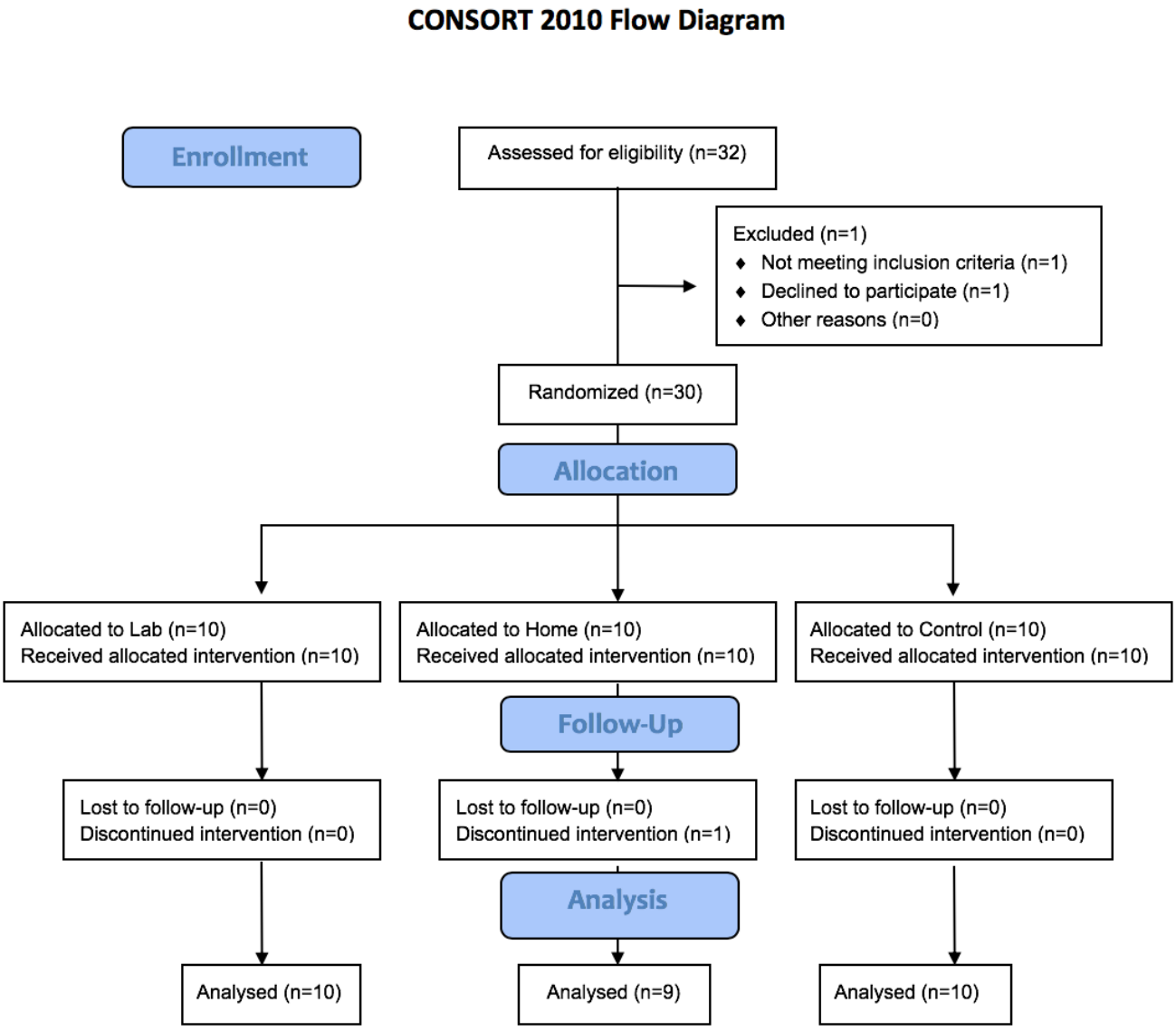


Figure 5.2: Study Consort diagram

5.4.3 Within group differences

Within group changes following a 4-week period of LHIT, HHIT or a CON intervention period are shown in Table 5.2.

	LHIT		HHIT		CON	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
AT (ml/kg/min)	14.89 (4.6)	17.09 (4.6)**	20.27 (5.8)	22.63 (5.4)**	18.22 (4.2)	17.86 (3.8)
VO_2 (ml/kg/min)	31.08 (7.9)	34.57 (8.4)**	36.06 (4.8)	39.19 (4.8)**	37.86 (8.8)	38.35 (9.0)
SBP (mmHg)	120 (11)	117 (9)	126 (6)	123 (8)	118 (14)	118 (12)
DBP (mmHg)	74 (7)	71 (6)	76 (8)	75 (7)	73 (7)	73 (11)
BMI (kg/m^2)	24.59 (4)	24.4 (4)**	25.54 (4)	25.23 (4)*	24.9 (4)	24.9 (4)
PA (degrees)	14.9 (3)	17.8 (2)**	13.1 (5)	15.3 (6)**	15.1 (2)	14.7 (1)
MT (mm)	2.36 (0.4)	2.43 (0.4)	2.31 (0.9)	2.30 (0.9)	2.49 (0.5)	2.53 (0.6)
FL (mm)	7.18 (2)	7.08 (2)	6.93 (3)	7.17 (3)	6.94 (2)	6.97 (2)
Glucose AUC	819.4 (110)	785 (102)	796 (159)	776.4 (155)	764.9 (122)	816.1 (117)
Insulin AUC	5445.4 (2421)	6914.4 (3769)	8154.8 (5145)	7525.9 (2698)	6330.4 (3392)	6193.4 (2233)
HOMA-IR	1.1 (0.7)	1.6 (1.0)	1.6 (0.7)	1.3 (0.5)	1.4 (0.7)	1.3 (0.5)

Table 5.2: Assessment parameters pre and post intervention.
*Data are presented as mean (SD). Analysis via repeated measures t-tests. *=p<0.05, **=p<0.01 versus pre-intervention in the same group.*

5.4.4 Cardiorespiratory fitness

5.4.4.1 Anaerobic threshold

There was a significant increase in AT within the LHIT (MD +2.2 (1.8) ml/kg/min; p=0.0038) and HHIT (MD +2.36 (1.7) ml/kg/min; *p*=0.0032) groups after the intervention period, with no significant change in the control group (*p*=0.65) (Table 5.2, Figure 5.3).

When compared to the control group, there was a significant increase in AT in the LHIT (MD +2.1 (0.34 to 4.03) ml/kg/min; p=0.02) and HHIT (MD +3.01 (1.17 to 4.85) ml/kg/min; p=0.002) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD -0.82 (-2.83 to 1.18) ml/kg/min; p=0.41) (Figure 5.3).

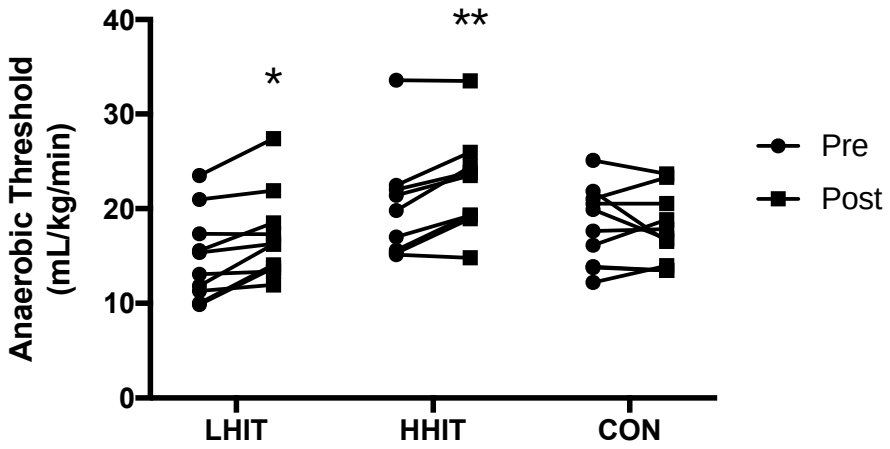


*Figure 5.3: Anaerobic threshold (ml/kg/min) pre and post intervention. *=p<0.05, **=p<0.01 versus pre-intervention when compared to control group*

5.4.4.2 VO_2peak

There was a significant increase in VO_2peak within the LHIT (MD +3.49 (2.3) ml/kg/min; *p*=0.001) and HHIT (MD +3.13 (2.2) ml/kg/min; *p*=0.0029) groups after the intervention period, with no significant change in the control group (*p*=0.53) (Table 5.2, Figure 5.4).

When compared to the control group, there was a significant increase in VO_2peak in the LHIT (MD +2.94 (0.64 to 5.25) ml/kg/min; p=0.01) and HHIT (MD +2.55 (0.34 to 4.76) ml/kg/min; p=0.03) groups after the intervention period. There was no significant difference between the LHIT and HHIT groups (MD +0.39 (-1.90 to 2.68) ml/kg/min; p=0.73) (Figure 5.4).

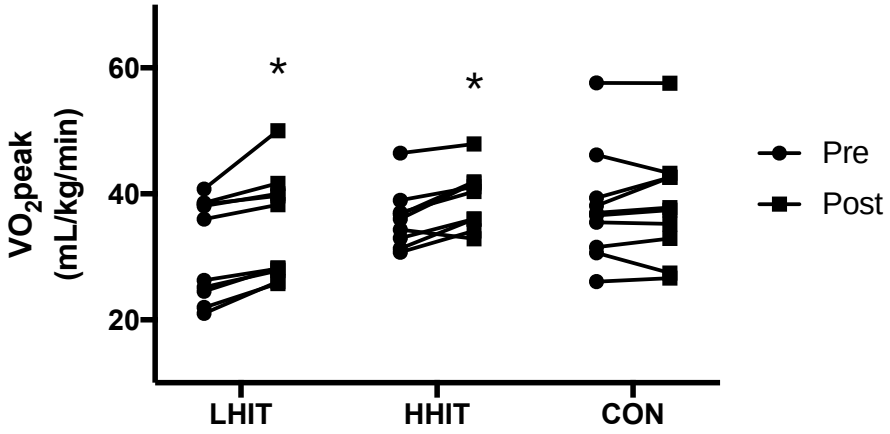


*Figure 5.4: VO_2Peak (ml/kg/min) pre and post intervention. * = p<0.05 versus pre-intervention when compared to control group*

5.4.4.3 Maximum wattage

There was a significant increase in WMax within the LHIT (MD +8.4 (6.1) W; *p*=0.0037) and HHIT (MD +8.2 (5.5) W; *p*=0.023) groups after the intervention period, with no significant change in the control group (*p*=0.46) (Table 5.2, Figure 5.5).

When compared to the control group, there was a significant increase in Wmax in the LHIT (MD +17.84 (6.7 to 29) W; *p*=0.001) and HHIT (MD +12.7 (1.66 to 23.74) W; *p*=0.02) groups after the intervention period. There was no significant difference in increase between the LHIT and HHIT groups (MD +5.14 (-5.78 to 16.06) W; *p*=0.56) (Figure 5.5).

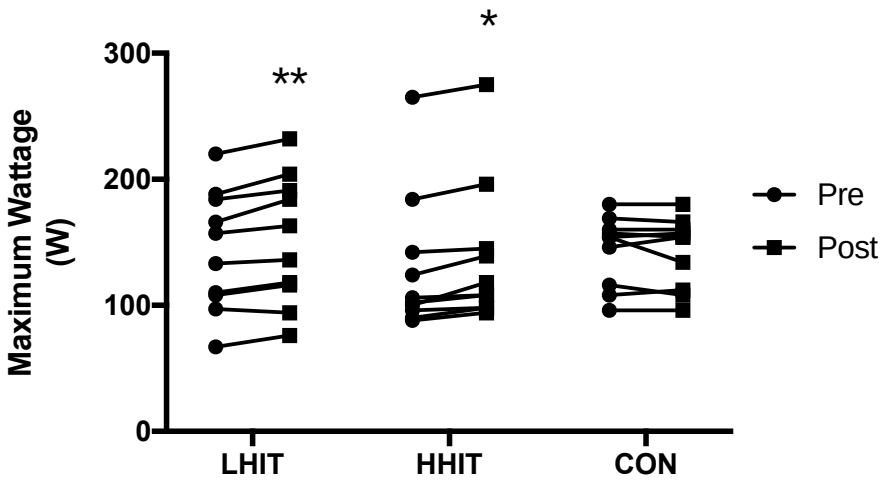


*Figure 5.5: Maximum wattage (W) pre and post intervention. ** = p<0.01, * = p<0.05 versus pre-intervention when compared to control group*

5.4.4.4 Comparison of CRF to older exercise groups

There was no difference between the older versus the younger group in AT for LHIT (MD + 0.34 (-1.29 to 1.96) ml/kg/min *p*=0.67). There was no difference between the older versus the younger group in VO_2peak for LHIT (MD 0.78 (-1.41 to 2.98) ml/kg/min; *p*=0.46).

There was no difference between the older versus the younger group in AT for HHIT (MD +0.23 (-1.66 to 2.11) ml/kg/min; *p*=0.80). There was no difference between the older versus the younger group in VO_2peak for HHIT (MD -0.71 (-3.51 to 2.09) ml/kg/min; *p*=0.60).

5.4.5 Body Mass Index

There was a significant decrease in BMI within the LHIT (MD -0.18 (0.2) kg/m^2; *p*=0.007) and HHIT (MD -0.17 (0.2) kg/m^2; *p*=0.027) groups after the intervention period, with no significant change in the CON group (*p*=0.4) (Table 5.2, Figure 5.6).

When compared to the control group, there was a significant decrease in BMI in the LHIT (MD -0.43 (-0.86 to 0.00) kg/m^2; p=0.05) and HHIT (MD -0.51 (-0.95 to -0.07) kg/m^2; p=0.03), groups after the intervention period. There was no significant difference in the magnitude of change between the LHIT and HHIT groups (MD 0.08 (95% CI -0.37 to 0.52) kg/m^2; p=0.72) (Figure 5.6).

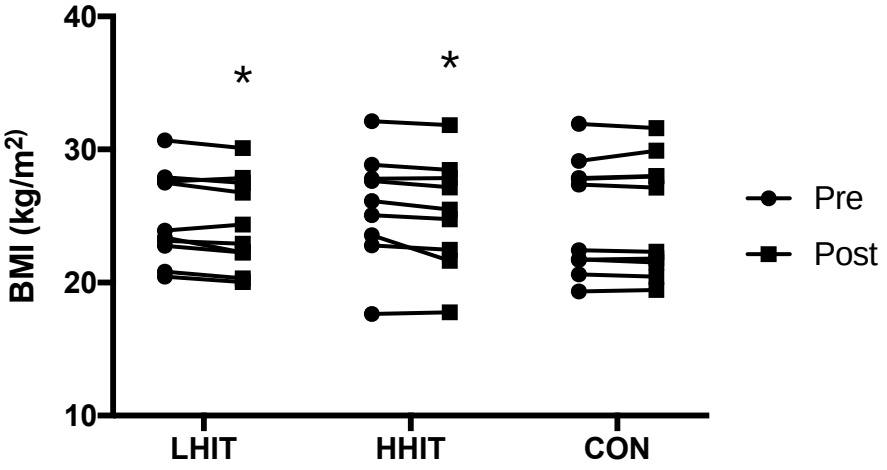


*Figure 5.6: BMI (kg/m2) pre and post intervention, * = p<0.05 versus pre-intervention when compared to control group*

5.4.6 Resting blood pressure

There were no significant changes in resting systolic or diastolic BP within any of the groups after the intervention period (Table 5.2).

When compared to the control group, there were no significant changes in systolic BP in the LHIT (MD -1.71 (-6.67 to 3.26 mmHg; p=0.49) or HHIT (MD -0.49 (-5.85 to 4.86) mmHg; p=0.85) groups after the intervention period.

Similarly, when compared to the control group, there were no significant changes in diastolic BP in the LHIT (MD -2.79 (-9.01 to 3.43) mmHg; p=0.37), or HHIT (MD -0.13 (-6.64 to 6.38) mmHg; p=0.97) groups after the intervention period.

5.4.7 Blood glucose concentrations

There were no significant changes in blood glucose AUC in response to a 75g oral glucose load within any of the groups after the intervention period (Table 5.2).

When compared to the control group, there were no significant changes in blood glucose AUC in the LHIT (MD -58.36 (-175.58 to 58.85) mmol/120min; p=0.31) or HHIT (MD -55.19 (-171.16 to 60.78) mmol/min; p=0.33) groups after the intervention period.

5.4.8 Plasma insulin concentrations

There were no significant changes in insulin AUC in response to a 75g oral glucose load within any of the groups after the intervention period (Table 5.2).

When compared to the control group, there were no significant changes in insulin AUC in the LHIT (MD +1127.61 (-1445.29 to 3700.51) uU/ml/120min; p=0.37) or HHIT (MD +494.3 (-2120.47 to 3109.08) uU/ml/120min; p=0.7) groups after the intervention period.

5.4.8.1 Insulin resistance

There were no significant changes in HOMA-IR within any of the groups after the intervention period (Table 5.2).

When compared to the control group, there were no significant changes in HOMA-IR in the LHIT (MD 0.51 (-0.11 to 1.12); p=0.1) or HHIT (MD -0.12 (-0.73 to 0.48); p=0.68) groups after the intervention period.

5.4.9 Muscle architecture

5.4.9.1 Pennation angle

There was a significant increase in *m. vastus lateralis* pennation angle within the LHIT (MD +2.9 (2)º; *p*=0.001) and HHIT (MD +2.2 (2)º; *p*=0.0025) groups after the intervention period, with no significant change in the control group (*p*=0.31) (Table 5.2, Figure 5.7).

When compared to the control group, there was a significant increase in *m. vastus lateralis* PA in the LHIT (MD +0.2 (0.13 to 0.27)º; p<0.001) and HHIT (MD +0.17 (0.09 to 0.24)º; p<0.001) groups after the intervention period. There was no significant difference in increase between the LHIT and HHIT groups (MD +0.03 (-0.04 to 0.11)º; p=0.36) (Figure 5.7).

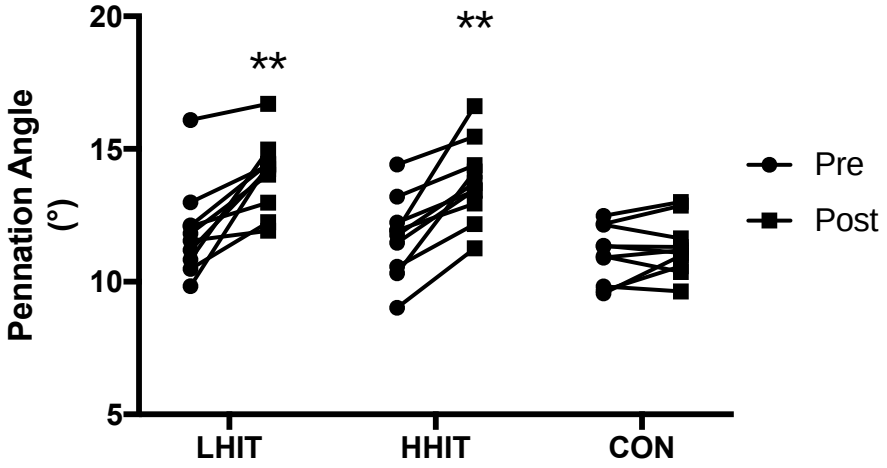


*Figure 5.7: Muscle pennation angle pre and post intervention. ** = p<0.01 versus pre-intervention when compared to control group*

5.4.9.2 Muscle thickness

There were no significant changes in the *m. vastus lateralis* muscle thickness within any of the groups (Table 5.2).

When compared to the control group, there were no significant changes in *m. vastus lateralis* MT in the LHIT (MD +0.03 (-0.13 to 0.19) mm; p=0.72) or HHIT (MD -0.05 (-0.21 to 0.12) mm; p=0.58) groups after the intervention period.

5.4.9.3 Fascicle length

There were no significant changes in the *m. vastus lateralis* fascicle length within any of the groups post intervention (Table 5.2).

When compared to the control group, there were no significant changes in the *m. vastus lateralis* FL in the LHIT (MD -0.07 (-0.62 to 0.48) mm; p=0.8) or HHIT (MD 0.59 (0 to 1.18) mm; p=0.06) groups after the intervention period.

5.5 Discussion

This study has shown that just four weeks of a time-efficient, equipment-free, bodyweight-based HIT programme is able to elicit improvements in the CRF, BMI and muscle architecture of younger adults. Our HIT protocol was however unable to elicit any improvements in resting blood pressure or indices of glucose and insulin metabolism in younger adults. Exploring the efficacy of our HIT protocol as a function of age, we have shown that there is no significant difference between younger and older adults when comparing the improvements in CRF for supervised versus unsupervised HIT.

Whilst HIT has been well documented to be a potent stimulus to improve CRF in a short period of time in younger adults (Gibala *et al.*, 2012; Gillen and Gibala, 2014; MacInnis and Gibala, 2017), the role of supervision and requirement for equipment during HIT in this age group has not been studied extensively (Blackwell *et al.*, 2017; Scott *et al.*, 2019). This study addresses the evidence gap and demonstrates that: 1) HIT is feasible in a non-laboratory environment; 2) HIT can achieve clinically relevant improvements in CRF; and 3) CRF improvements can be delivered using an equipment-free, bodyweight-based HIT protocol with no direct feedback in younger adults. Compared to the existing literature on 'home-based' (unsupervised) HIT in younger adults, our findings are novel in that our study was randomised, our HIT protocol relied entirely on bodyweight exercises, there was no direct feedback and our participant group were younger, healthy volunteers with few known medical problems, as many of the individuals in this target demographic would be expected to be. Our HIT programme was well received by all participants and we experienced no serious adverse events or injuries during completion of the four-week programme, irrespective of the supervision received. The improvements we report in this study of younger adults provides further validation, beyond our study of older individuals, that our HIT programme is feasible in all adult age groups and that the exercises employed generate sufficient intensity to achieve the benefits of HIT (MacInnis and Gibala, 2017). In addition, we show equal comparative efficacy of this programme for improving the CRF of both young and older adults. This data provides more evidence against the notion that HIT is difficult to achieve outside of a laboratory environment or without the use of expensive specialist equipment such as cycle ergometers (Hardcastle *et al.*, 2014).

Higher fitness levels at an earlier age are associated with a lower future risk for all-cause mortality and adverse cardiovascular events (Valle *et al.*, 2015; Shah *et al.*, 2016). As such, the significant improvements in AT, VO_2peak and maximum wattage that we report after just four weeks of intervention provide further evidence that HIT as a modality can produce rapid

improvements in CRF and potentially mitigate future disease risk. As discussed in section 4.5, AT cannot be subjectively influenced and as such provides a particularly reliable validation of changes in CRF (Agnew, 2010). That our younger participants improvement in AT was greater than that observed in our older cohort (who had already improved further than the clinically significant target of 1.5 ml/kg/min (Dunne *et al.*, 2016)), further validates our equipment-free method of HIT for improving CRF in younger adults, and dispels any notion of this training programme only being effective in those with low baseline CRF.

The ~10% increase in VO_2peak we report for both of our young HIT groups is consistent with those observed after a five-week, cycle ergometer-based study comparing HIT to MICT in overweight and obese young women, where HIT training was found to increase CRF by +7.9%, with MICT eliciting improvements of +11.7% (Kong *et al.*, 2016). The improvements using our HIT programme were achieved in one week less. Our results are also superior to a six-week, cycle ergometer-based HIT study which employed a four-by-30 second HIT regimen in overweight or obese young males. This study reported only a 2.8% increase in VO_2peak with HIT (Fisher *et al.*, 2015) and concluded that with their particular HIT protocol six weeks of MICT using continuous cycling (+11.1%) was superior to HIT for improving CRF. That our HIT protocol involved over double the duration of high-intensity time (5 min vs. 2 min) per session may explain the discrepancies between these studies, as session frequency of three days per week and target intensity of 85% maximum predicted HR for HIT were similar. Our results are also superior to a meta-analysis of low volume HIT using up to 13 training sessions which reported a 6.2% and 3.6% improvement in the VO_2peak of young, active, non-athletic males and females respectively (Weston *et al.*, 2014). The majority of studies in this meta-analysis were fully supervised and had a requirement for equipment. As such, that the improvements we report were achieved using an entirely equipment-free protocol, further validate it as a useful method to improve CRF in younger adults. In addition, by not using equipment and taking into account the much-reduced time commitment, when compared to MICT and some other forms of HIT, over the longer term our HIT regime may also achieve improved adherence given equipment and time are commonly cited barriers to exercise (Foster *et al.*, 2015).

As discussed in section 4.5, an increase of one MET or 3.5 ml/kg/min (Jetté, Sidney and Blümchen, 1990) in VO_2peak is associated with a 15% and 19% lower risk of all-cause and CVD mortality, respectively (D. Lee *et al.*, 2011). Again, as was seen in our older cohort, the significant increase in VO_2peak we report for both the young exercise groups is close to this level of clinical significance, with no difference in the improvement achieved between the age groups for either form of HIT (laboratory versus home). This data again supports our HIT programme as a feasible method for improving CRF in young adults and reducing the

risk of all-cause mortality in later life. There is also evidence to suggest that the exercise habits and health benefits gained during early adulthood are often carried forward into later life (Guthold *et al.*, 2020). Despite our improvements in CRF parameters of young and older adults with just 4-weeks of our HIT protocol, further larger-scale studies into this modality of HIT are still needed to assess the retention of these adaptations and whether further improvements are possible after four weeks.

BMI is another parameter known to be closely associated with cardio-metabolic disease risk. We report a modest improvement in BMI for both exercise groups following completion of intervention, mirroring the improvement seen in our older adults. As an elevated BMI is a major contributor to development of the MetS (Healy *et al.*, 2008), and a lower BMI is associated with reduced risk of developing cardiometabolic disorders in this age group (Kassi *et al.*, 2011), these changes may offer addition protection from future chronic disease, in addition to those associated with improvement in CRF.

Muscle mass is known to be associated with whole body health, especially in older adults (Franchi *et al.*, 2018). Although in this study we saw no changes in muscle thickness, (Franchi *et al.*, 2018), other changes in muscle architecture were observed. Specifically, we report improvements in the PA of both our young and older individuals (to the same degree), with both formats of HIT. From a functional perspective, PA is associated with force and contractile capability, with an increase in PA following an intervention thought to represent an increase in cross-sectional area and associated strength (Lee *et al.*, 2015; Monti *et al.*, 2020). Whilst improvements in PA have been shown following training modalities commonly associated with hypertrophy and strength gains, e.g. resistance and plyometric exercise training (Monti *et al.*, 2020), PA has been less well studied following HIT. Significant improvements in PA for both exercise groups in both ages, despite no changes in MT and FL, may suggest subtle hypertrophic responses in the lower limbs, that are not detectable by MT. This suggestion would fit with the increase in lean mass on DXA seen in our older HIT groups and with previous reports of HIT being able to produce hypertrophy in both young (Fisher *et al.*, 2015) and older (Boereboom *et al.*, 2016) adults.

Despite many studies reporting improved blood glucose and insulin sensitivity in young, non-diabetic adults (Ciolac *et al.*, 2010; Metcalfe *et al.*, 2012; Fisher *et al.*, 2015; Shepherd *et al.*, 2015; Kong *et al.*, 2016), we did not demonstrate any significant improvements in indices of glucose and insulin metabolism. In section 4.5 we discussed the concept that individuals with higher basal insulin and metabolic abnormalities, such as raised BMI may have a greater response to HIT and that many studies had seen significant changes in insulin sensitivity in overweight and or metabolically impaired adults (Jelleyman *et al.*, 2015).

However, Ciolac *et al* were able to show improvements in the insulin sensitivity of young, healthy women with a family history of hypertension using a supervised HIT. However, the thrice weekly treadmill protocol used consisted of much longer training sessions (40 minutes) than ours and a 4-times longer intervention duration of 16-weeks (Ciolac *et al.*, 2010). Similarly, a 15-week, cycle-based intermittent sprint study in healthy young females of normal BMI was also able to reduce insulin resistance (Trapp *et al.*, 2008). Babraj *et al* were able to show improvements in glucose and insulin metabolism in healthy young males following just two weeks of a supervised, cycle-based sprint protocol. However this protocol was similar to the Wingate-type exercise discussed in section 1.10.2, using 4-6 efforts per session for 6 sessions (Babraj *et al.*, 2009). This type of sprint exercise is extremely demanding, difficult to achieve outside of a laboratory setting and does not appeal to many non-athletic individuals (Gillen and Gibala, 2014). It would also be extremely difficult to replicate the intensity of these sessions with bodyweight exercises. As we have previously discussed in Chapter 4, where the same lack of effect on parameters related to insulin sensitivity was observed in older adults, it may be that the shorter time-frame utilised for our well-tolerated, lower than Wingate, for example, intensity protocol is simply not long effort to elicit changes in glucose and insulin metabolism in healthy individuals and further studies using our protocol for a longer time period is scope for future work.

A number of studies have shown HIT to be an excellent way to improve blood pressure in young adults (Ciolac *et al.*, 2010; Gillen *et al.*, 2014). However, we failed to demonstrate any changes in resting systolic or diastolic blood pressure in either of the younger HIT exercise groups after four weeks intervention, contrary to our findings in older adults. A six week, cycle ergometer based study in young, overweight or obese males also failed to demonstrate improvements in resting systolic or diastolic blood pressure via HIT (Fisher *et al.*, 2015). Contrary to these findings, a 16 week treadmill-based intervention study in young normotensive women demonstrated improvements in both systolic and diastolic blood pressure with HIT, with the magnitude of these improvements greater than that seen with MICT over the same intervention duration (Ciolac *et al.*, 2010). Another interval running study, with a 12-week intervention duration, reported that in young, non-athletic men systolic blood pressure was significantly reduced. However, the exercise in this study was much higher than in the other studies, and five out of eight participants experienced muscle injuries (Nybo *et al.*, 2010). Based on our current findings and the previous literature, it may well be that in normotensive, younger participants, a longer duration of HIT, especially at a tolerable intensity, is necessary for eliciting improvements in resting systolic and diastolic blood pressure.

Home-based studies involving HIT have been relatively poorly studied owing to previously held notions that HIT was difficult and even unsafe to perform outside of a supervised, laboratory-based environment (Hardcastle *et al.*, 2014). Despite this, in recent years, given the growing popularity and research evidence base surrounding HIT, more work has begun to explore HIT in non-laboratory environments (Blackwell *et al.*, 2017; Scott *et al.*, 2019). Our findings provide further evidence that home-based HIT is safe and effective for improving CRF in both young and older adults, and that supervision is not essential for implementation of an equipment-free HIT protocol. Although our self-reported levels of adherence in our home-based HIT groups was high, the use of eHealth technologies may offer motivation, via feedback, and improve adherence in exercise regimes, especially over longer periods. For example, in a recent unsupervised, equipment-free HIT study, Scott *et al.* reported improvements in cardiometabolic health with good levels of adherence in younger diabetic adults using a 12-week protocol. In this study heart rate was monitored remotely with feedback and instruction provided to participants via a mobile phone application (Scott *et al.*, 2019). Whilst unsupervised HIT studies have not been extensively investigated, the application of HIT in this way may prove to be a crucial cost-effective public health tool to battle the rise of inactivity, cardiometabolic disorders and the associated later poor health outcomes in younger adults.

5.5.1 Limitations

This randomised control trial was adequately powered by a prior power calculation to draw valid conclusions of its primary outcome of changes in CRF. However, the small sample size and the loss of one participant from analysis may still have led to a possibility of type 2 errors.

Our participants were healthy young adults who mainly displayed an interest in the project due to the primary outcome of improving CRF. This may have created selection bias within our final cohort. Whilst the number of repetitions achieved was a good measure of exercise effort, data on heart rate variability were not recorded and this is scope for future work.

Body composition changes as measured by DXA scan were not available for this study and this is again scope for future work to fully assess the changes in body composition achievable by our HIT programme.

Hormonal changes during menstrual cycles cause many physiological effects including changes in the thermoregulatory, respiratory and renal systems (Janse de Jonge, 2003). Controlling for stage of menstrual cycle for the acute study days and during the four weeks of exercising would not have been feasible for this study, however we recognise that this may have had an effect on performance during CPET and during interval exercises.

Another limitation of this study, as with many studies of HIT regimens, is that there is no evidence for the long-term effects of these interventions. Future studies will need to focus on whether the HIT protocol is sustainable, and if the gains made in CRF are sustained with this form of HIT and indeed whether further improvement is possible beyond four weeks.

5.6 Chapter conclusions

Inactivity and sedentary lifestyles in association with reduced CRF in younger adults have been shown to increase the risk of cardiometabolic disorders and all-cause mortality in later life. The cardioprotective effects of regular physical activity are clear and extend across all ages. Designing equipment-free exercise interventions with a reduced time commitment may be more appealing to younger adults and encourage more participation in regular exercise in this age group.

A 4-week, time-efficient, equipment-free HIT programme is able to elicit improvements in the CRF and BMI of younger adults with the magnitude of these improvements being similar when compared to older adults. Furthermore, this programme is equally successful when performed in an unsupervised setting such as the home. The application of unsupervised HIT may be useful in counteracting the effects of the rise of sedentary behaviours and consequent cardiometabolic disorders in younger adults that will be carried forward into benefits later in life.

Chapter 6: A pilot study to explore the acute effects of phytonutrients upon vascular and metabolic responses to feeding in older adults

6.1 Chapter overview

The whole-body health benefits of regular exercise, as well as its ability to protect against the effects of sarcopenia in later age, have been discussed in the previous chapters. Whilst regular exercise remains the most effective strategy to combat the changes in physiology associated with advancing age, given the previously explored barriers to exercise in the elderly and the population as a whole, more attention is now being given to alternative strategies, i.e. nutritional supplements, to improve overall health and mitigate sarcopenia. In relation to skeletal muscle 'health', improving blood flow to muscle tissue may lead to improved oxygen and nutrient delivery, thereby potentially improving the maintenance of muscle mass and function in older adults who may not be able to exercise due to co-morbidity. A large number of pre-clinical studies support the benefits of various plant polyphenol-enriched extracts for improving blood flow to different tissues, with a smaller number of studies offering evidence for these supplements to also improve aspects of metabolic health (e.g. insulin sensitivity). In this chapter, the effects of three different phytonutrients on macrovascular blood flow and metabolic responses to acute feeding will be assessed.

6.2 Introduction

Skeletal muscle is the most abundant tissue type in humans, making up approximately 40% of total body weight and containing 50-75% of all body proteins (Hoppeler and Fluck, 2002). As discussed in detail in section 1.3 of this thesis, muscle tissue also has crucial roles in generating mechanical energy for force and power during exercise, as well as being a metabolically active tissue type, maintaining core temperature and an important source of storage for carbohydrates and amino acids (Frontera and Ochala, 2015). Muscle contractions also increase energy utilisation and activate numerous metabolic pathways in an effort to meet the muscles' increased demand for energy (Casey, Curry and Joyner, 2008).

Muscle mass maintenance is achieved through diurnal sinusoidal fluctuations in muscle protein synthesis (MPS; anabolism) and breakdown (MPB: catabolism) rates that are eventually counterbalanced such that net muscle protein balance remains zero by the end of each day (Burd, Gorissen and van Loon, 2013). The loss of skeletal muscle mass and function with ageing in the process of sarcopenia, as discussed in detail in section 1.3.5, is a well-known phenomenon, with reported loss of leg muscle mass of 1-2% and loss of strength 1.5-5% per year, reported beyond the age of 50 years (Cruz-Jentoft *et al.*, 2019). In extreme cases, this loss can be as much as a total of ~50% in the 8-9th decades of life (Wilkinson, Piasecki and Atherton, 2018). Given the important functional and metabolic roles of muscle tissue, as well older individuals representing a growing proportion of the population, sarcopenia represents a major, global health problem. Clinically, sarcopenia manifests as high personal and social burdens, increasing the risk of falls and fractures, impairing the ability to perform activities of daily living and eventually leading to loss of independence (Dos Santos *et al.*, 2017). Severe and prolonged muscle loss leads to frailty, increased risk of hospitalisation and long-term dependence on social care services (Cruz-Jentoft *et al.*, 2019). These burdens manifest as high costs to healthcare systems, with future estimates for healthcare budgets in the billions (Pinedo-Villanueva *et al.*, 2019). To give a specific example of the healthcare related cost associated with falls and fractures in the elderly, so called ‘fragility fractures’ are estimated to cost £4.4 billion in the UK alone (Public Health England, 2020).

The loss of muscle mass with ageing represents a major shift in whole body protein distribution and a partial loss of a primary protein reservoir that may be utilized to supply amino acids for synthesis of new body proteins (Atherton and Smith, 2012). The stressed state, seen in illness such as sepsis, cancer, trauma and chronic disease, many of which are

more common with advancing age, imposes greater demand for amino acids from MPB (Wolfe, 2006b). These illnesses are often associated with a rapid and extensive loss of muscle mass, strength and metabolic function (Wolfe, 2006b), which creates a significant problem for those with pre-existing deficiencies in muscle mass such as the elderly population. Decreased muscle mass and function result in a greater challenge for cells to repair themselves following a stressor to the body. An example of this is during hospitalisation; if there is pre-existing deficiency, a further acute loss of muscle mass and function may push an individual over a threshold that makes adequate recovery unlikely to occur, resulting in morbidity or even mortality (Wolfe, 2006b). Despite substantial investigation and progression into the understanding of the mediators and mechanisms involved in muscle atrophy, there is still not an effective, universally accepted treatment by which muscle wasting can be prevented or reversed, with this especially true in unwell patients (Alamdari, O'Neal and Hasselgren, 2009) where resistance exercise training, the most effective sarcopenia countermeasure available, cannot be performed.

Muscle blood flow is purported to be a key link in the interplay and regulation of systemic and local muscle metabolism (Casey, Curry and Joyner, 2008). Due to the ability of muscle to increase its metabolism by more than 100-fold, the rise in blood flow to active muscles can be vast and is generally proportional to the rise in metabolism (Casey, Curry and Joyner, 2008). When compared to younger adults, ageing is associated with a 20-30% reduction in supine resting limb blood flow, discussed in detail in section 1.12, representing increased vascular resistance (Donato *et al.*, 2006). Increased vascular resistance and endothelial dysfunction are classical hallmarks of vascular disturbances associated with ageing (Heiss *et al.*, 2015). With ageing, there is an increase in stiffening collagen content and calcification in the wall arteries and a reduction in elastic elastin content (Figure 6.1), leading to an increased wall thickness to lumen ratio and therefore increased stiffness (Jani and Rajkumar, 2006).

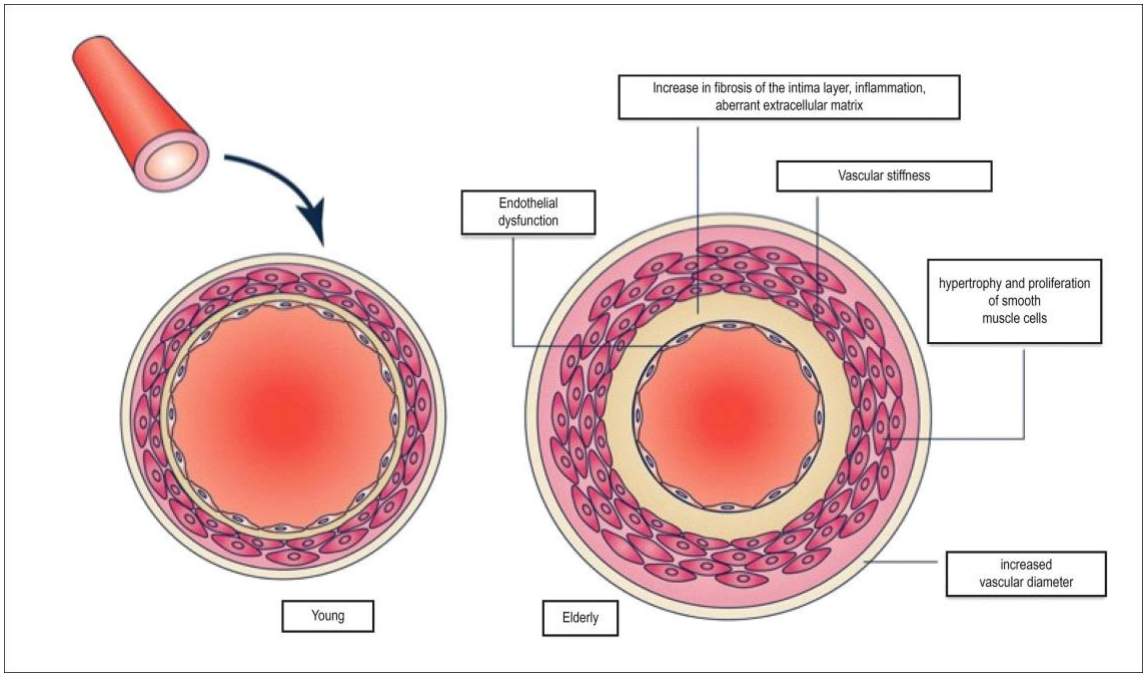


Figure 6.1: Pathophysiology of vascular ageing (Costantino et al., 2016)

Another hallmark of vascular disturbance associated with advancing age is the reduction in circulating nitrite concentrations. Circulating nitrates are released from the vascular endothelium in the form of NO which acts directly on vascular smooth muscle and is therefore responsible for vascular smooth muscle relaxation (Jin and Loscalzo, 2010) which is essential for maintaining vessel compliance and improving endothelial function, even in pre-existing atherosclerosis (Hodgson and Croft, 2010). The resulting vasodilation and microvascular recruitment, whilst inhibiting vasoconstriction and decreased arterial compliance, improves muscle nutrient and oxygen delivery (Keske *et al.*, 2015) and hence maintains muscle health. As well as reduced NO concentrations, there is an increase in the vasoconstrictor endothelin, also released from the endothelium, as it is no longer regulated by the reduced circulating NO concentrations (Barton, 2014). The resultant reduction in circulating nitrite concentrations and increase in circulating endothelin concentrations with ageing leads to reduced vascular compliance, with this suggested to be the main mechanism behind vascular disturbances in older age (Sindler *et al.*, 2011). These changes in vascular function promote vasoconstriction and subsequent decreased capillary number, further inducing a lowered LBF (Zempo, Isobe and Naito, 2017). The decline in vascular endothelial function and subsequent limb blood flow (Anton *et al.*, 2006), as seen in ageing is a major risk factor for CVD (Akazawa *et al.*, 2012). In disease states such as hypertension and diabetes, this process is exaggerated, increasing the risk of CVD (Jani and Rajkumar, 2006).

Aside from the link between age-related declines in blood flow and CVD risk, decreased blood flow to appendicular regions may also have a direct and substantial role in the development of a number of other chronic diseases, most notably type 2 diabetes. The link between blood flow and insulin is well-established, with insulin often referred to as a vasodilatory hormone due to its known effect of increasing blood flow by binding to receptors on the vascular endothelium (Keske *et al.*, 2016). Similarly, the common association between endothelial dysfunction and insulin resistance has led to the proposal that endothelial dysfunction may be an important factor in the development of insulin resistance (Clark *et al.*, 2003). Julius *et al.* proposed a hemodynamic link between these factors which suggested that a decreased skeletal muscle blood supply would subsequently reduce 'nutritional flow', i.e. the blood flow in intimate contact with myocytes, resulting in insulin resistance in skeletal muscle (Julius *et al.*, 1991). Given that insulin is suggested to enhance glucose uptake in 3 ways; i) microvascular recruitment, ii) trans-endothelial transport, and iii) sarcolemmal transport (Keske *et al.*, 2016), reduced LBF may act to, or be representative of, reduced microvascular recruitment leading to decreased peripheral glucose uptake and insulin resistance (Phillips *et al.*, 2012). As such, improving LBF may reduce the risk of developing insulin resistance and subsequently T2DM in middle-aged and older adults (Dinenno *et al.*, 2001) although this may be contingent on the translation of increased limb blood flow to microvascular blood flow. As well as facilitating the delivery of oxygen and nutrients to muscle tissue, limb blood flow also reflects capacity to clear waste metabolites, free radicals and in particular atherogenic lipids which have all been shown to be involved in the development of cardiovascular and metabolic diseases such as MetS (Dinenno *et al.*, 2001; Goodwill and Frisbee, 2012). Finally, sarcopenia is not only associated with a loss of muscle mass and function, but also a loss of muscle quality. This loss of quality is associated with an increase in extra and intra-myocellular lipid deposition which has been linked to the development of insulin resistance (Wilkinson, Piasecki and Atherton, 2018), and may also be a consequence of reduced blood flow resulting in reduced lipid clearance.

Continuing to focus on sarcopenia, nutrition is a major determinant of MPS, with nutrition and contractile activity, i.e. exercise, the primary anabolic stimuli for skeletal muscle. The anabolic effects of nutrition are principally driven by the transfer and incorporation of amino acids captured from dietary protein sources into skeletal muscle proteins (Atherton and Smith, 2012). However, this rise is only transient, lasting around 1.5 hours (Atherton and Smith, 2012) when, even in the presence of available amino acids, returns to basal levels (Breen and Phillips, 2011). In addition, it is well established that when compared to younger individuals, older adults exhibit reduced MPS responses to both exercise (Kumar, Selby, *et al.*, 2009) and feeding (Cuthbertson *et al.*, 2005; Wall *et al.*, 2015), even in response to

supplementary amino acid ingestion (Cuthbertson *et al.*, 2005; Katsanos *et al.*, 2005); a concept referred to as 'anabolic resistance' (Burd, Gorissen and van Loon, 2013; Wall *et al.*, 2015). This concept is likely a key physiological mechanism underpinning sarcopenia, however the underlying mechanisms of this resistance are not yet fully understood (Breen and Phillips, 2011). One potential mechanism behind anabolic resistance is a reduced vasodilatory response to insulin older adults (Meneilly *et al.*, 1995), with this reduction observed independent of insulin resistance (Skilton *et al.*, 2005). Supporting the potential for this reduced vascular compliance to have a role in reduced anabolic responses, Timmerman *et al.* reported improvements in MPS following restoration of nitric oxide (NO) dependent vasodilatation with sodium nitroprusside infusion in combination with insulin, suggesting enhancing muscle perfusion improves amino acid availability (Timmerman *et al.*, 2010). This link is not however supported by others. In a collection of studies, Phillips and colleagues reported that enhanced (microvascular) blood flow responses to nutrition via RET (Phillips *et al.*, 2012) or cocoa flavanols (Phillips *et al.*, 2016) were not associated with increased anabolic responses, with these findings reported across both young and older adults. Despite these somewhat equivocal findings, age-related decreases in LBF, and subsequent reduction in oxygen and nutrient delivery, is still hypothesized by some to be a major factor in the aetiology of sarcopenia (Mitchell *et al.*, 2013), as the dynamic equilibrium of protein turnover is disturbed in favour of negative balance, leading to muscle wasting (Dinenno *et al.*, 2001).

Beyond providing energy, and the aforementioned role of amino acids for stimulating anabolic responses, a wealth of evidence exists showing the protective effects of regular consumption of certain food types, mainly fruits and vegetables, against common medical conditions, including coronary heart disease (Joshipura *et al.*, 2001), obesity (Slavin and Lloyd, 2012) and hypertension (Slavin and Lloyd, 2012). Based on this, natural products derived from these health-promoting foods known as "nutraceuticals" have in the last decade received extensive attention from both health professionals and the population as a whole (Zhao, 2007; Nasri *et al.*, 2014). A broader, detailed insight into these products is available in section 1.11 of this thesis. By definition, a nutraceutical is a substance derived from natural or bioactive phytochemicals which not only supplements the diet for nutrition but has medicinal properties for promoting health and even preventing disease (Kalra, 2003; Zhao, 2007; Nasri *et al.*, 2014). The best-known examples of nutraceuticals are the omega-3 fatty acids found in fish oils which have been shown to have excellent cardiovascular protective properties (Massaro *et al.*, 2010), and the plant sterols and stanols used to lower cholesterol, often commercially available in the form of yoghurt-based drinks (Berger, Jones and Abumweis, 2004). In relation to the nutraceutical compounds explored in this chapter, a large number of pre-clinical studies support various plant polyphenol-enriched extracts for

improving vascular parameters such as endothelial function (Massaro *et al.*, 2010) and reducing platelet aggregation (Bahadoran, Mirmiran and Azizi, 2013).

However, few human studies have assessed the effects of these compounds on aspects of vascular function in relation to feeding responses. Of the human studies that have been conducted, cocoa flavanols (CF) compared to our other two compounds of green tea extract (GTE) and curcumin (CU) (all compounds have been outlined in detail in thesis section 1.13) have been the most widely studied in relation to vascular function. Despite this, the majority of human work based on CF and vascular function has focussed on central cardiac function (De Palma *et al.*, 2016), brain blood flow/cognition (Grassi *et al.*, 2016) and basal (rested/fasted) blood vessel stiffness, with little work assessing the impact of cocoa on peripheral vascular aspects in response to nutrition (Phillips *et al.*, 2016). To our knowledge, none have explored the impact of this compound on vascular and subsequent metabolic responses associated with human health, i.e. postprandial glucose and insulin profiles. Similarly, the limited human studies using GTE and CU have primarily focussed on CVD risks (Peters, Poole and Arab, 2001) such as hypertension and dyslipidaemia (Tenore *et al.*, 2015), endothelial dysfunction (Nakayama *et al.*, 2014), and insulin resistance (Brown *et al.*, 2009), with no insight into their effects on peripheral vascular and metabolic responses to nutrition. There is considerable scientific evidence which suggests that the regular consumption of CF may improve vascular health and reduce risk of cardiovascular disease (Erdman Jr *et al.*, 2008), improve cognitive function (Grassi *et al.*, 2016) and reduce arterial stiffness (West *et al.*, 2014).

Epigallocatechin 3-gallate (EGCG) is the most abundant and potent green tea catechin and has been extensively studied for its beneficial health effects as a nutraceutical agent (Kim, Quon and Kim, 2014) and may be effective in decreasing blood cholesterol levels (Tenore *et al.*, 2015) and reducing the progression of existing atherosclerotic lesions (Chyu *et al.*, 2004). Finally, curcumin is a phenolic compound present in the rhizomes of turmeric which has been used by Asian communities for over 300 years and has long been theorised to possesses antibacterial (Moghadamtousi *et al.*, 2014), cardiovascular protective, anti-inflammatory (Gupte *et al.*, 2019) and antioxidant properties (Srivastava and Mehta, 2009).

The molecular and cellular mechanisms underlying the impact of organic polyphenols on cardiovascular outcomes, although not fully understood, are thought to involve signalling pathways that stimulate NO production (Hodgson and Croft, 2010) with resulting vasodilation and microvascular recruitment, whilst inhibiting serotonin mediated vasoconstriction, therefore improving muscle nutrient and oxygen delivery (Keske *et al.*, 2015).

In relation to the metabolic responses to nutrition that may be improved by our nutraceuticals, and/ or related to their ability to improve blood flow responses to nutrition, it must be considered that blood is the primary carrier of metabolites including a variety of nutrients (carbohydrates, lipids and amino acids), hormones, electrolytes, metabolic by-products and organic wastes (Ivanisevic *et al.*, 2015). Indeed, the concept of arterial blood supplying tissues with nutrients and oxygen, and venous blood carrying the metabolic by-products and carbon dioxide from tissues is a well-established concept and arterio-venous balance (AVB) is one of the best examples of metabolite transport *in vivo (Ivanisevic et al., 2015)*. In simple terms, if the difference is positive, there is a net uptake; and if it is negative, there is a net release. By simultaneously measuring the blood flow rate into a limb, as well as the AVB of a metabolite, the net uptake can be calculated (Guo and Jensen, 2013) using the Fick principle, which states that uptake is equal to the product of AVB and LBF (Clark *et al.*, 2003).

In summary, the numerous health benefits of regular exercise on cardiovascular and muscle health have been extensively reported. For example, previously our research group has shown that resistance exercise training can restore age-related reductions in limb blood flow responses to feeding (Phillips *et al.*, 2012). However, exercise prescription is not always appropriate for the very elderly and/ or those with comorbidity, and it is this demographic which needs to be protected from the numerous negative effects of sarcopenia. Despite the many benefits of plant-derived polyphenols on aspects of human health, little has been done to explore the effects of these compounds on vascular responses to nutrition (Phillips *et al.*, 2016). Whilst unlikely to provide the same benefits for maintaining muscle mass in the elderly, polyphenols may be a useful tool in combatting the negative effects of sarcopenia. In this randomised control trial, we shall explore the effects of CF, GTE and CU (all plant polyphenol-enriched extracts) for improving limb blood flow and glucose uptake responses to nutrition in older adults.

6.3 Experimental methods

6.3.1 Ethical approval

Ethical approval was applied for from the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee prior to any subjects being recruited into the study (2-1704). This included ethical approval for the use of ionising radiation in the form of dual-energy x-ray absorptiometry (DXA). The study was also prospectively registered with clinicaltrials.gov, identifier NCT number: NCT03213340. Full details of the ethical approval and amendments can be seen in Appendix 7.

6.3.2 Recruitment

Participants were recruited through a variety of methods. Firstly, the study was advertised via a poster in local community clubs as well as the local newspaper. Two volunteer banks were also targeted for recruitment; the Royal Derby Hospital Trust Voluntary Services Team and the University of Nottingham Clinical Physiology Volunteer Database of past participants who had consented to having their personal details stored on a secure database for the purpose of being contacted for future studies. Local societies and charity groups were also contacted with recruitment presentations given at their respective monthly meetings.

6.3.3 Inclusion criteria

The inclusion criteria for this study were:

- Male or female
- Age 65 and over
- Full capacity to consent

6.3.4 Exclusion criteria

The exclusion criteria for this study were:

- Participation in a formal exercise regime (more than once a week) or other routine strenuous physical activity
- BMI <18 or >35 kg/m^2
- Active cardiovascular disease:

    - Uncontrolled hypertension (BP > 160/100)
    - Angina
    - Heart failure (class III/IV)
    - Significant cardiac 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
    - Diabetic patients needing medication
    - Uncontrolled thyroid disease
    - Cushing's Disease
    - Hyper and hypo parathyroidism
- Active malignancy
- Clotting dysfunction
- History of DVT
- Significant musculoskeletal or neurological disorders
- Family history of early (<55y) death from cardiovascular disease
- Participation in a research experiment within the last 3 months
- Surgery within past 3 months
- Smokers
- Active inflammatory bowel disease or renal disease
- Known allergy or intolerance to any of the study ingredients
- Subjects regularly taking over the counter supplements containing green tea extracts, curcumin or cocoa flavanols

6.3.5 Screening visit and consent

Participants who displayed an interest in study participation were emailed the study participant information sheet (Appendix 8). Having had sufficient time (minimum 48h) to fully evaluate this information, they were then invited for a screening visit at the University of Nottingham Royal Derby Hospital Centre. Participants were asked to fast from midnight to ensure fasting blood tests could be taken as described later in this section. They were also asked to refrain from drinking alcohol 24 hours before this visit. Where possible, to ensure participants were not involved in a formal exercise training regime (an absolute exclusion criterion), the International Physical Activity Questionnaire-Short Form (IPAQ-SF) was completed as a screening tool (P. H. Lee *et al.*, 2011) via telephone before the screening visit. If this was not possible, it was completed at the screening visit.

During the screening visit, participants were first given a thorough explanation of the study. Having had the opportunity to ask any questions, participants were then asked to provide written informed consent to participate (Appendix 9). Participants were then assigned a linked-anonymised recruitment code. The screening visit then included a detailed medical history from the participant, including allergies. Following this, a full medical examination of abdomen, upper and lower limbs was performed. Anthropometric measures of height and weight were taken to calculate the participant's BMI. Having sat for five minutes, blood pressure (BP) was measured from the left arm where possible (Beaney *et al.*, 2018) using an automated sphygmomanometer. Three readings were taken one minute apart (Beaney *et al.*, 2018) and the mean systolic and diastolic BP recorded. A resting 12-lead electrocardiogram (ECG) (CardioDirect 12, Spacelabs Healthcare, Washington, USA) was then performed to rule out pre-existing cardiac rhythm abnormalities. Participants with abnormal BP readings, ECG indications of cardiovascular disease, or a BMI outside the exclusion criteria were excluded at this stage. Finally, baseline blood tests were performed for measurement of full blood count (FBC), urea and electrolytes (UEs), liver function tests (LFTs), lipid profile, thyroid function tests (TFTs), fasting glucose, glycated haemoglobin (HbA1c) and clotting to rule out any contraindications for inclusion in the study as per the exclusion criteria outlined above. Any abnormal findings were discussed with the participant and with their GP via a letter recommending for further investigation and follow up.

Upon satisfying the criteria above, participants were asked to complete baseline assessments of physical function at the Clinical Physiology Exercise Suite at the University of Nottingham Royal Derby Hospital Centre. First, hand grip strength (HGS), an accepted biomarker of overall strength, health status and predictor of multimorbidity and disease-

specific mortality (Bohannon, 2019) was performed for both the dominant and non-dominant hand. Whilst seated, the forearm of the tested hand was positioned parallel with the ground creating a 90 degree angle between forearm and upper arm and three measurements of maximal voluntary contraction were taken using an isometric dynamometer (Camry EH101, Zhongshan Camry Electronic Co. Ltd, Guangdong, China) with one minute of rest between each trial. The highest recorded reading for each hand was used in analysis (Gale *et al.*, 2007).

Next participants were asked to perform the Short Physical Performance Battery Tests (SPPBT), namely standing balance (tandem, semi-tandem and parallel feet), walking speed (4m) and chair stand time (five times, with arms crossed over the chest), which are well established tools to assess lower extremity physical performance status and overall functional capability, providing a measure of the biological age of an older individual (Pavasini *et al.*, 2016). Finally participants were asked to perform the “timed up-and-go” (TUG) test, a validated test of functional performance and screening tool for risk of falls, with a faster time (for rising from a chair walking six meters and returning to sit in the chair) indicating a better functional performance (Barry *et al.*, 2014).

6.3.6 Acute study day

Having fulfilled all the necessary criteria for inclusion, participants were asked to attend for their first acute study day, Visit 1 (V1). The same fasting requirements as for the screening visit (from midnight) were observed for V1. Participants were asked to refrain from taking any medications that may impact blood flow on the day prior to and on the day of testing where safe to do so, these included ACE inhibitors, calcium channel blockers, Sildenafil and decongestants such as ephedrine and Sudafed. Participants were also asked to refrain from any exercise and from drinking alcohol for 48 hours prior to V1. Medications and allergies were confirmed prior to commencement of any measurements on V1.

6.3.6.1 Randomisation and blinding

This was a randomised controlled, single blinded crossover trial with participants randomised to either treatment or placebo for V1 with participants blinded to which treatment they were receiving. Randomisation was facilitated using sealedenvelope.com (Sealed Envelope Ltd., London UK).

6.3.6.2 Protocol overview

The overall protocol for the study can be seen in Figure 6.2.

Time (Mins)												
	-120	-60	0	15	30	60	90	120	150	180	210	240
	Fasted	Test/ Placebo**	Meal				Fed					
DXA	X*											
Line insertion	X											
LBF	X				X	X	X	X	X	X	X	X
AVB	X			X	X	X	X	X	X	X	X	X

*Figure 6.2: Study overview. *=V1 only, **=Cocoa 30min prior to meal*

In brief, participants attended the research unit at ~0800h. After current medications and allergies were confirmed to be as per the screening visit, participants had whole-body composition assessed by a dual-energy x-ray absorptiometry (DXA) scan. Participants were then taken to the clinical investigation suite of the research unit where they were positioned supine on a clinical bed, in which they remained for the duration of their study visit. To facilitate measures of A-V balance for glucose and assessment of plasma insulin, participants first had a cannula inserted into a vein on the back of their right hand. A second cannula was then inserted into the femoral artery of the right leg. Further detail on these procedures is provided in the sections below.

As can be seen in the schematic above, the protocol was identical for all V1 visits, apart from one exception. In the cocoa groups (treatment and placebo), their experimental supplement was provided 30 minutes before the liquid meal compared to 60 minutes in the green tea and curcumin groups. In addition, the DXA scan was only performed at the start of V1 (not V2).

6.3.6.3 Retest day

All participants that successfully completed V1 were asked to observe a wash-out period of between 7 and 14 days before retest at V2. As outlined above, the DXA scan was not

repeated at V2. Participants received the alternative treatment to V1 (supplement or placebo) on V2.

6.3.6.4 DXA

Participants had their body composition measured using a whole-body DXA scan (Lunar Prodigy 2, GE Medical Systems, Buckinghamshire, UK). This facilitated characterisation of the participants across the groups via measurement of whole-body mass, total fat mass (TF), body fat percentage (FM%) and lean muscle mass (LMM). In line with University of Nottingham policy, all DXA scans were performed by an Ionising Radiation Medical Exposure Regulations (IR(ME)-R) trained operator. Participants were asked to void their bladder immediately prior to scanning to prevent distortion of the images from excess urine and were provided with a clinical gown to wear to ensure no metal or plastic items were about their person. Immediately prior to scanning, participants were again fully informed of and verbally consented to the minimal radiation exposure, amounting for less than two days of natural background radiation (NBR) during the scan, and for a comparison, a normal chest x-ray amounts to three days (National Health Service (NHS), 2019a). Measurements were calculated using the integrated DXA software as detailed in section 4.3.8.

6.3.6.5 Peripheral line insertion

A 22-gauge intravenous cannula (BD Venflon™ Pro, Becton Dickson, New Jersey, USA) was inserted in a retrograde position into a vein on the dorsum of the hand. The cannula was connected to a 3-way tap to facilitate repeated blood sampling and a slow continuous infusion of 0.9% sodium chloride (Baxter International Inc., Illinois, USA) was attached to maintain line patency and to allow periodic flushing of the cannula after blood sampling to prevent the line from clotting. The cannulated hand was then placed into a hand warming box and heated to 55°C to allow arterialisation of the venous blood (Liu *et al.*, 1992). A baseline sample of 4 mL of blood was taken after the cannulated hand had been in the hotbox for a minimum of 15 min, with subsequent sampling times as per the schematic outlined above.

6.3.6.6 Central line insertion

To facilitate measurement of arterio-venous (AV) balance (in combination with the hot-hand sampling outlined above), a femoral venous catheter was inserted using a standard operating procedure in the research unit. Verbal consent was first obtained from the

participant and the possible complications again discussed as per the original consent process. The right leg was abducted at the hip and the knee flexed slightly to allow exposure of the groin. Under ultrasound (US) guidance (iU22, Philips Healthcare, Guildford, UK) the course of the common femoral vein (CFV) and the surrounding structures was mapped (Figure 6.3) and marked using permanent marker. Under sterile conditions, the groin was then prepped with 0.5% Chlorhexidine Gluconate solution (Ecolab Inc., Minnesota, US) ensure aseptic conditions were adhered to.



Figure 6.3: Ultrasound mapping of the common femoral vein. SV, Saphenous vein; CFV, Common Femoral Vein; CFA, Common Femoral Artery

Whilst the leg was drying, the venous cannula and introducer (Leadercath, Vygon, Ecouen, France) were primed by flushing with 0.9% sodium chloride solution. The end of the US probe was covered in probe gel and in an aseptic manner, the probe cover was applied and secured with the provided sterile elastic bands. Once dry, sterile US gel was applied to the groin. The groin was reimaged and the anatomical landmarks confirmed using the marks drawn prior to prepping. Under US guidance, up to 5ml of 1% lidnocaine (B. Braun Medical Ltd, Melsungen, Germany) was infiltrated under the skin at the proposed puncture site and into the tissues surrounding the common femoral vein and given approximately one minute to take effect. 5ml of 0.9% sodium chloride solution was drawn up into a syringe and the introducer loaded onto the syringe and primed with 1ml of solution. After testing the effects of the anaesthetic block, the skin was pierced with the introducer and under US guidance the vein was punctured, with entry confirmed by being able to easily draw back venous blood into the syringe. Keeping the needle in place, the syringe was removed from the

introducer and the Seldinger introducer wire (Leadercath, Vygon, Ecouen, France) was passed through the introducer ensuring a smooth, easy advancement. Where there was resistance, the leg was repositioned or the wire gently rotated and where there was heavy resistance the procedure was abandoned. Once the wire was advanced, the introducer was removed over the wire leaving the wire in the CFV. The primed venous catheter was then passed over the wire under US guidance and advanced to 10-15 cm. The wire was removed from the catheter leaving the line in situ. The line was connected to a primed 3-way tap and a slow continuous infusion of 0.9% sodium chloride (Baxter International Inc., Illinois, USA) was then attached to maintain line patency and to allow periodic flushing of the cannula after blood sampling to prevent the line from clotting. The line was secured to the skin using a transparent sterile dressing.

At the end of the study day the line was removed by gently withdrawing the femoral cannula and pressing down firmly over the puncture site with sterile gauze for a minimum of 10 minutes. Participants were giving written instructions on how to care for puncture sites and what to do in case of spontaneous bleeding when at home.

6.3.6.7 Arterio-venous balance

Blood was drawn from the retrograde cannula simultaneously with blood from the femoral venous line by two investigators to allow for measurement of AV balance. 3ml of blood was drawn from each line to expel blood which had been diluted by the continuous 0.9% sodium chloride infusion and discarded. A 4ml sample was then collected for analysis, after which the lines were flushed with 5ml of 0.9% sodium chloride and the slow continuous infusion restarted. Blood glucose concentrations were measured immediately from both samples with a near-patient glucose analyser (YSI Life Sciences, Ohio, USA). The remaining sample was placed on ice and after centrifugation for 20 minutes at 4°C, the plasma was aliquoted and frozen at -80°C for future analysis of insulin. Samples were taken at 15, 35, 65, 95, 125, 155, 185, 215 and 245 minutes after feeding.

6.3.6.8 Leg blood flow

Macrovascular blood flow was measured using doppler US (iU22, Philips Healthcare, Guildford, UK) of the contralateral femoral artery to the cannulated femoral vein. Participants were laid supine to measure mean blood velocity and arterial lumen diameter. Measurements were made 2-3cm proximal to the bifurcation of the femoral artery to minimize the effect of turbulence (Phillips *et al.*, 2012) and the isonation angle kept at less

than 60° in order to minimize the error that may be induced in the velocity flow estimate (Casey, Curry and Joyner, 2008). The integrated software then calculated LBF as a product of the artery cross sectional area and the mean blood velocity over a minimum of 3 cardiac cycles (Phillips *et al.*, 2012) (Figure 6.4). A mean value from three measurements taken at each time point (in litres per minute (cc/min x 1000)) was used for analysis (Phillips *et al.*, 2012). Measurements were taken at baseline with the probe position marked, and then at 25, 55, 85, 115, 145, 175, 205 and 235 minutes after feeding using the same probe reference mark and technique.

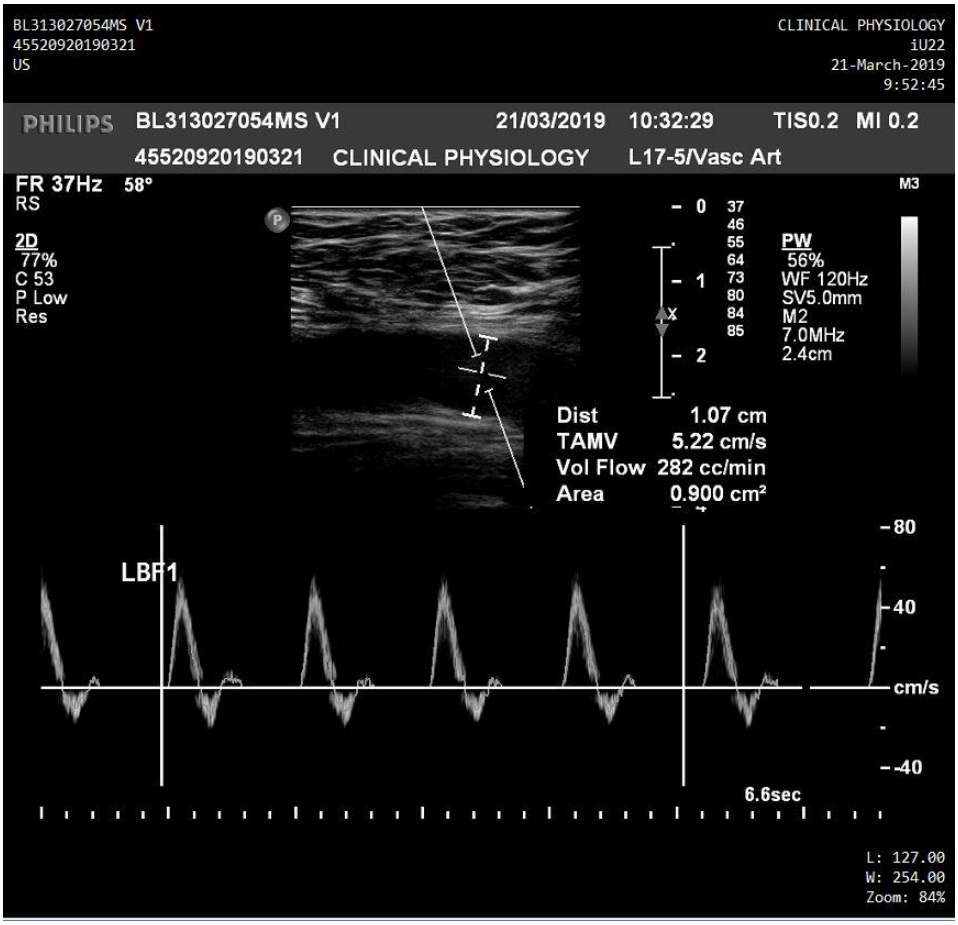


Figure 6.4: Doppler ultrasound image of femoral artery blood flow. Dist, Distance between the two callipers; TAMV, Time averaged mean velocity; Vol Flow, Calculated blood volume flow; PW, pulse wave ultrasound (settings).

6.3.6.9 Glucose uptake

Limb glucose uptake was calculated by using the Fick principle, which states that glucose uptake is equal to the product of AVB and LBF (Clark *et al.*, 2003). By this principle, increasing flow or cellular permeability to glucose will increase glucose uptake.

6.3.6.10 Measurement of plasma insulin

Plasma insulin concentrations were measured using an ultrasensitive enzyme linked immunosorbent assay (ELISA) (Mercodia AB, Uppsala, Sweden), according to manufacturer's instructions. Samples were thawed at room temperature and then vortexed to ensure thorough mixing of defrosted samples. Samples 2 to 5 post-feeding were diluted 1:8 using the ELISA kit "Calibrator 0" (Mercodia AB, Uppsala, Sweden) to ensure that the measured concentration fell within the reference range of the assay, all other samples were not diluted.

In brief, 25μL of plasma and calibrator solutions were pipetted into the plate wells before enzyme conjugate solution was added to each well and the plate was incubated on a plate shaker for 1 hour at room temperature. The plate was then manually washed six times with pre-prepared wash buffer solution. Substrate TMB was then added to each well and the plate incubated for 30 minutes at room temperature. Stop solution was then added and the plate mixed on a shaker for approximately 5 seconds to ensure mixing of the substrate complex and stop solution. Finally, the fully prepared plates were read at 450nm using an optical plate reader (Thermo Scientific Multiskan Ascent, ThermoFisher Scientific, USA) to allow calculation of the insulin concentrations against the calibrator concentration curve.

6.3.6.11 Study treatments

On each study visit, once all baseline measures had been taken, participants were asked to ingest the study treatment or placebo dependant on which study day they were randomised to (Table 6.1).

Cohort	Study Treatment	Description	Administration
1A	Control	Chocolate chips 0mg flavanoids	33g orally 30min before meal
1B	Experimental	Chocolate chips ~500mg flavanoids	33g orally 30min before meal
2A	Control	Placebo capsules 0mg EGCg	4 capsules orally with water 60min before meal
2B	Experimental	Green tea extract ~500mg EGCg	4 capsules orally with water 60min before meal
3A	Control	Placebo capsules 0mg curcumin	2 capsules orally with water 60min before meal
3B	Experimental	Formulate curcumin ~500mg curcumin	2 capsules orally with water 60min before meal

Table 6.1: Study treatment administration

The required dose of cocoa flavonoid was 500mg and this equated to 33g of the cocoa flavonoid chips (Abbott Nutrition, Columbus, Ohio, USA), ingested 30min before administration of the liquid meal. The placebo cocoa chips contained no cocoa flavonoids, but were matched for appearance, taste and volume.

Green tea extract containing 500mg of EGCg was ingested as decaffeinated capsules (SunPhenon®, Taiyo International Inc., Minneapolis, Minnesota, USA) 60min before administration of the liquid meal. Again, the green tea placebo capsules were outwardly matched.

500mg of formulate curcumin was ingested as capsules (Verdure Sciences®, Noblesville, Indianapolis, USA) also 60min before administration of the liquid meal, with an outwardly matched placebo capsule for the group also (Capusgel®, Greenwood, South Carolina, USA).

The difference in timing between the cocoa trials and other two supplements was based on the formulation of the supplements. Green tea and curcumin, being given in capsule form, arguably needed a longer time to be broken down compared to cocoa in chocolate chip form. As a between-group analysis was not one of the primary aims of the study, this did not negatively impact the study design.

Having observed the required time after ingestion of the treatment (as per Table 1), participants were given a liquid feed in the form of four ounces 120ml of Ensure® Plus (Abbott Nutrition, Columbus, Ohio, USA) with only allowed water *ab libitum* for the remainder of the study. The liquid feed represents a commonly used oral nutritional supplementation (ONS) in clinical and care settings. In addition, the provision of a mixed-nutrient liquid meal (versus, for example an isolated protein supplement), offers more pragmatic insight into the potential role of the tested nutraceuticals on responses to feeding, as most individuals consume meals of combined foods.

6.3.6.12 Data analysis

Calculated glucose and insulin levels were plotted against the 15-minute time intervals and concentrations for the area under the curve (AUC) were subsequently calculated.

6.3.7 Statistical Analysis

6.3.7.1 Power Calculation

The sample size for this study was not intended to provide efficacy analysis, but rather to assess trends that could be studied in a subsequent larger study. Based on previous work done by our research team assessing changes in vascular perfusion following cocoa supplementation in response to intravenous amino acid feeding (Phillips *et al.*, 2016), it was calculated that 12 participants per cohort would be required to highlight any trends for supplement-induced vascular changes in response to feeding.

6.3.7.2 Group analysis

As this was a RCT, significance testing of baseline differences was not performed as per the CONSORT 2010 statement (de Boer *et al.*, 2015). Effects are reported as number (%), mean (SD) or +/- mean difference (MD) (SD). Two-way repeated measures ANOVA with Sidak's post hoc analysis was used to compare condition differences over time. Analysis for product versus placebo intervention change was performed using repeated measures t-tests. The level of significance was set at $p<0.05$. All analyses were performed using GraphPad Prism for Mac OS X Version 7.0d (GraphPad Software, La Jolla, San Diego, California USA).

6.4 Results

6.4.1 Participant demographics

Thirty participants aged age 71 (5) years, of which 14(47%) were male, were recruited to this study. Demographics can be seen in Table 6.2.

Group	Mean Age (years)	Male / Female	BMI (kg/m^2)
Cocoa (n=12)	72 (4)	6/6	27 (3)
Green Tea (n=12)	74 (4)	5/7	26 (2)
Curcumin (n=12)	73 (3)	6/6	25 (3)

Table 6.2: Participant demographics

6.4.2 Study adherence

The study Consort diagram can be seen in Figure 6.5. In total, 64 healthy volunteers were screened for this study. 25 were excluded before randomisation: 23 not meeting the study criteria, 1 deciding not to progress to V1, and 1 excluded on the basis of abnormal blood tests who was referred to their general practitioner. 39 participants therefore went on to randomisation.

One participant was found to have an elevated SBP at V1 and was therefore excluded. Two participants declined to progress to V2 leaving 36 participants who completed the study, 12 in each group (Figure 6.5).

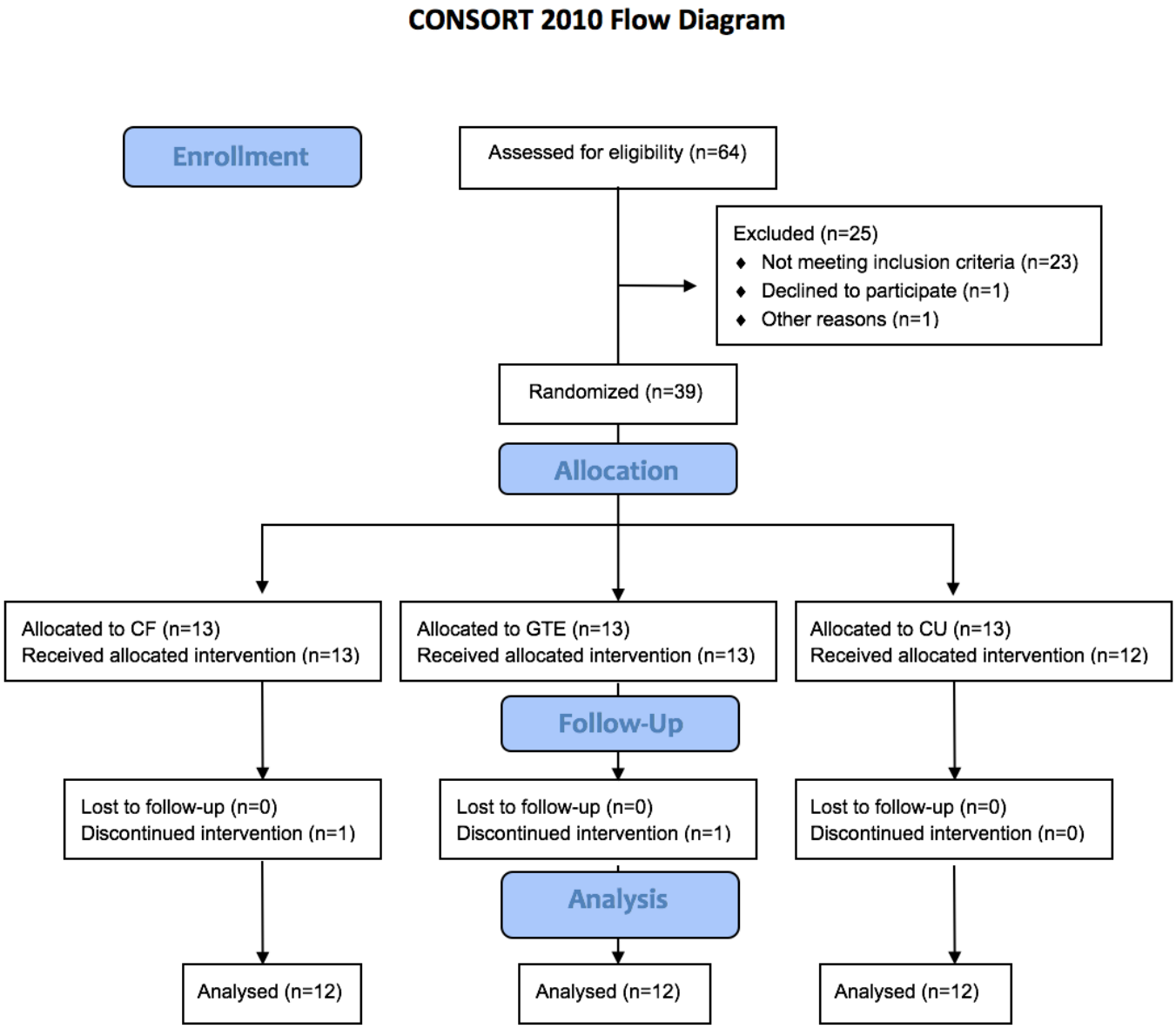


Figure 6.5: Study Consort diagram

6.5 Results - Cocoa

6.5.1 Leg blood flow

There was no significant change in LBF AUC post CF supplementation (Table 6.3, Figure 6.6).

	CF	Placebo	Change	*p*
LBF (ml/min)	85505 (27151)	77725 (21778)	7780 (18442)	0.2

Table 6.3: Leg blood flow AUC following cocoa supplementation or placebo with a mixed macronutrient liquid feed

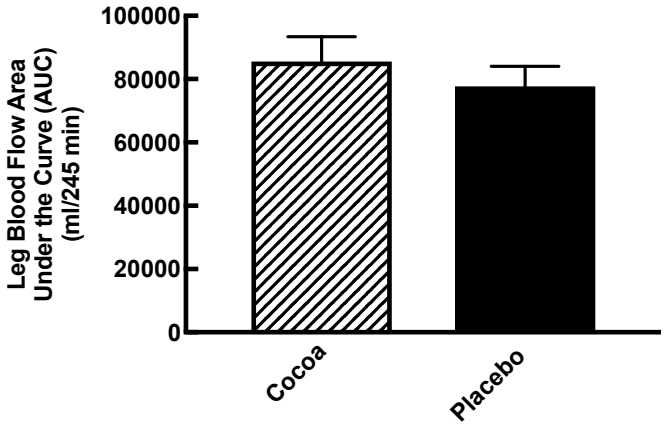


Figure 6.6: Leg blood flow AUC following cocoa supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

6.5.2 Glucose and insulin concentrations

The increase in arterial (A) (Figure 6.7) and venous (V) (Figure 6.8) glucose following cocoa supplementation was significantly lower at 15 (A: MD -0.88 (0.3) mmol; $p<0.01$, V: MD -0.8 (0.3) mmol; $p<0.01$) and 35 (A: MD -0.67 (0.3) mmol; $p<0.05$, V: MD -0.65 (0.3) mmol; $p<0.05$) minutes post feeding.

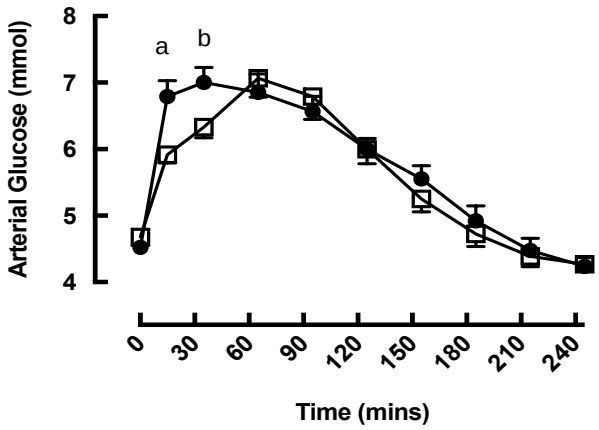


Figure 6.7: Arterial blood glucose concentrations at baseline and after a mixed-macronutrient liquid feed and cocoa (square) or placebo (circle) supplementation (given 30 min before feed). N=12. a = p<0.01, b = p<0.05

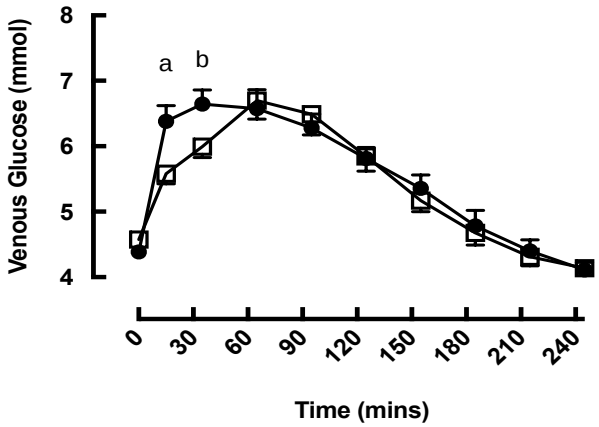


Figure 6.8: Venous glucose concentrations at baseline and after a mixed-macronutrient liquid feed and cocoa (square) or placebo (circle) supplementation (given 30 min before feed). N=12. a = p<0.01, b = p<0.05

There were no significant differences in postprandial area under the curve for arterial or venous blood glucose, AVB, glucose uptake or plasma insulin with cocoa or placebo supplementation (Figure 6.9). A composite of this data is summarised in Table 6.4.

	CF	Placebo	Change	*p*
Arterial Glucose (mmol)	1423 (71)	1388 (103)	35.3 (108)	0.3
Venous Glucose (mmol)	1340 (79)	1369 (79)	28.9 (105)	0.4
AV Balance (mmol)	52.3 (25)	61.6 (21)	-9.3 (24)	0.2
Glucose Uptake (mmol/ml)	16862 (7478)	18732 (7654)	-1871 (9136)	0.5
Insulin (uU/ml)	4340 (1313)	4585 (1420)	-245 (689)	0.2

Table 6.4: Glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following cocoa supplementation or placebo with a mixed macronutrient liquid feed

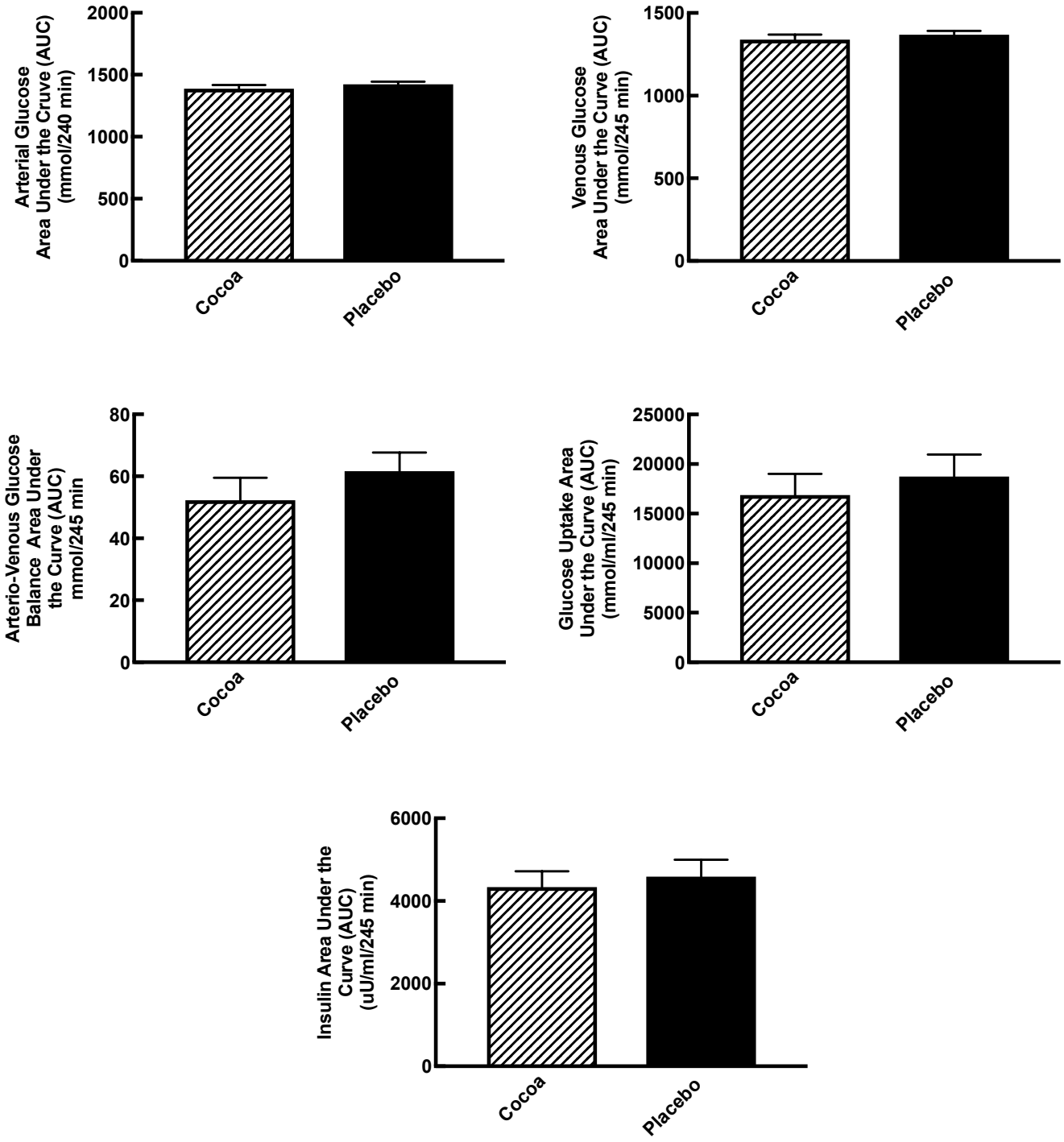


Figure 6.9: Arterial and venous glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following cocoa supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

6.6 Results - Green tea

There was no significant change in LBF AUC post GTE supplementation (Table 6.5, Figure 6.10).

	GTE	Placebo	Change	*p*
LBF (ml/min)	72717 (7893)	74968 (22305)	-2251 (22261)	0.7

Table 6.5: Leg blood flow AUC following green tea extract supplementation or placebo with a mixed macronutrient liquid feed

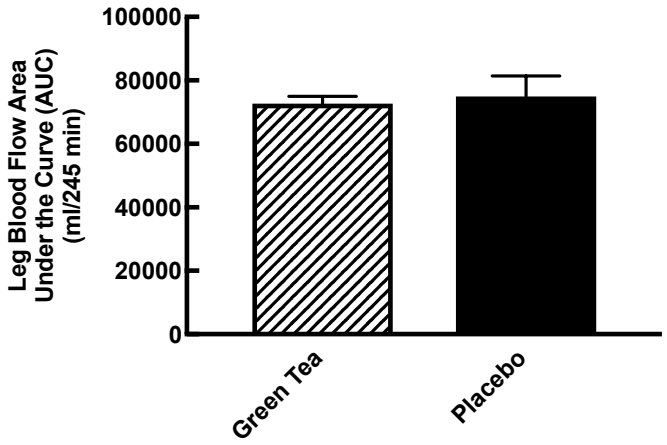


Figure 6.10: Leg blood flow AUC following green tea supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

There were no significant differences in postprandial area under the curve for arterial or venous blood glucose, AVB, glucose uptake or plasma insulin with GTE or placebo supplementation (Figure 6.11). A composite of this data is summarised in Table 6.6.

	GTE	Placebo	Change	*p*
Arterial Glucose (mmol)	1309 (86)	1275 (101)	34 (86)	0.2
Venous Glucose (mmol)	1261 (83)	1233 (91)	28 (84)	0.3
AV Balance (mmol)	58.2 (24)	48.3 (19)	9.9 (32)	0.3
Glucose Uptake (mmol/ml)	14838 (5823)	13758 (6005)	1080 (8650)	0.7
Insulin (uU/ml)	2732 (962)	2600 (918)	132 (697)	0.5

Table 6.6: Glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following green tea extract supplementation or placebo with a mixed macronutrient liquid feed

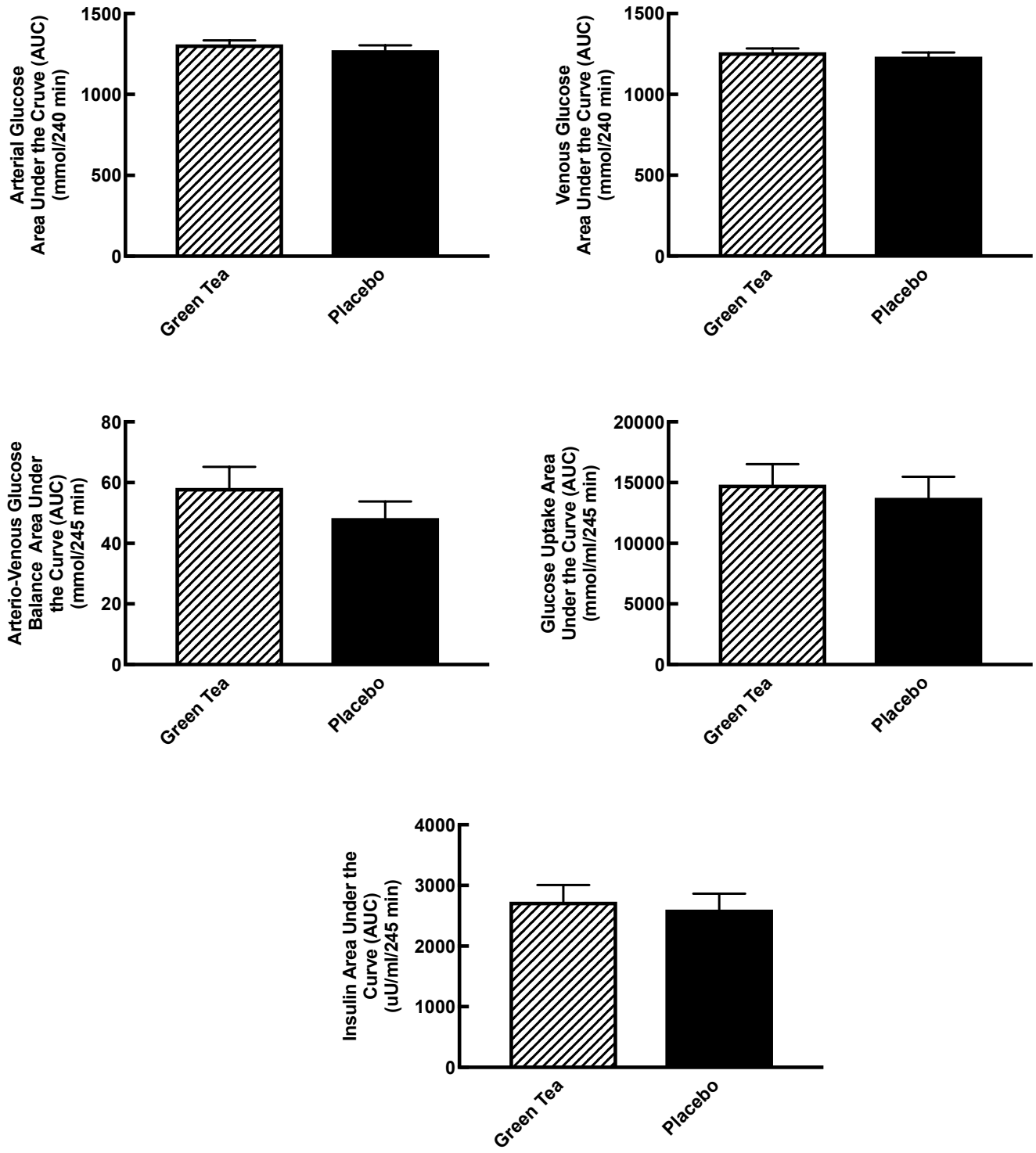


Figure 6.11: Arterial and venous glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following green tea extract supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

6.7 Results - Curcumin

There was no significant change in LBF AUC post CU supplementation (Table 6.7, Figure 6.12).

	CU	Placebo	Change	*p*
LBF (ml/min)	69219 (19190)	74293 (9342)	-5074 (20161)	0.4

Table 6.7: Leg blood flow AUC following curcumin supplementation or placebo with a mixed macronutrient liquid feed

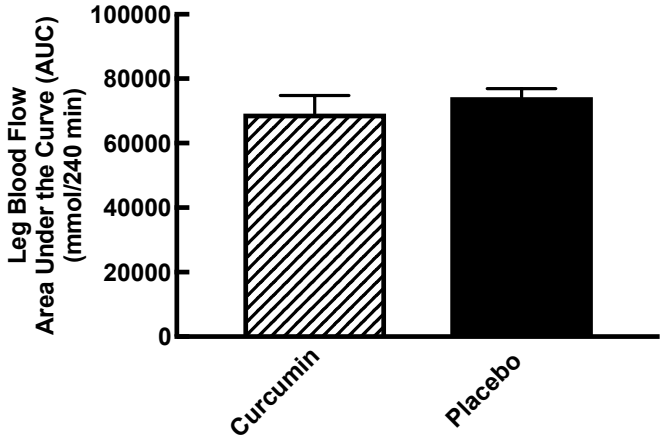


Figure 6.12: Leg blood flow AUC following curcumin supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

There were no significant differences in postprandial area under the curve for arterial or venous blood glucose, AVB, glucose uptake or plasma insulin with CU or placebo supplementation (Figure 6.13). A composite of this data is summarised in Table 6.8.

	CU	Placebo	Change	*p*
Arterial Glucose (mmol)	1263 (84)	1258 (81)	5 (53)	0.7
Venous Glucose (mmol)	1236 (86)	1223 (81)	13 (49)	0.4
AV Balance (mmol)	39.6 (13)	40.2 (12)	-0.6 (19)	0.9
Glucose Uptake (mmol/ml)	10520 (6036)	11974 (3440)	-1454 (7932)	0.5
Insulin (uU/ml)	4000 (1666)	3614 (1324)	386 (1118)	0.3

Table 6.8: Glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following curcumin supplementation or placebo with a mixed macronutrient liquid feed

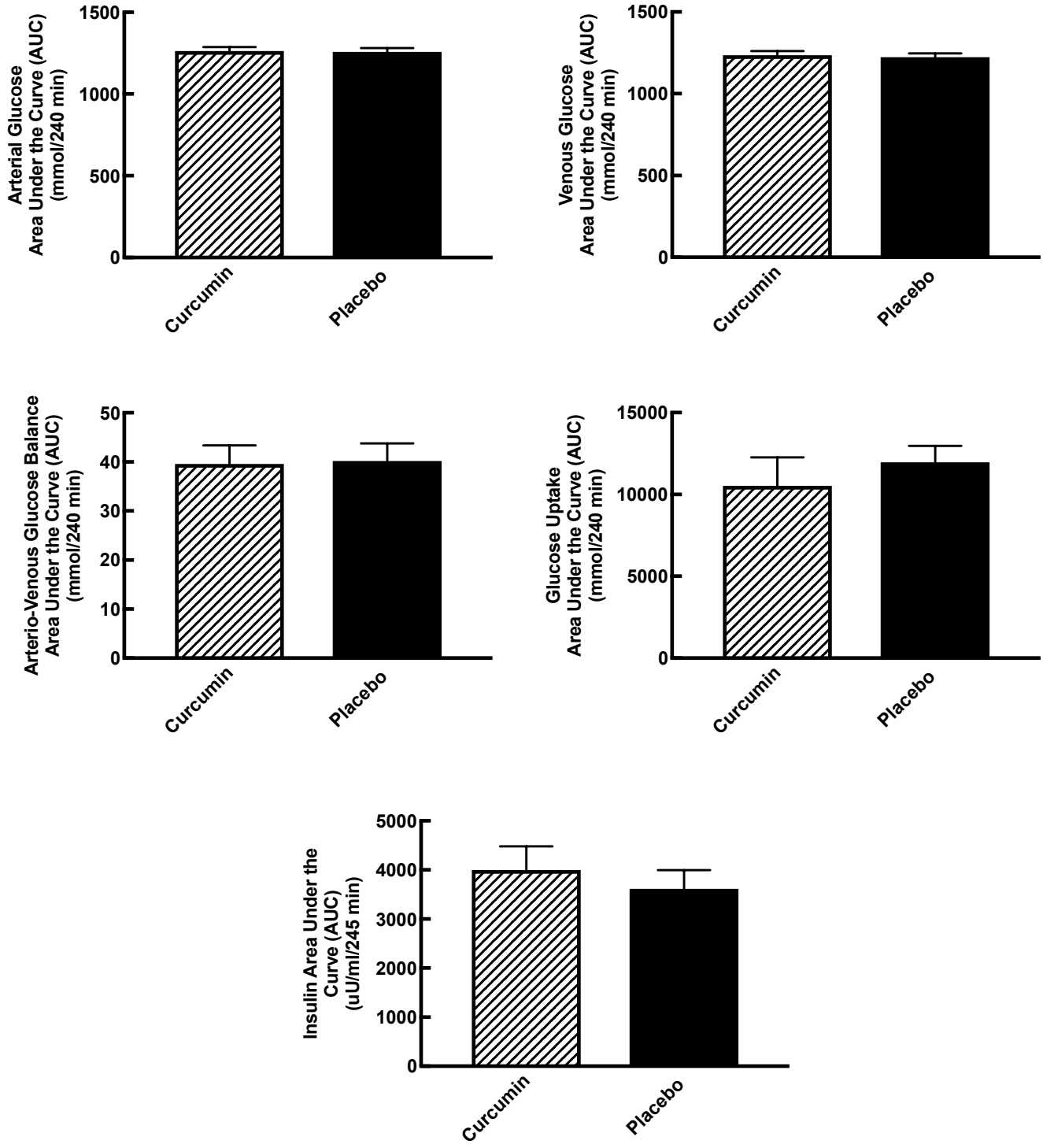


Figure 6.13: Arterial and venous glucose, arteriovenous balance, glucose uptake and insulin AUC (units/245mins) following curcumin supplementation (hatched) or placebo (black) with a mixed macronutrient liquid feed

6.8 Discussion

From the work presented in this chapter, we report that acute supplementation with cocoa, green tea extract nor curcumin improves muscle blood flow in response to oral feeding in older adults. In addition, we do not report improvements in arterio-venous balance, insulin concentration or glucose uptake, suggesting that nutrient delivery is also unchanged following acute supplementation with these compounds in response to oral feeding.

Cocoa flavanols

Whilst the exact mechanisms behind flavanol-mediated increases in blood flow are unknown (Corti *et al.*, 2009), CF have been shown to increase activation of endothelial NO, thereby inducing vasodilatation in blood vessels (Fisher *et al.*, 2003). When coupled with their antioxidant, anti-inflammatory and reduction in platelet aggregation effects, they promote improved vascular function and potentially reduce the effects of cardiovascular mortality (Marsh *et al.*, 2017). They have also previously been shown to reduce insulin resistance (Grassi *et al.*, 2012) and blood pressure (Corti *et al.*, 2009); both of which are associated with reduced CV disease risk. In this study we did not find any improvement in LBF responses to feeding following acute CF supplementation. This finding is in contrast to previous work by our research group showing that a similar dose of CF did result in improved LBF in older men in response to feeding, however this response did not result in improved MPS despite observed improvements in muscle microvascular blood flow also (Phillips *et al.*, 2016). It must however be noted, that the feeding regime in the previous study by Phillips *et al.* was a primed constant infusion of amino acids and dextrose solution (Phillips *et al.*, 2016) compared to the mixed macronutrient liquid feed used herein. In addition, the cocoa supplementation was given at the start of 'feeding' in the previous study, compared to our protocol of it being given 30 min prior. The oral feed was chosen in this study, as it was deemed more realistic in a "real-world", i.e. home or clinical, setting and therefore provides a more realistic assessment of the likelihood of success of the three compounds in improving LBF for older adults in the home setting. However, this feeding regime may be insufficient to mount a vascular response, even in combination with a supplement, in individuals who as discussed previously may already have blunted vascular responses to feeding (Burd, Gorissen and van Loon, 2013; Wall *et al.*, 2015).

As well as LBF, regular consumption of CF has been shown to improve cerebrovascular blood flow in older individuals, independent of feeding, which could be a significant finding in the treatment of cerebrovascular ischaemic syndromes such as stroke and dementia

(Sorond *et al.*, 2008). A non-controlled study by Sorond *et al.* administered 900mg of CF, nearly double the concentration that we administered acutely, daily for two weeks and saw an 8% increase in middle cerebral artery flow (measured by transcranial doppler) after one week and 10% after two weeks (Sorond *et al.*, 2008). Although not measured in our study, based on the results from Sorond *et al*. it may be that the dose we have administered acutely in this study was again not sufficient to mount a response to feeding in the older individuals in our study. Further research should explore the potential impact of CF on brain blood flow and associated clinical parameters.

In this acute study, whilst there was a reduced increase in arterial and venous concentration at 15 and 35 minutes following CF supplementation, we do not report any differences in A-V blood glucose or plasma insulin concentrations in response to oral feeding with CF or a placebo supplement. This is not supportive of the findings of a 15-day interventional crossover study which compared the effects of daily dark (containing approximately 500mg of CF) and white chocolate (containing theoretically minimal amounts of CF) supplementation in healthy young subjects. This study found dark chocolate to significantly reduce fasting plasma glucose and insulin concentrations (and subsequently HOMA-IR), as well as reduced response to an OGTT (Grassi *et al.*, 2005). Although the mechanisms behind this improvement are not fully understood, the authors concluded that the improvements in insulin sensitivity following dark chocolate supplementation were secondary to the protective effects of CF (Grassi *et al.*, 2005). The acute versus chronic nature of our study compared to that by Grassi *et al.* is the obvious difference and this may explain the somewhat disparate findings.

Green tea extract

Second only to water, tea is one of the most widely consumed beverages worldwide (Yang *et al.*, 2004). Green tea in particular has been used in Eastern medicine for many years, with some evidence to suggest in can reduce inflammation, improve blood flow, treat infectious disease and purify the body (Sumpio *et al.*, 2006; Naumovski *et al.*, 2019). As discussed previously, Epigallocatechin 3-gallate (EGCg) is the most abundant and potent green tea catechin and has been extensively studied for its beneficial health effects (Sumpio *et al.*, 2006; Kim, Quon and Kim, 2014). In particular, EGCg is a potent stimulator of NO (Keske *et al.*, 2015) and whilst there are many laboratory studies which have shown this, few studies in human as to the effects of EGCg on endothelial function exist (Lorenz *et al.*, 2017). In addition, and of particular relevance to advancing age, a focus of this chapter, there are also a number of *in vitro* studies using aged rats which have shown that EGCg and GTE have both osteoprotective (Dvoretskiy *et al.*, 2020) and muscle protective properties (Alway *et al.*,

2015; Dvoretskiy *et al.*, 2020); suggesting that they may have a role to play in the treatment and prevention of sarcopenia, although this is yet to be determined in human trials.

In our study of older adults, we report no significant changes in LBF or glucose uptake with GTE compared to placebo prior to nutrition, suggesting that acute GTE supplementation does not improve muscle nutrient delivery in response to feeding in older adults. Based on recent work by Fox *et al.*, it may be that the dose of GTE was too low to elicit improvements in these parameters and/or the older adults cannot improve. Fox *et al.* conducted a study in young healthy males and found increased brachial artery blood flow following 600mg but not 300mg of GTE versus placebo in response to RET (Fox *et al.*, 2020); as such our 500mg dose may not have been sufficient. The authors of this study did sensibly note that they could not confirm whether the resultant hyperaemia of GTE would result in improvements in muscle mass gains beyond those elicited by RET alone (Fox *et al.*, 2020), with more long-term GTE supplementation studies assessing the impact on muscle mass and function, needed to investigate the potential for this group of phytonutrients as tool against sarcopenia.

In this study we report no significant differences in glucose or insulin concentrations in response to feeding with prior GTE supplementation versus placebo. This replicates the findings of previous studies investigating the impact of acute GTE supplementation on response to feeding in healthy young participants (Josic *et al.*, 2010), patients with T2DM (Ryu *et al.*, 2006) and also of eight weeks GTE supplementation in middle-aged overweight or obese men (Brown *et al.*, 2009). Our findings are also partly in keeping with a recent systematic review which concluded that whilst GTE is shown to reduce fasting glucose, there is no significant effect on fasting insulin (Xu *et al.*, 2020).

Curcumin

Curcumin has been shown to be a potent anti-inflammatory (Gupte *et al.*, 2019) and antioxidant (Srivastava and Mehta, 2009) compound, as well as possessing a host of other health-beneficial properties including hypertension, inflammatory bowel disease, diabetes and cancer (Mohanty and Sahoo, 2017). These actions, in particular anti-inflammatory, are thought to be secondary to the unique ability of CU to inhibit the action of NFκB in various catabolic conditions, such as prolonged illness and sepsis (Alamdari, O'Neal and Hasselgren, 2009) and the treatment of burns (Dewar *et al.*, 2011), which may provide the basis for the development of treatments for proteolytic catabolic states such as sarcopenia. In addition, *in vitro* and animal studies have shown CU to be a potent vasodilator, thereby improving microvascular tissue bed nutrient and oxygen delivery (Dewar *et al.*, 2011),

however studies in older adults are lacking. In one of the first studies investigating the effects of CU supplementation on vascular endothelial function in middle-aged and older adults, Santos-Parker *et al.* reported a 37% increase in forearm blood flow following 12 weeks of 2g CU supplementation daily when compared to placebo (Santos-Parker *et al.*, 2017). This improvement was reported to be secondary to increases in NO bioavailability and reductions in vascular oxidative stress (Santos-Parker *et al.*, 2017). Although we do not report any improvements in muscle macrovascular blood flow responses to feeding following acute supplementation with CU in older adults, the dose of CU administered in the study by Santos-Parker *et al.* was four times the amount given in our study and for a much longer period compared to our acute, single dose supplementation. As such it may be that our dose was not sufficient to elicit change and/or this change may not have been observed in the femoral (versus brachial) artery, even with a prolonged and higher dose. The dose administered by Santos-Parker *et al.* was possible owing to the non-toxic profile of CU supplementation, even at such doses (Alamdari, O'Neal and Hasselgren, 2009). Although not assessing vascular responses to feeding, the work of Akazawa *et al.* suggests that a dose of 2g per day may not be needed to elicit vascular improvement. In a study of post-menopausal women who were administered 150mg of CU daily for eight weeks, flow mediated dilatation, an accepted measure of endothelial function (discussed in detail in section 1.13.1) significantly improved when compared to baseline, with the magnitude of improvement comparable to a second group who performed eight weeks of moderate aerobic exercise (Akazawa *et al.*, 2012). As such, the authors concluded that CU supplementation could provide a novel strategy to improve CVD risk for patients who are unable to perform regular exercise (Akazawa *et al.*, 2012). In relation to our study, the impact of this lower dose when given chronically would suggest that it was the acute nature of our CU dose, opposed to the dose *per se*, that limited its impact. However, in a randomised crossover trial in healthy middle-aged men, a single serving of a curry meal containing 4g of CU was able to acutely improve postprandial brachial artery blood flow and FMD compared to a control meal (Nakayama *et al.*, 2014). Bringing these findings together it seems that a much higher dose of CU acutely or a prolonged lower dose is needed to elicit changes in blood flow, and this is scope for future studies in older adults.

That we report no significant differences in glucose or insulin concentrations in response to feeding with or without CU supplementation is in keeping with the findings of Nakayama *et al.* who also did not report any changes in insulin and glucose concentrations following a single dose of 4.5g of CU supplementation and oral feeding (Nakayama *et al.*, 2014). Similarly, Santos-Parker *et al.* also did not report any significant changes in glucose or insulin concentrations, or indeed HOMA-IR, following 12 weeks of CU supplementation (Santos-Parker *et al.*, 2017), but this was not in response to nutrition. Only one study reports

a beneficial effect of a CU containing supplement on postprandial insulin responses, with this study conducted in healthy, overweight, middle-aged men. However, this trial investigated the effects of a high antioxidant spice blend containing 2.79g of CU in addition to other spices, so the beneficial findings cannot be attributed solely to CU (Skulas-Ray *et al.*, 2011).

6.8.1 Limitations

This randomised control trial was powered by a prior power calculation which was not intended to provide efficacy analysis, but rather to assess trends that could be studied in a subsequent larger study. As such the calculated sample size may have meant that overall the study was underpowered.

Aside from avoidance of the compounds involved, we did not impose any strict dietary restrictions on our participants, and as such the baseline flavanol intake of our participants may have influenced our results. In addition, we did not include analysis of bioavailability of each compound for this study.

Whilst muscle delivery is the main outcome from this study, we do not report on improvements in MPS and given the disparate findings between blood flow and MPS measures in previous studies (Phillips *et al.*, 2016), MPS should be both acutely and chronically measured using tracers to accurately assess the benefit of these compounds on combatting sarcopenia.

LBF measures via ultrasound are highly operator dependent, even when performed according to a detailed SOP, and this may have impacted our data. As discussed previously, maintaining a constant isonation angle for each measurement is crucial to achieving accurate, reliable measurements (Casey, Curry and Joyner, 2008). Our method for measurement was freehand and did not utilise a fixed mechanism, which would have been technically very difficult to maintain for numerous timepoints, but which may have again enhanced reliability. This was however the reason for analysing the average of three measurements.

Our participants were healthy volunteers with relatively few medical co-morbidities. We recognise that the effects of these compounds may be different in the very elderly and co-morbid individuals and further study of the effects of these compounds in these cohorts is warranted if suggestions of positive impacts are achieved in future work.

A final limitation of this study, as with many human studies investigating the effects of phytonutrients, is that there is no evidence as to the long-term effects of these compounds on improving health. Further, long-term studies investigating the benefits as well as the risks of long-term supplementation with these compounds are needed to ensure the development of safe treatments for the general public.

6.9 Chapter conclusions

The effects of sarcopenia represent a growing public health concern due to the advancing age of the general population. Vascular disturbances with age, and the resultant reduced limb blood flow, may be a key mechanism in the pathogenesis of sarcopenia by reducing the concentrations of oxygen and nutrients delivered to muscle tissue. Exercise is proven to protect against and even prevent these negative effects, however exercise prescription is not appropriate for the very elderly and infirm.

Alternative methods in the form of plant-derived nutraceuticals may be a novel strategy to combat sarcopenia. Whilst having been shown to be potent compounds to improve health in pre-clinical trials, acute, single dose supplementation of cocoa, green tea extracts and curcumin do not improve macrovascular blood flow in response to oral feeding and are therefore unlikely to protect against sarcopenia when administered in this method. Further research into these compounds, including their long-term effects, are needed to ensure individuals affected by sarcopenia and frailty who cannot exercise, and represent a growing proportion of the population, have alternative strategies to improve and maintain health.

Chapter 7: Thesis overview, discussion and future directions

An ageing population will mean a greater prevalence of age-related conditions and an increase in the amount of ill health. Geriatric syndromes such as frailty and falls have a significant impact on the quality of life of older adults and may lead to disability. Physiological decline and reduced functional reserve due to ageing is likely to affect several systems leading to multi-morbidity, increasing the risk of needing institutional care. This already is, and will continue to have, significant socioeconomic consequences when considering the treatment of chronic disease and comorbidity, and their implications for hospitalisation and social care for the older adult (Fried *et al.*, 2001; Beard *et al.*, 2016).

Sarcopenia, the involuntary, age-associated loss of skeletal MM and function, has now been redefined as a disease of muscle constituting muscle failure (Cruz-Jentoft *et al.*, 2019). This syndrome can increase the risk of falls and frailty and other negative health outcomes, further reducing the independence of older adults. This effect is compounded in older, non-active adults, who may already have reduced MM due to a negative balance in MPS to MPB, a well described phenomenon in ageing (Breen and Phillips, 2011). In addition, there is substantial evidence to show that there is a natural decline in CRF with advancing age. Lower levels of CRF have been demonstrated to be an important marker of functional limitations and is directly associated with frailty in older adults, as well as being a major risk factor for premature mortality (Kodama *et al.*, 2009). There are no guidelines for managing low CRF in older adults, and strategies for improving low CRF and maintaining MM in this age group need to be developed.

Exercise has consistently been shown to be an excellent modality for improving CRF and MM. When considering individual exercise modalities, AE has been shown to be excellent for improving cardiovascular parameters (Brook *et al.*, 2016), whilst RET is superior at developing and maintaining MM (Snijders *et al.*, 2019). HIT has emerged as a time-efficient exercise strategy for primarily improving CRF, but also a number of other health related facets, such as lowering BP, reducing total body adiposity, increasing lean MM and improving insulin sensitivity (Laursen and Jenkins, 2002; Gibala and Little, 2010; Little *et al.*, 2011). To date, the majority of studies comparing the efficacy of these exercise modalities for improving health, especially HIT, have been performed in the laboratory setting with the need for supervision. In order to make these interventions more accessible to the general public, effective exercise regimens need to be unsupervised and home-based.

Whilst the merits of exercise have been well explored, sarcopenia has worse clinical manifestations in those older adults who cannot exercise (Iolascon *et al.*, 2017) and are therefore at higher risk of losing muscle mass (Cuthbertson *et al.*, 2005; Breen and Phillips, 2011). Polyphenols may serve as a means to maintain muscle mass in this group of

individuals. Nutraceutical agents represent a rapidly growing area in the health market, and whilst this sector is highly lucrative, treatments are rarely soundly scientifically based on the background of high powered RCTs. Much evidence exists as to the ability of nutraceuticals to improve cardiometabolic health, such as plant stanols (Berger, Jones and Abumweis, 2004) and omega-3 fish oils (Massaro *et al.*, 2010). Cocoa flavanols, green tea extract and curcumin supplements have been well studied *in vitro* (Kalra, 2003; Han, Shen and Lou, 2007; Nasri *et al.*, 2014), with only cocoa being more widely studied in smaller human trials (Heiss *et al.*, 2015; Grassi *et al.*, 2016). These studies have shown the potent action of nitric oxide in increasing blood flow, even reversing the effects of years of damage from atherosclerosis (Grassi *et al.*, 2012) and HTN (Ried, Fakler and Stocks, 2017). The effects of these compounds on improving muscle mass and combatting sarcopenia have not been extensively studied.

Chapter 3 reviewed the health benefits of participation in regular exercise for adults. A worryingly high percentage of adults are not however meeting the recommended weekly amounts of exercise. The barriers to exercise participation, and more importantly adherence, were also discussed, with “lack of time” being a chief barrier for both older and younger adults (Trost *et al.*, 2002). Interest in optimizing the magnitude of adaptation from exercise, whilst minimizing the time and effort, is therefore a topic of considerable interest within the exercise-for-health community. Home-based exercise interventions may provide promising strategies to overcome these barriers. Home-based interventions to improve CRF had not been previously systematically reviewed. This was performed in Chapter 3 which concluded that home-based exercises have been poorly studied with inconsistent comparisons to supervised regimes.

Chapter 4 expanded upon the risks of sedentary behaviour and physical inactivity in older adults, including the risks of reduced CRF and comorbidity. We reported improvements in the CRF, body composition and cardiometabolic health of older adults after completion of a four week, equipment-free, bodyweight-based HIT programme. This study was the first, to our knowledge, to examine the effects of an unsupervised, home-based HIT programme in older adults and was well received with a 100% completion rate by all exercising participants.

In Chapter 5 the worrying rates of sedentary behaviour and physical inactivity amongst younger adults and the association with obesity, poor cardiometabolic health and premature mortality were discussed. Exercise in this age group is again associated with a myriad of positive health outcomes and protects against the predisposition to the previously mentioned conditions, as a direct solution to combat inactivity (Ekelund *et al.*, 2016). We reported

improvements in the CRF, BMI and muscle architecture of younger adults after completing four weeks of the same intervention. In addition, we reported equal comparative efficacy of this programme for improving the CRF of both young and older adults. The success of this study provides further evidence that HIT is feasible in a range of adult age groups.

Chapter 6 expanded on the review of plant derived nutraceutical agents in Chapter 1 as a tool to maintain MM in older adults who cannot exercise due to pre-existing comorbidity or frailty. Improving muscle blood flow may increase the delivery of oxygen and nutrients to muscle tissue, thereby maintaining MM (Phillips *et al.*, 2016). Our results did not show improvements in muscle nutrient delivery following acute supplementation with CF, GTE or CU. This may have been because of the acute delivery of each compound and that prolonged delivery is needed to elicit the health benefits that supplementation with these compounds can have as seen in other studies (Sorond *et al.*, 2008; Santos-Parker *et al.*, 2017).

This thesis has discussed the positive merits of encouraging younger individuals to engage in regular exercise, which can prevent the development of serious medical conditions such as diabetes, hypercholesterolaemia and hypertension, leading to improved and sustained health in later life (Hwang *et al.*, 2018; Guthold *et al.*, 2020). It has also discussed the benefits of encouraging older adults to engage in regular exercise to ensure they maintain CRF and muscle mass (Kumar, Atherton, *et al.*, 2009), thereby reducing the risk of CVD, sarcopenia and premature mortality as they age, to maintain independent, fulfilling lives. This thesis has shown that four weeks of an equipment-free HIT programme can improve the CRF of both young and older adults, making it a feasible and time efficient modality to improve fitness across a range of ages. Furthermore, and for the first time in older adults, these improvements have been shown to be achievable irrespective of the supervision received during exercise sessions. Owing to the multiple benefits of HIT discussed, translating HIT away from the laboratory environment is critical for delivering these health improvements to clinical populations. Application of HIT with a reduced time commitment in the unsupervised or home-based setting may serve as an excellent methodology to ensure younger and older adults adhere to regular exercise, as our regimen overcomes many of the barriers to starting and adhering to exercise discussed above and in Chapter 3.

Future work based on the results from the studies within this thesis should include studies which examine the longer-term effects of HIT on CRF, MM and other facets of cardiometabolic health, and whether the encouraging results we report can be sustained and even improved on. With HIT already being supported by the NHS as a feasible methodology for improving and maintaining health (National Health Service (NHS), 2018), the results from

the two studies in younger and older adults could provide the basis for a nationally approved HIT protocol, which could be easily adapted into a web-based application and downloaded onto mobile devices. With studies expanding on the use of HIT as a method of prehabilitation for patients undergoing major abdominal surgery (Boereboom *et al.*, 2016; Blackwell *et al.*, 2020), our HIT regimen may serve as an adjunct for improving health in individuals who have a reduced time to surgery pathway, such as those with colorectal cancer. A home-based approach with little supervision would also require less resources from the NHS. The application of our regimen and its success in improving CRF in a limited timeframe may also aid to improve CRF in patients awaiting less time crucial surgical procedures, such as daycase and benign surgical procedures, which has been shown to decrease the rate of complications post-operatively (Older, 2013; Snowden *et al.*, 2013).

Future studies based on the three compounds used in Chapter 6 should focus on prolonged administration of the products, which may yield better results for improving blood flow and maintaining muscle. If to be used as agents to target sarcopenia, MPS should be measured in both an acute (tracers) and chronic (Dueterium Oxide; D2O) setting, with muscle function (an important facet of sarcopenia) also assessed in a chronic study.

Just 15 minutes of exercise, three times a week, without even needing to leave the home can improve health irrespective of age. Being able to perform these exercises at home may be a key method to fight against the rise of sedentary behaviour, inactivity and the associated poor health outcomes. The battle however, as it always has been, lies in encouraging the general public to get up and get exercising. For those individuals who genuinely cannot exercise, a highly lucrative market exists promoting the use of plant-based supplements to improve health. Whilst these compounds have excellent potential for the development of life-changing therapies, high-powered studies are lacking and need to be carried out to ensure this group of individuals do not suffer from poor health and can lead more fulfilling lives.

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Appendices

Appendix 1: Search strategies for systematic review

CINAHL

(exp *EXERCISE/ AND ((supervis* OR unsupervis*).ti,ab OR (lab*OR home*).ti,ab)) [Publication types MetaAnalysis OR Randomized Controlled Trial OR Systematic Review][Human age groups All Adult] ((exp *EXERCISE/ AND ((supervis* OR unsupervis*).ti,ab OR (lab*OR home*).ti,ab)) AND exp "RANDOMIZED CONTROLLEDTRIALS"/) [Human age groups All Adult]

EMBASE

((exp *EXERCISE/ AND ((supervis* OR unsupervis*).ti,ab OR (lab*OR home*).ti,ab)) AND ("systematic review").ti,ab) [Human age groups Adult 18 to 64 years OR Aged 65+ years] [Humans]

MEDLINE

(exp *EXERCISE/ AND ((supervis* OR unsupervis*).ti,ab OR (lab*OR home*).ti,ab)) [Document type Meta-analysis OR Randomized Controlled Trial OR Review] [Human age groups Adult OR Aged]

Appendix 2: Ethical approval for older adult HIT 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
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

27 June 2017

Mr TS Sian,
Clinical Research Fellow
c/o Dr Bethan Phillips
Division of Medical Science and Graduate Entry Medicine
School of Medicine
University of Nottingham
Royal Derby Hospital
Uttoxeter Road
Derby
DE22 3DT

Dear Mr Sian

Ethics Reference No: C16122016 **– please always quote**	
Study Title: A randomised control trial to assess the efficacy of short-term, home-based high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness (Home HIT).	
Short Title: Home HIT	
Chief Investigator/Supervisor: Dr BE Phillips PhD, Assistant Professor, Division of Medical Science and Graduate Entry Medicine.	
Lead Investigators/student: Mr Tanvir Singh Sian MRCS, Specialist Registrar in General Surgery, Surgical Research Fellow, Division of Medical Science and Graduate Entry Medicine.	
Other Key Investigators: Dr PJ Atherton PhD, Professor, Dr K Smith PhD, Principal Research Fellow, Dr JP Williams, Associate Professor and Consultant Anaesthetist, Mr JN Lund, Associate Professor and Consultant Surgeon, Division of Medical Science and Graduate Entry Medicine.	
Type of Study: Physiology, exercise	
Proposed Start Date: 01/2017	**Proposed End Date:** 31/01/2019 24 mths
No of Subjects: 36	**Age:** 18+years
School: SoMS GEM	

Thank you for your letter dated 19 June 2017 responding to the comments made by the committee and the following revised documents were received:

- FMHS REC Application form version 1.3: 08.06.2017
- Study Proposal, version 1.3: 08.06.2017
- Participant Information Sheet, version 1.3: 08.06.2017
- Consent Form version 1.2: 08.06.2017

These have been reviewed and are satisfactory and the study has been given a favourable opinion.

A favourable opinion is given on the understanding that the conditions set out below are followed:

Please can you submit a copy of the FMHS REC Application form signed off by Mrs Aline Nixon, IRMER Radiation Practitioner when this has been done for our records please.

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.

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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

Appendix 3: Participant information sheets for HIT studies

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Division of Medical Sciences and Graduate Entry Medicine

School of Medicine

Healthy Volunteers Information Sheet

Study Title: A randomised control trial to assess the efficacy of short-term, home-based high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness (Home HIT).

Name of Investigators:

Mr Tanvir Sian | Dr Beth Phillips | Mr Jonathan Lund

Dr Ken Smith | Dr John Williams | Prof Phil Atherton

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of your consent form and this leaflet.

Background:

Previous studies have shown that as people become less fit, they are at increased risk of suffering a complication if undergoing surgery. We also know that measuring how fit someone is, is better at predicting the risk of a complication than using a person's age alone. Lots of research has shown that exercise can reverse some of the age-related declines in fitness, however most work so far has used long sessions of continuous exercise over a long period of time. We have recently shown that High-Intensity Interval Training (HIT), where people cycle on an exercise bike very hard for a minute, followed by a short rest and then repeated over a 15 minute session can more rapidly improve a person's fitness. However whether this training that was conducted in a supervised laboratory setting can be as effective when adapted to be done at home is not yet known.

We aim to recruit up to 36 healthy volunteers aged 55 years and over to assess the efficacy of a 4-week home-based HIT programme in improving general fitness compared to lab-based HIT. If the results from this study show that home HIT is a promising method to improve fitness in the time frame dictated by many surgical situations (4-weeks), we plan to study the effects of home HIT in patients who have been diagnosed with abdominal cancer to improve their fitness pre-surgery.

In addition, we also wish to see if our adapted form of HIT, which uses no specialist equipment, is effective at improving fitness in younger individuals. This will allow us to assess if the results we observe in older individuals are age-dependent or applicable to the population as a whole.

Why have you been chosen?

In order for this study to be successful, healthy volunteers have been chosen who fit the entry criteria and do not have significant on-going medical problems. We are inviting up to 36 participants aged 55 years and over to take part in addition to 30 younger individuals aged 18-35 years.

Do you have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Would an inconvenience allowance be paid?

There is no inconvenience allowance associated with this study. You will however receive information about your fitness and body composition (muscle and fat).

What does the study involve?

The location for this study is The Medical School at the Royal Derby Hospital. The study consists of: 1) a screening visit to assess your suitability for the study, 2) a baseline visit to assess your initial fitness, body composition and muscle structure, 3) 4 weeks exercise training in the laboratory (or a control period, see below) and 4) a re-assessment visit where your baseline visit measures will be repeated.

Study Groups

There will be two groups of younger individuals as shown below. One group will be asked to perform the exercise and one group will not.

1) Laboratory-HIT:

Subjects in this group will train three times each week in the laboratory for four weeks, unless more sessions are needed to catch-up on any missed in the previous week. You will receive encouragement and feedback during each session.

2) Control Group:

This group will undergo the screening, baseline and reassessment visits as outline below but will not be required to perform the exercise regime.

Screening Visit

Prior to being enrolled onto the study you will be asked to attend the Medical School at approx. 0900h for a screening visit to assess your suitability for the study, to give you the opportunity to ask questions about the study and to ask you to provide written consent for your participation in the study. We ask that you attend this session fasted from midnight, although you may drink water freely. This visit will last approximately 60 min and include:

i. a small blood sample to measure health parameters, including liver and kidney function
ii. measures of your height and weight
iii. an assessment of your heart, including an electrocardiogram (ECG) to monitor your heart and a blood pressure check

Baseline Study Day

Once we have the received the results of your blood tests and on the premise that all of your results are within normal range, we will enrol you on to the study.

On your first study day we will ask you to attend (again fasted from midnight) at approximately 0900h for a dual-energy x-ray absorptiometry (DXA) scan. The DXA scan provides us with information about the type of tissue under the skin (fat, muscle etc.) and about your bone quality. An additional leaflet providing more information about DXA is enclosed with this document.

After the DXA scan you will have an ultrasound (US) scan of your thigh. An US machine uses soundwaves which humans cannot hear and provides information about the structure of the thigh muscles. It does not use any x-rays or any other form of radiation. A small amount of cold jelly is applied before the scan and wiped off afterwards.

After the US scan we will take a very small blood sample to assess your fasting insulin and glucose. From this we can calculate your insulin resistance (a risk factor for metabolic diseases, e.g. diabetes) and see if this is improved with the exercise training. We will also assess your body's ability to process sugars. You will be asked to consume a sugary drink and we will then take regular blood samples from a cannula (fine plastic tube) from a vein in the back of your hand over 2 hours. During this time, your hand will be placed in a warmed box. After this we will provide you with a snack and a drink and you will rest for 30-minutes.

After the rest period you will be asked to complete a set of questionnaires asking about your physical activity levels. You will then be asked to complete a short set of muscle function assesments such as hand grip strength.

Your final assessment on this visit will be a cardio-pulmonary exercise (CPET) test. Using an exercise bike, this test is considered the gold-standard measure of whole-body fitness. We will first adjust the seat height of the bike to make sure you are comfortable. We will then fit a facemask that will collect the air that you breathe in and out during the test. You will also be connected to an ECG machine and blood pressure monitor. The test will last approximately 12-18 minutes in total, including 2 minute warm up and recovery phase. The intensity that you are cycling against will get gradually harder as the test progresses and we will ask you to cycle until you cannot maintain a set speed any more.

If you are in the HIT group you will first be shown the HIT exercises at the end of the baseline visit, however all of your training sessions will be instructed and supervised.

Training Sessions

You will be required to perform your exercise programme up to 5 times a week for 4 weeks, although three times each week will be the aim.

If you are in the HIT group you will be excluded from the study if you:

- Are unable to attend for more than 3 consecutive sessions
- Miss more than 6 sessions in total
- Fail to complete the set exercise regime on 6 sessions or more

The HIT sessions will consist of a 2-minute warm up, 5 x 1-minute high intensity exercise bouts separated by 90 seconds active recovery and then a 2-minute recovery period.
The warm up and recovery phases will consist of light on-the-spot jogging and the active recovery will be walking.

The high intensity bouts will consist of:

1. Star jumps
2. Standing squats
3. On-the-spot sprint
4. Standing squats, and
5. Star jumps

The pyramid arrangement of exercises is designed to help maintain intensity with the first bout of star jumps and mountain climbers giving a target number to achieve in HIT bouts 4 and 5.

Alternative exercises can be provided if the above cannot be completed due to pre-exising limitations (e.g. wrist problems and mountain climbers).

Reassessment Study Day

After 4 weeks of exercise training you will be asked to return to the Medical School for the tests in the Baseline Study Day to be repeated. In addition, we will ask you to complete a questionnaire about your thoughts on the exercise programme you have completed.

What do I have to do?

We are studying the effects of different exercise training programmes on physical fitness. With the exceptions outlined above, we ask that you maintain your diet and physical activity levels as normal.

What is the drug or procedure that is being tested?

This study assesses the effects of equipment free HIT in a 4-week period for improving physical fitness. No other drug or procedure is being tested.

What are the side effects or risk of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known. Blood tests can result in bruising around the area where the needles have been, although this procedure will only be performed by individuals trained and experienced in this technique. HIT is intense exercise, albeit for short periods. Especially after the initial sessions you may experience some delayed muscle soreness in the days following. If you feel unwell doing any of the exercise sessions, stop immediately and contact a member of the research team.

What are the possible benefits of taking part?

We cannot promise the study will help you, but you may see improvements in your resting heart rate, blood pressure and physical fitness. Participation in the study will also provide you with some information about your body composition from the DXA scan. You will also receive a pack at the end of the study containing data about your fitness and how you have responded during the trial. Overall it is hoped that the information we gain from this study will give us a better understanding of fitness changes in response to HIT and if this can happen with home HIT. This may help us design better guidelines for exercise training in older people and potentially help improve a patient's fitness prior to undergoing surgery.

What are the possible disadvantages and risks of taking part?

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.

What are the exclusion criteria for this study?

For the young subject groups of this study we are recruiting healthy volunteers aged between 18 and 35 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid a disturbance

allowance or having an invasive procedure (e.g. blood sample >50ml), you would not be eligible to take part.

You would also be unsuitable if you have particular medical conditions or are taking certain medications. If you are interested in this study please discuss these further with the study doctor.

What if something goes wrong?

If you suffer any symptoms or side effects, you should report them immediately to the study doctor (see end of sheet for contact details). If you have a concern about any aspect of this study, you should ask to speak to the study team who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator Dr B Phillips. If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary, Mrs Louise Sabir, University of Nottingham, Faculty of Medicine & Health Sciences Research Ethics Committee c/o School of Medicine Education Centre, B Floor, Medical School, QMC Campus, Nottingham University Hospitals, NG7 2UH. E-mail: louise.sabir@nottingham.ac.uk. In the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study. In addition, all medical practitioners taking part in the research have personal medical negligence cover.

Will my taking part in this study be kept confidential?

We respect your right to privacy and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement will carry details of your name and address, but no health related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to University staff with direct responsibility for ensuring the study is conducted appropriately.

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised 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.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any

information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept 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. 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 and only direct members of the research team will have access to your personal data.

You can find out more about how we use your information and read our privacy notice at:

https://www.nottingham.ac.uk/utilities/privacy.aspx

What will happen to any samples I give?

We will seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?

In the current study we will not be using your samples for genetic testing.

What will happen to the results of the research study?

Data collected during the study will be published in the scientific literature enabling other health professionals to use the information. You will not be identified in any publication. There is usually a delay of up to one year from the study completion before this occurs. Should you wish to be informed of publications resulting from this study, please inform the study team.

Who is funding the research?

This study is funded by the University of Nottingham.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

Contact for Further Information

Thank you for your interest in this study. For further information please contact Mr Tanvir Sian at tanvirsian@gmail.com or on 01332 724640. The research team is alternatively contactable at The University of Nottingham, Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, Royal Derby Hospital, Derby, DE22 3DT.

Appendix 4: International Physical Activity Questionnaire-Short Form (IPAQ-SF)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 1 minute at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**
_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix 5: Consent form for HIT studies

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Division of Medical Sciences and Graduate Entry Medicine
School of Medicine

Healthy Volunteers Consent Form

Title of Study: A randomised control trial to assess the efficacy of short-term, home-based high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness (Home HIT).

Name of Researchers:

Mr Tanvir Sian	Mr Jonathan Lund	Dr Ken Smith
Dr Beth Philips	Dr John Williams	Prof Phil Atherton

Name of Participant:

Please initial box

1.	I voluntarily agree to take part in of the above study.	☐
2.	I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.	☐
3.	I agree to the above investigators contacting my general practitioner to make known my participation in the study where relevant.	☐
4.	I agree to comply with the reasonable instructions of the supervising investigator and will notify them immediately of any unexpected unusual symptoms or deterioration of health.	☐
5.	I authorise the investigators to disclose the results of my participation in the study but not my name.	☐
6.	I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published.	☐
7.	I understand that relevant sections of data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this	☐

study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential.

8. I understand and agree that blood samples will be taken for screening tests prior to being enrolled in the study. ☐

9. Consent for storage and use in possible future research *(Optional)*
I agree that the samples I have given and the information gathered about me can be stored by researchers at the School of Medicine, for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Dr Phillips and colleagues, who ran the first study, including researchers working for commercial companies. Any samples or data used will be anonymised, and I will not be identified in anyway. ☐

10. I authorise the investigators to disclose to me any abnormal test results. ☐

11. I understand that I can ask for further instructions or explanations at any time. ☐

12. I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐

13. I confirm that I have disclosed relevant medical information before the study. ☐

14. I have not been a subject in any other research study in the last three months, which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (e.g. venepuncture >50ml, endoscopy) or exposure to ionising radiation. ☐

15. I confirm that I have not been exposed to more than 5 mSv of ionising radiation in the last 12 months. ☐

Name of Participant:…… **Date:**…….. **Signature:**……..……

Study Identifier:……

Name of Person

Taking Consent:…… **Date:**…….…… **Signature:**…….

Name of Principal

Investigator:…… **Date:**…….. **Signature:**…….

2 copies: 1 for participant, 1 for the project notes

Appendix 6: Ethical approval for young adult HIT 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
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

13 December 2017

Mr TS Sian,
Clinical Research Fellow
c/o Dr Bethan Phillips
Assistant Professor
Division of Medical Science and Graduate Entry Medicine
School of Medicine
University of Nottingham
Royal Derby Hospital
Uttoxeter Road
Derby. DE22 3DT

Dear Mr Sian

Ethics Reference No: C16122016 **– please always quote**	
Study Title: A randomised control trial to assess the efficacy of short-term, home-based high-intensity interval training (HIT) for improving indices of cardiorespiratory fitness (Home HIT).	
Short Title: Home HIT	
Chief Investigator/Supervisor: Dr BE Phillips PhD, Assistant Professor, Division of Medical Science and Graduate Entry Medicine.	
Lead Investigators/student: Mr Tanvir Singh Sian MRCS, Specialist Registrar in General Surgery, Surgical Research Fellow, Division of Medical Science and Graduate Entry Medicine.	
Other Key Investigators: Dr PJ Atherton PhD, Professor, Dr K Smith PhD, Principal Research Fellow, Dr JP Williams, Associate Professor and Consultant Anaesthetist, Mr JN Lund, Associate Professor and Consultant Surgeon, Division of Medical Science and Graduate Entry Medicine.	
Type of Study: Physiology, exercise	
Proposed Start Date: 01/2017	**Proposed End Date:** 31/01/2019 24 mths
No of Subjects: 36 + 36 (18-35yrs)	**Age:** 18+years
School: SoMS GEM	

Thank you for notifying the Committee of amendment no 1: 11.10.2017 as follows:

- Addition of 2 groups of young people 18-35 years as the current study is looking at an older age group and the novel training mode of equipment free HIT has not been assessed in younger people. This will help inform if any differences in fitness gains seen in older people after cycling HIT compared to equipment free HIT are a function of age.

and the following revised documents were received:

- FMHS REC Notice of Amendment form dated 11.10.2017
- Participant Information Sheet (young) version 1.4 11.10.201

These have been reviewed and are satisfactory and the study has been given a favourable opinion.

A favourable opinion is given on the understanding that:

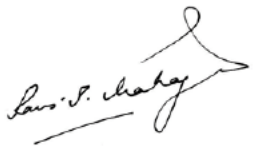
1. The protocol agreed is followed and the Committee is informed of any changes using a notice of amendment form (please request a form).

University of Nottingham
UK | CHINA | MALAYSIA

2. The Chair is informed 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

Appendix 7: Ethical approval for Abbott 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
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham, NG7 2UH

6 July 2017

Professor Philip Atherton
Professor of Clinical, Metabolic and Molecular Physiology
Division of Medical Sciences and Graduate Entry Medicine
School of Medicine,
Royal Derby Hospital,
Uttoexter Road
Derby, DE22 3DT

Dear Professor Atherton

Ethics Reference No: 2-1704 **– please always quote**	
Study Title: A pilot study to explore the acute effects of phytonutrients upon muscle microvascular blood flow (perfusion) in response to feeding in older adults.	
Chief Investigator/Supervisor: Prof Philip Atherton, Professor of Clinical, Metabolic and Molecular Physiology	
Lead Investigators/student: Dr Ushnah Din, Clinical Research Fellow/ PhD Student	
Other Key Investigators: Dr BE Phillips, Assistant Professor of Clinical, Metabolic and Molecular Physiology, Dr Kenneth Smith, Principal Research Fellow,Mrs Amanda Gates, Clinical Measurement Technician Division of Medical Sciences and Graduate Entry Medicine	
Type of Study: A Scientific study funded by Abbott Nutrition, physiolology, muscle biopsies, DXA, green tea, curcumin, cocoa.	
Proposed Start Date: 1.5.17	**Proposed End Date:** 1.5.19 24mths
No of Subjects: 45	**Age:** 65-85 years
School: Medicine, Derby	

Thank you for submitting the above application which has been considered by the Committee at its meeting on 24th April 2017 and the following documents were received:

- FMHS REC Application form and supporting documents version 1.0: 20.04.2017
- IRMER Practitioner Signed form for DEXA dated 25.04.2017

These have been reviewed and are satisfactory and the study 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. 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 Lomasgatri

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix 8: Participant information sheets for Abbott study

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Division of Medical Sciences and Graduate Entry Medicine,
School of Medicine

Healthy Volunteers Information Sheet

Study Title: A pilot study to explore the acute effects of phytonutrients upon muscle microvascular blood flow (perfusion) in response to feeding in older adults.

Name of Investigators:

Prof Philip Atherton	Mrs Amanda Gates
Dr Bethan Phillips	Dr Ushnah Din
Prof Kenneth Smith	Mr Tanvir Sian

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you decide to take part you will be given a copy of this leaflet and a copy of your signed and dated consent form.

Background:
Ageing is associated with a gradual reduction in muscle mass and strength, and is also one of the main risk factors for cardiovascular and metabolic (e.g., diabetes) disease. Recent research has shown that ageing is also associated with reduced blood flow to muscle. Based on this, developing ways to improve blood flow to throughout the body may improve nutrient (protein and carbohydrates) delivery and utilisation in older adults, potentially reducing muscle mass losses and reducing cardio-metabolic disease risk.
It has already been shown that improved blood flow via exercise training has a positive impact on health, including reduced cardiovascular and metabolic disease risk. However, exercise training is not appropriate for all (e.g. those will social and/ or physical limitations) so alternative interventions which are applicable to a wider population (i.e. nutritional supplements) need to be developed.
We have recently shown that the active ingredient in cocoa (cocoa flavanols) can improve muscle and limb blood flow in older men; however, we do not yet know the effects of cocoa on brain and central (heart) blood flow, nutrient uptake and utilisation; or if these findings were also present in women.
Therefore, in this study we aim to recruit 36 older individuals (aged 65-85 years; males and females) to study the effects of 3 different nutrient supplements (green tea, curcumin and cocoa) with known active ingredients, on different aspects of blood flow and nutrient uptake.

Why have you been chosen?
In order for this study to be successful, volunteers will be chosen who fit the entry criteria and do not have significant on-going medical problems. Volunteers will be non-smokers of normal/ slightly over-weight, who do not participate in a formal exercise regime.

Do you have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Is there a cost to take part?
There will be no cost to you for taking part in the study.

Would an inconvenience allowance be paid?
Yes – you will be paid £100 for each of the two study visits. At the follow-up visit after study visit 2 you will asked to provide feedback about your study experience and will be asked to provide your bank details so that your inconvenience allowance can be paid.

What does the study involve?
The location for this study is the Clinical, Metabolic and Molecular Physiology research laboratories of the University of Nottingham Medical School at the Royal Derby Hospital. The study consists of a screening visit, 2 study (experimental) visits and 2 follow-up visits. The total duration of your study involvement will be 21 to 55 days, dependent upon the scheduling of your visits.

Screening Visit
Prior to being enrolled onto the study you will be asked to attend the Medical School at approx. 9am, fasted from ~9pm the night before, for a screening visit to: 1) assess your suitability for the study; 2) give you the opportunity to ask questions about the study, and 3) ask you to provide written consent for your participation in the study. This visit will last approximately 60 minutes, during this time we will:

i. take a small amount of blood to measure health parameters, including fasting glucose, liver and kidney function
ii. measure your height and weight
iii. conduct a physical examination, including an ECG to monitor the electrical activity of your heart, a blood pressure check and an assessment of your hand-grip strength and walking speed.
iv. Ask you to complete a short questionnaire about your daily activity levels.

Study Visit 1
Once we have the received the results of your blood tests and on the premise that all your results are within the normal range, we will enrol you onto the study and randomly assign a subject number that corresponds to one of three supplement groups: Green Tea, Curcumin or Cocoa.

On your first study visit we will ask you to attend at approximately 9am (again fasted from 9pm the night before) for a DXA scan.

DXA scan-
DXA is short for dual-energy x-ray absorptiometry. The DXA scan provides us with information about the type of tissue under the skin (fat, muscle etc.) and about your bone quality. An additional leaflet providing more information about the DXA is enclosed with this document. We will provide you with a copy of your DXA scan.

After the DXA scan, baseline measurements will be made of the blood flow in:
1) your leg (LBF) per se;

2) your upper and lower leg muscles (via contract enhanced ultrasound (CEUS));
3) your forearm (as flow mediated dilation (FMD));
4) your heart, and
5) your brain.

A baseline muscle sample (biopsy) will be taken from the front of your thigh (*vastus lateralis muscle*) and baseline blood samples will be taken (see **Figure 1** for study visit outline).

	Screen	Visit 1 and Visit 2									
		-60 m	0	30m	60m	90m	120m	150m	180m	210m	240m
		Fasted	Meal + Test or Placebo				Fed				
Grip/ Gait	↑										
DXA		↑*									
Leg BF		↑		↑	↑	↑	↑	↑	↑	↑	↑
CEUS		↑		↑	↑		↑		↑		↑
FMD		↑			↑		↑				
Cardiac BF		↑				↑					
Brain BF		↑			↑		↑				
Blood (A-V)		↑		↑^ ↑	↑	↑	↑	↑	↑	↑	↑
Biopsy		↑						↑			

Figure 1. Study visit outline

Leg, heart and brain blood flow-
Leg blood flow, heart (cardiac) blood flow and brain blood flow will be measured in regional blood vessels by simple ultrasound; a non-invasive technique (similar to a pregnancy scan) that involves cool gel being applied to your groin, chest and forehead, respectively.

Muscle blood flow-
The muscle blood flow (via CEUS: contrast enhanced ultrasound) requires a cannula (a narrow, flexible plastic tube) to be inserted into a vein in your arm; once in place this same cannula will be used for all CEUS measurements. This cannula allows us to infuse microbubbles into your body that can be seen by ultrasound; we can then track these bubbles to assess how much blood flow is reaching your muscles. This technique has been used in clinical and research settings for many years, and has been performed hundreds of times in our laboratory.

Forearm blood flow-
The blood flow measurement in your forearm (via FMD: flow mediated dilation) is to assess how compliant your blood vessels are to a challenge (i.e., how much they will open to allow more blood flow). Again, using simple ultrasound, the blood flow in a forearm vessel will be measured before and after a blood pressure cuff is inflated around the arm for 5 minutes, with blood flow occlusion from the cuff acting as the challenge.

Blood sampling (arterialised and venous)-
In order to assess nutrient uptake and utilisation, arterialised and venous sampling will be used. To do this, 2 further cannulae will be inserted; one into the back of your hand (which will be placed in a warmed box for the duration of the study day), and the other into a large vessel at the top of your leg (the femoral vein). All cannulae insertions will be performed by a qualified medical doctor who is skilled in these procedures, with local anaesthetic given to freeze the

skin before insertion of the cannula at the top of your leg. These insertions allow us to take blood samples periodically during the day.

Muscle biopsy-
The muscle sample (biopsy) will be taken from the front of your thigh after local anaesthetic has been injected to freeze the area. A small (0.5-1 cm) cut will then be made through the skin and underlying tissue, before a small piece of muscle (about the size of an orange pip), is taken with specially designed tweezers. With the local anaesthetic you should feel no pain during this procedure but you may feel movement or pushing sensations. To obtain the required amount of muscle the tweezers are usually reintroduced through the initial cut 2-3 times. A stitch is placed in the skin afterwards to aid healing.

Feeding-
After the baseline measures you will receive a vanilla flavoured drink containing mixed nutrition similar to that of a standard meal (carbohydrate, protein and fat). You will also receive your supplement (Green Tea, Curcumin or Cocoa) or a placebo.

Repeat measurements-
Leg blood flow and blood samples will then be taken every 30 minutes throughout the remaining 4 hours. CEUS will occur five times during this period. FMD and brain blood flow will be measured twice, with one more measure of heart blood flow and one more muscle biopsy (two muscle biopsies in total on each study visit).

Overview-
The total testing period for this day will be ~6 hours: 30-minutes preparation, 1 hour of baseline measures, 4 hours of post-feeding measures and 30 minutes post-study monitoring.

Follow-up Visits
Five to seven days after your study visits you will be asked to attend a short (~30 minute) follow-up visit to check the biopsy sites and remove the stitches. At the follow-up visit after study visit 2 you will also be asked to provide feedback about your study experience.

Study Visit 2
Your second study visit will take place 7-14 days after study visit 1 and will be exactly like study visit 1, except there will be no DXA scan and you will be provided with the supplement or placebo that you did not receive in study visit 1.

What do I have to do?
We are studying the acute effects of three different nutritional supplements on blood flow and nutrient uptake in healthy older adults. With the exception of fasting on the night before your screening and study visits and abstaining from alcohol for 48 hours before these visits, please do not modify your usual diet or activity levels for this study.

What is the drug or procedure that is being tested?
We are testing the acute effects of three different nutritional supplements: Green Tea, Curcumin and Cocoa on blood flow and nutrient uptake.

What are the side effects or risk of any treatment or procedures received when taking part?

Research studies often involve some risks, not all of which may be currently known.

Blood sampling

The risks involved in venous cannulation and blood sampling are very small. The femoral vein lies next to the femoral artery, therefore to aid correct placement and reduce risks, ultrasound imaging will be used to locate the vein. It is rare to cause damage to the vessels using this technique; however, should this occur, pressure would be applied over the site for ten minutes until bleeding had stopped. Occasionally there may be bruising under the skin due to leakage from a blood vessel on withdrawal of the cannula, but this is uncommon with good practice and pressure will be applied to all sites when the cannulae are removed to minimise this risk. As with all procedures through the skin there is a slight risk of infection, although the use of sterile techniques minimise this risk.

Muscle biopsies

In our experience, muscle biopsies are generally well tolerated by subjects. Scarring is hardly perceptible and fades with time to near invisibility. The muscle biopsies are usually painless at the time they are being taken, because of the local anaesthetic we use. However, some discomfort can be felt during the procedure, and at the biopsy sites when the anaesthetic has worn off (usually 6-12 hours later). However, this is usually mild (rated as less than 2/10 by most past participants). Some muscle tenderness and stiffness is usually felt for 1 to 2 days afterwards and this can be alleviated by over-the-counter painkillers, such as Paracetamol. There may be some bruising associated with the biopsy procedure, although this should clear within a week. There is a slight risk of infection at biopsy sites, and damage to small nerves in the skin is possible during the biopsy procedure. These nerves usually grow back with restoration of normal sensation and infection risk is minimal when our aftercare procedures are followed.

Muscle blood flow measurements (CEUS)

Sonovue®, the fluid containing the microbubbles for CEUS, is widely used within the NHS for a range of diagnostic procedures. It has no commonly identified side effects. However, less common side effects include a rash, itch, dizziness, nausea (feeling sick), flushing, back pain and chest pain. More than 250 administrations of Sonovue® have been carried out by our research group without any problems. However, if you experience any problems when you are given this infusion, you should immediately inform the research team.

Forearm blood flow measurements (FMD)

Inflation of a blood pressure cuff around the arm for 5-minutes during the FMD measurements may cause some discomfort and a 'pins and needles' like sensation may be felt in the hand towards the end of this period and/or on release of the cuff. This discomfort is almost always transient and disappears soon after the cuff is released.

Liquid meal and supplement

Like all new foods that are introduced into your body, you may experience gas, bloat, diarrhea or constipation as a result of consuming the meal.

What are the possible benefits of taking part?

We cannot promise the study will help you individually, but you will be helping with research that has the potential help older people in the future. Participation in the study would provide you with a basic medical check-up and a copy of your DXA scan. You will also be invited to our annual research celebration event.

What are the possible disadvantages and risks of taking part?

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.

What are the exclusion criteria for this study?
We are recruiting healthy volunteers between the ages 65 to 85 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; or having an invasive procedure (e.g. blood sample >50ml) you would not be eligible to take part.

You would also be unsuitable if you have particular medical conditions or are taking certain medications. If you are interested in this study please discuss these further with the research team.

What if something goes wrong?
If you suffer any symptoms or side effects, or have a concern about any aspect of this study, you should ask to speak to the research team who will do their best to answer your questions. The contact details are given at the end of this information sheet. If you have a complaint on your treatment by a member of staff or anything to do with the study, you can initially approach the lead investigator, Professor Philip J Atherton. If this achieves no satisfactory outcome, you should then contact the Ethics Committee Secretary:
Mrs Louise Sabir, University of Nottingham, Faculty of Medicine & Health Sciences Research Ethics Committee c/o School of Medicine Education Centre, B Floor, Medical School, QMC Campus, Nottingham University Hospitals, NG7 2UH. E-mail: louise.sabir@nottingham.ac.uk.
If during the study, any injury that occurs as a direct result of the study procedures, Abbott Nutrition agrees to pay reasonable medical expenses to treat the injury, provided that you have followed the directions of the research team. In addition, in the unlikely event that you suffer injury to yourself or damage to your property as a result in taking part in this research, the University does have an insurance policy to cover harm arising as a result of the defect in the design of the study. In addition, all medical practitioners taking part in the research have personal medical negligence cover.

Can the study be stopped?
There may be unforeseen circumstances in which your participation in the study may be terminated by the research team or Abbott Nutrition.

Can I withdraw from the study?
You can withdraw from the study at any time without giving reason. To do this please inform the lead investigator in writing at the address on the last page of this document. Once you withdraw, all information gathered will be kept and used, but no new data will be collected about you once you stop.

May I have access to my records and make copies?
You have the right to access and make copies of your study records as applicable by privacy laws. However, to ensure valid results, we will ask that you agree not to review or make copies of some of your records until after the study is completed.

Will my taking part in this study be kept confidential?
We respect your right to privacy and we will take measures to safeguard confidentiality. A single form, on which you are asked to sign to give consent for involvement will carry details of

your name and address, but no health-related details. This is kept securely in a locked cabinet within the Medical School. Access to this cabinet is restricted to personnel directly involved in the study and to staff with direct responsibility for ensuring the study is conducted appropriately. We will follow current ethical and legal practice and all information about you will be handled in confidence. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

All 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.

As it is anticipated that the results of this study will be published in scientific journals, this study will be registered at http://www.ClinicalTrials.gov. This website will not include any information that can identify you and you can access the website at any time.

What will happen to any samples I give?
We will to seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

Some of these future studies may be carried out by researchers other than the current research team. This may include researchers working for commercial companies. Any samples or data used will be anonymised so that you could not be identified in any way. If you do not agree to this, any remaining samples will be disposed of in accordance with the Human Tissue Authorities code of practice.

Will any genetic tests be done?
In the current study, we will not be using your samples for genetic testing.

What will happen to the results of the research study?
Data collected during the study will be published in the scientific literature enabling other health professionals to use the information, it may also be used for a research students PhD thesis. You will not be identified in any publication. There is usually a delay of at least one year from the study completion before this occurs. Should you wish to be informed of publications resulting from this study, please inform the research team.

Who is funding the research?
This study is funded by Abbott Nutrition.

Who has reviewed the study?
This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee.

You will be notified by research study staff, in a timely manner, if significant new information becomes available that may determine if you want to continue in the study.

Contact for Further Information
Thank you for your interest in this study. For further information please contact:

Dr Ushnah Din, Clinical Research Fellow
mzxusd@exmail.nottingham.ac.uk
01332 724687

or

Mrs Amanda Gates, Clinical Measurement Technician
Amanda.gates@nottingham.ac.uk
01332 724687

The lead investigator for this study, Prof Philip J Atherton, is contactable at:
Philip.atherton@nottingham.ac.uk
01332 724725

All of these researchers are alternatively contactable at The University of Nottingham, Division of Medical Sciences and Graduate Entry Medicine, School of Medicine, Royal Derby Hospital, Derby, DE22 3DT.

Division of Medical Sciences and GEM

School of Medicine

The University of Nottingham

Human Tissue Act Management:

Healthy Volunteer Consent Form (HVCF)

Project Title: A pilot study to explore the acute effects of phytonutrients upon muscle microvascular blood flow (perfusion) in response to feeding in older adults.

Name of Investigators: PJ Atherton, BE Phillips, K Smith, A Gates, U Din

Please read this form and sign it once the investigator above-named, or their designated representative, has explained fully the aims and procedures of the study to you

Please Initial

- I voluntarily agree to take part in this study. ☐
- I confirm that I have been given a full explanation of the study and that I have read and understand the information sheet (version number …… dated……) for the above study and have had the opportunity to ask questions. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐
- I agree to comply with the reasonable instructions of the research team and will notify them immediately of any unexpected unusual symptoms or deterioration of health. ☐
- I give permission for the University of Nottingham and Abbott Nutrition to collect, store, analyse and publish information obtained from my participation in this study. I understand my personal details will be kept confidential. ☐
- I understand that data collected in the study may be looked at by responsible individuals from the University of Nottingham, Abbott Nutrition and regulatory authorities where it is relevant to my taking part in this study. ☐
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others (outside the University of Nottingham or Abbott Nutrition) it will be made anonymous. Data will be kept for 7 years after all results of this study have been published. After this time it will be disposed of in line with University policy. ☐
- I understand that the tests or procedures are carried out for research only and not for clinical diagnostic purposes. However, if the study investigator should feel it necessary to inform my GP of my participation in the study, an adverse event or abnormal test result, I understand I am giving my consent to do so. ☐
- I confirm that I have disclosed relevant medical information before the study. ☐
- *I shall receive an inconvenience allowance of £100 per acute study. If I withdraw from the study for medical reasons not associated with the study a payment will be made to me proportional to the length of the period of participation, but if I withdraw for any other reason, the payment to be made, if any, shall be at the discretion of the supervising investigator. ☐

Human Tissue Act Management:

Healthy Volunteer Consent Form (HVCF)

- I have not been a subject in any other research study in the *last three months*, which involved: taking a drug, being paid a disturbance allowance**,** having an invasive procedure (e.g. venepuncture >50ml, endoscopy) or exposure to ionising radiation. ☐
- I confirm that I have not been exposed to more than 5 mSv of ionising radiation in the *last 12 months*. ☐
- **OPTIONAL** - I agree to my tissue/ blood samples being shared with other researchers including those working for commercial companies (not including Abbott Nutrition), for example a drug company researching into the human body and drug development. I understand that I would not gain commercially. ☐
- **OPTIONAL** - Where tissue is removed from my body for the purposes of this study but not used for this study and when the ethical approval for this project expires, I agree to the future and continued storage and use of this tissue for future research into normal functioning of the human body and disease. Such stored tissue will fall under a Licence of the Human Tissue Act and tissue records as specified under the Licence will be maintained for 25 years. ☐

Personal Data:

- **OPTIONAL** - I agree to my contact details being stored for the purpose of being invited to participate in future research studies. ☐

Participant Name: ……

Signature: …… **Date:** ……..……

I confirm that I (investigator) have fully explained the purpose of the study and what is involved to:

……..

I have given the above named a copy of this form together with the information sheet.

Investigator Name: ……

Signature: ……. **Date:** ……..……..

PI Signature: …….. **Date:** ……

Study Volunteer Number: ……

2 copies: 1 for the participant, 1 for the study file.