An investigation into the effect of skeletal muscle metabolic function & cardio-respiratory fitness on exercise capacity in the presence and absence of disease

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Declaration

I, Siana Jones confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

It is uncommon for cardio-respiratory fitness and skeletal muscle metabolic function to be assessed independently within an exercise test. Exercise capacity may require adaptation of one or other of these systems to a greater or lesser extent and the presence of disease may affect them to different extents. The physiological mechanisms underpinning the decline in exercise tolerance with age/disease and the benefits of exercise-training as a preventative therapy for some diseases are not fully understood.

In this thesis functional capacity was assessed in terms of oxidative capacity. Near-Infrared Spectroscopy (NIRS) measures microvascular changes in oxygenated and deoxygenated haemoglobin and can be used to estimate oxidative capacity in skeletal muscle when combined with arterial occlusions. Oxidative capacity of skeletal muscle was determined as part of 3 studies; (1) in a group of older adults (>65 years old) with or without type 2 diabetes (T2D), (2) in a tri-ethnic group of older adults from the same cohort with the objective of determining ethnic difference in oxidative capacity independently of T2D and (3) in a group of young adults before and after a period of endurance training in preparation for their first marathon.

NIRS measurements of muscle oxidative capacity revealed poorer function in older adults with diabetes (57.5±6.8 versus 38.7±2.6 s, p=0.02) and poorer oxidative capacity in South Asian older adults independently of T2D (difference (95%CI): 10.1 (2.3, 17.9) s, p=0.011). In young healthy men and women, skeletal muscle oxygen consumption post-exercise increased with endurance training (p<0.01) despite no improvement in cardio-pulmonary peak$\dot{V}O_2$ (p=0.81). Faster marathon completion time correlated with cardio-pulmonary peak$\dot{V}O_2$ ($r_{partial}$=-0.55, p< 0.01) but not oxidative capacity.

Skeletal muscle oxidative capacity can be measured in old and young adults using NIRS combined with arterial occlusion performed immediately following exercise testing. People

with T2D have poorer oxidative capacity compared to people without and South Asians have poorer oxidative capacity compared to Europeans which African Caribbean's and Europeans had similar skeletal muscle oxidative capacity. Skeletal muscle metabolic adaptions occur following 6 months of endurance training. Although the cardio-pulmonary system is limiting for running performance, skeletal muscle changes can be detected despite no significant improvement in cardio-pulmonary function.

Impact statement

In this thesis Near-infrared Spectroscopy (NIRS) is employed to investigate skeletal muscle oxidative capacity in two distinct populations. The knowledge and expertise gained here could be put to beneficial use in the future through: (I) application of NIRS to further understand oxidative capacity in other populations (for example heart failure patients) (II) application of NIRS to understand different aspects of skeletal muscle function (for example vascular function) and (III) alignment with technical research departments to gain insight for development of novel NIRS devices.

Exercise capacity and skeletal muscle NIRS measurements were carried out here in a longitudinal population-based cohort study of older adults; the scope for this dataset reaches far beyond the results presented here. Measurements were conducted as part of a clinic day where a whole host of cardiovascular, anthropometric and cognitive measurements were also carried out on the same individuals. Therefore, locally, this dataset provides a valuable resource for future academic publications. More widespread impact could occur with data sharing or sharing of testing methods developed here.

Changes in haemoglobin/myoglobin at rest, during exercise and in the recovery phase from exercise were collected with a high sampling frequency (10Hz) from young and old participants with and without disease. These data are available for a greater number of participants than measurement of oxidative capacity was carried out in, therefore, this provides an immense resource with which development of novel algorithms designed to probe various different aspects of skeletal muscle function could be developed. This is likely to be of use within the field of cardio-metabolic health but shows potential for development in other fields where skeletal muscle is important; for example, sports science/medicine or units dedicated to understanding rare skeletal muscle myopathies.

Understanding the technology and its limitations is a valuable step towards developing more sophisticated NIRS devices. It is important that expertise in physiology is gained from this work which can be translated to develop the use of NIRS as a tool for investigating skeletal muscle. Furthermore, perhaps, through allowing these data to be available to developers (academic and commercial), the channels for discussion regarding the true physiological effects being detected in the NIRS signals could be opened. This could lead to development of improved understanding, development of improved devices and perhaps, devices made specific to task.

Some of the work presented in this thesis has contributed to the academic literature by way of three peer-reviewed publications (see section: ‘Publications associated with this thesis’). Impact could further be brought about by disseminating other results presented here in scientific journals, for example, the ethnic differences in skeletal muscle oxidative capacity which have not previously been described in older adults.

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Publications associated with this thesis

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European Society of Cardiology (ESC); 2015. **A 6-minute stepper test (6-MST) is a feasible protocol for assessing exercise capacity in older adults which also allows physiological changes to be accurately monitored throughout exercise**

Physiology Society Meeting; 2016. **Assessing exercise capacity in older adults using a self-paced 6-minute stepper test (6-MST)**

Artery Research; 2016. **Near Infrared Spectroscopy (NIRS) can detect improvements in arterial function following 6-months of marathon training**

Diabetes UK; 2017. **Reduced exercise capacity in patients with type-2 diabetes is associated with poorer skeletal muscle metabolic recovery**

British Microcirculation Society and Cell Adhesion Society (BMS/CAS); 2017. **Type 2 diabetes is associated with reduced exercise capacity, reduced oxygen consumption and poorer skeletal muscle oxidative capacity in older adults**

Artery Research; 2017. **Near Infrared Spectroscopy (NIRS) can detect differences in microvascular reactive hyperaemia in the presence of hypertension**

Physiology Society Skeletal Muscle Symposium; 2017. **Type 2 diabetes is associated with reduced exercise capacity, reduced oxygen consumption and poorer skeletal muscle oxidative capacity in older adults**

Acronyms and abbreviations

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
CK	Creatine Kinase
COPD	Chronic obstructive pulmonary disease
CPET	Cardio-Pulmonary Exercise Test
CVD	Cardiovascular disease
CW	Continuous wave
Deoxy-Hb	Deoxygenated haemoglobin
ECG	Electrocardiogram
ETC	Electron Transport Chain
HCO_3^-	Bicarbonate
METs	Metabolic equivalents
NIRS	Near-infrared spectroscopy
Oxy-Hb	Oxygenated haemoglobin
PA	Physical activity
PCr	Phosphocreatine
PURE	Prospective urban rural epidemiology
RER	Respirator exchange ratio

SABRE	Southall and Brent revisited (study)
6MST	Six minute stepper test
6MWT	Six minute walk test
T2D	Type 2 Diabetes

Chapter 1: General Introduction

1.1 Exercise capacity

1.1.1 Definition of exercise capacity

Exercise capacity can be defined as the maximal or sub-maximal amount of physical exertion that an individual can sustain during a designated exercise test.(1) Exercise capacity is a facet of physical function; physical function (or physical capability) describes ability to perform the physical tasks of everyday life.(2) Good exercise capacity indicates, but is not a requirement for, good physical function. Thus, having good physical function does not necessarily imply good exercise capacity. This relationship is depicted in figure 1.1 which is a simplified model of the factors that influence physical function and exercise capacity. Figure 1.1 is not exhaustive; the key components which influence physical function; exercise capacity, body composition, and psychological factors are shown in pink rectangles and behaviors which influence physical function and exercise capacity are in blue ovals. Cardio-respiratory function is given as a determinant of exercise capacity and skeletal muscle metabolic capacity as components of Cardio-respiratory function in figure 1.1; these terms are central to this thesis and will be defined and described in detail in subsequent sections (‘Cardio-respiratory function’ and ‘Skeletal muscle oxidative capacity’, respectively).

Exercise training is distinct from physical activity. Physical activity can be defined as a behavior involving any bodily movement, produced by skeletal muscles, that requires energy expenditure(WHO:http://www.who.int/topics/physical_activity/en/); exercise training is purposeful, repeated exercises which have the objective of improving capacity (performance) in a given exercise; for example running speed of a specified distance (Figure 1.1).(3) Physical function can be assessed through self-report in questionnaires or,

objectively, via functional tests such as the timed up-and-go test or standing balance often designed for older adults.(2, 4) Quinn et al, have published an extensive clinical review of physical function tests for older adults.(4) Exercise tests vary in design; this will be described in detail in the following section.

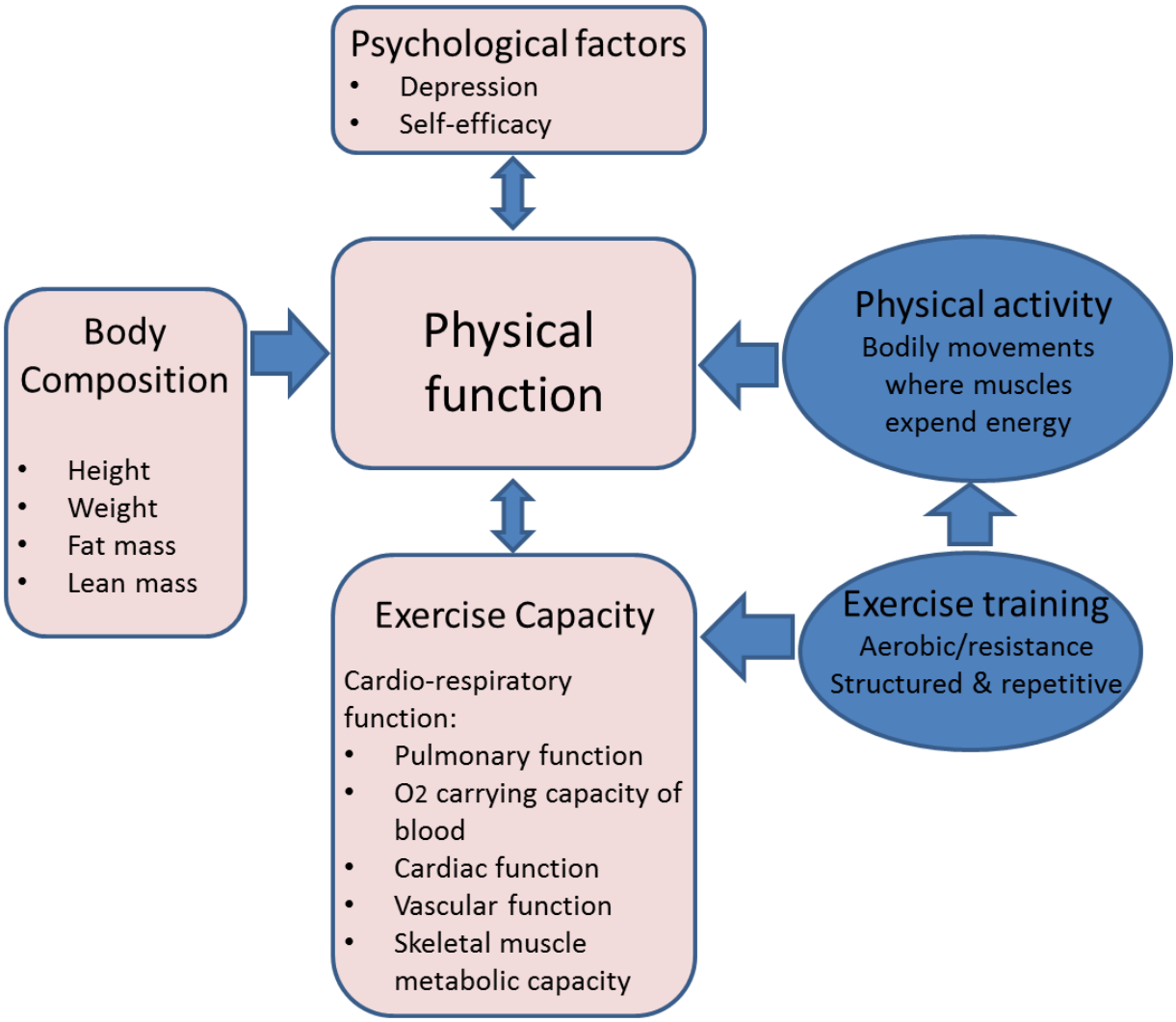


Figure 1.1 A simple conceptual diagram representing the key components that influence physical function. Features inside pink rectangles are factors that influence physical function, including exercise capacity. Behaviours which influence physical function and exercise capacity are inside blue ovals. For simplification the arrows highlight the main relationships and the diagram is not exhaustive.

It is greatly valuable to understand factors which influence physical function in older adults because, with ageing, loss of physical function can lead to loss of independent living.(2, 5) Loss of independent living in older adults is associated with increased social and economic costs.(6) As the older adult population is predicted to increase in numbers in the future, so too is the cost of care.

Exercise capacity declines with age and as a result of many disease processes.(7, 8) Evidence overwhelmingly supports the role of exercise-training to prevent early onset of some disease processes and attenuate age-related declines in physical function.(5, 9) However, the physiological mechanisms underpinning the decline in exercise tolerance with age/disease and the benefits of exercise-training as a preventative therapy for some diseases are not fully understood. Understanding the factors which contribute to the age-related decline in exercise capacity is likely to provide insight into therapeutic/preventative targets.

1.1.2 Measuring maximal exercise capacity

Treadmill and cycle ergometers

The gold standard exercise test protocol is a maximal incremental (graded) workload test. Most common is the continuous exercise testing protocol which involves incrementally increasing the workload (intensity) of the exercise via either speed and/or gradient of the treadmill or resistance on the pedals of the cycle ergometer until the participant can no longer continue (self-reported exhaustion).(3, 10) Discontinuous versions of this test are also used where recovery between loads is given, such as the Naughton test which intersperses 3-minute bouts of treadmill walking at increasing gradients with 3-minute recoveries. Both continuous and discontinuous tests have been show to produce similar values for $\dot{V}O_2$max.(11) The obvious advantage of a continuous test is that it can be carried out relatively quickly.

An incremental exercise test can, theoretically, be conducted using any modality where the workload can be measured and adjusted. Modalities commonly used for maximal exercise testing are treadmill and stationary cycling (cycle ergometer) based tests. These modalities permit well-controlled increments in load to be delivered in a laboratory or clinic environmental where conditions such as temperature and humidity can be controlled. A further advantage of conducting tests in the laboratory or clinic, is that inclusion of continuously monitored gas exchange can be easily carried out. This permits measurement of changes in oxygen consumption ($\dot{V}O_2$), the highest achieved oxygen consumption ($\dot{V}O_2max$) indicates cardio-respiratory fitness. Assessment of cardio-respiratory fitness will be discussed in detail in the subsequent section '*Measuring cardio-respiratory fitness*'.

Treadmill tests are an effective tool for evaluating maximal exercise capacity in healthy participants because walking is a form of exercise which most individuals are generally familiar with and able to undertake. Variations in the grade of the treadmill can be applied to increase the workload without having to increase the speed greatly; as in the Naughton testing protocol.(3, 11) Other example maximal treadmill running protocols include the: Astrand, Bruce, Balke, Ellestad and Harbor protocols.(12) Some protocols maintain speed and increase gradient; such as the Astrand or Balke protocols, while others combine changes in gradient and speed such as the Bruce protocol. Protocols can be smoothed so that the steps of increase in workload are small but more frequent, this is called a ramp protocol. One disadvantage of treadmill testing is its effect on gait mechanics, especially in older participants who are more likely to feel unstable, thus reducing the accuracy of the energy expenditure measurement(13)

The protocols for a maximal cycle ergometer are also graded; workload is typically increased via the resistance on pedals with the revolutions per minute kept constant. Tests can be performed on an upright or supine ergometer. Most commonly, an upright

ergometer is selected, supine testing is useful if, for example, cardiac measurements using ultrasound are collected during exercise. An advantage of a cycle exercise test is that it does not affect gait mechanics in the way that a treadmill test does and the participant will have better stability on the stationary bike. This should improve uptake in older or frailer individuals. A large population-based study, carried out in Germany, described 97.4% uptake of a submaximal cycle exercise test in its adult participants (18-65 years old).(14) In contrast, a Danish study, that specifically enrolled older adults (>60 years old), examined 436 men and 366 women and found that, of all the women invited to exercise, only 56% were willing to undertake the cycle exercise test.(15)

If exercise tests are carried with the objective of comparing fitness between individuals who are likely to have different fitness levels, the duration of the graded exercise test should be considered. If the starting workload is too low and the increments are too small the participant will have to spend a long time exercising before maximum is reached. If the starting load is too high and the increments too big the duration will be considerably shorter. The test duration is important because, with longer duration, factors such as fatigue have a stronger influence on the participants' self-reported termination of the test. Therefore, it is necessary for the person conducting the test to select a starting workload appropriate for the individual. Selection is typically done based on the weight and sex of the participant.

Field tests

Field tests are also used to assess exercise capacity; these can include running on a designated course or running between points at increasing paces.(10) For example, the maximal multi-stage 20-meter shuttle run test (sometime called the beep test) can be used to determine maximum running speed achievable over 20 meters to indicate exercise capacity.(16) Field tests are useful as they permit exercise to be carried out in a 'real-world' environment and, usually, only basic equipment is necessary, making them cheaper

to conduct. The limitations are that physiological changes are less easy to accurately measure. Portable devices for monitoring gas exchange, heart rate, ECG changes and blood pressure have been developed but they require the participant to carry/wear the equipment during the test which could influence the results. Aside from a basic heart rate monitor, this equipment is likely to encumber effort. Measuring exercise-induced changes in blood pressure is not only important to ensure safety during an exercise test,(17) but also, exercise blood-pressure is a useful indicator of cardiovascular health.(18)

Although exercise testing provides an assessment of exercise capacity it is also commonly used clinically to diagnose pathologies such as arrhythmias or coronary disease. In this later context, the exercise test is referred to as an *Exercise-stress test* where exercise-induced cardiac changes during the test act as a diagnostic marker.(19) In the case of an exercise–stress test, termination of the test may be based on the presence of diagnostic markers. ECG monitoring is always recommended for maximal exercise tests in cardiology, therefore, these tests must be carried out in a clinical environment.(2)

In older adults maximal exertion protocols become increasingly more difficult with the onset of co-morbidities and increased frailty. Aside from the balance-related difficulty of treadmill walking, the risks associated with maximal testing become greater in older adults and medical supervision is recommended.(20) This makes the process of maximal exercise testing relatively labor-intensive. There are also several absolute contraindications to maximal exercise testing which are more commonly associated with older adults, for example; a recent cardiovascular event such as a myocardial infarction (MI) in the past 5 days or unstable angina, severe aortic stenosis or diseases of the aorta such as an aortic aneurysm (or repair) and uncontrolled arrhythmias. Uncontrolled hypertension is among several relative contraindications that should also be considered prior to maximal exercise testing.(17) These become more frequently present with age. Submaximal exercise testing is a valuable alternative to maximal exercise testing if the

populations under investigation are older in age or likely to include individuals at risk of adverse events.(21)

1.1.3 Measuring sub-maximal exercise capacity

Sub-maximal exercise tests can be either predictive or performance tests.(21) Predictive tests are designed to permit prediction of maximal cardiorespiratory fitness. Performance tests involve measuring the responses to a standardized activity; for example a stair climb or a timed performance of running or walking a specified distance.

Treadmill and cycle ergometers

A variety of methods for determining $\dot{V}O_2$max from sub-maximal exercise tests have been described.(22) Treadmill and cycle ergometer protocols can be modified to assess sub-maximal exercise capacity. The Modified Bruce treadmill protocol employs an additional initial phase with a lower workload than the initial stage of the maximal Bruce protocol and halves the increments in workload making the test feel smoother to the participant. The test is terminated when 85% of the predicted heart rate is reached. Predictive equations were subsequently developed using data from tests conducted in various groups (active and sedentary men, healthy adults and patients with cardiac disease). These equations use the exercise time achieved during the test which is a marker of the workload achieved, thus, exploiting the linear relationship between workload and oxygen uptake.(23, 24) Modification for the Bruce protocol allows it to be more suitable for less-able individuals; however, the disadvantages associated with stability on the treadmill persist. Pearson's r values for the correlation between predicted and measured $\dot{V}O_2$max were reported between 0.87-0.94 depending on the participant group tested.(25)

Ebbeling et al developed the Single-Stage Submaximal Treadmill Walking Test which involves treadmill walking at one speed and gradient.(26) The predictive equations developed from this test were established using the data from a sample of healthy adults aged 20-59 years old. Although the test itself is a useful means for assessing exercise

capacity in people who are less able or prone to fatigue, the value of predictive equations for use in older or disease populations is dubious.

The Astrand-Ryhming nomogram can be used to estimate $\dot{V}O_2$max from a submaximal Cycle Ergometer test (the Astrand-Ryhming cycle test).(27) It uses the measured heart rate and the workload achieved on the final stage for the estimation of $\dot{V}O_2$max.The nonogram has undergone several modifications to make it suitable for use in a wider variety of study populations than previous predictive equations permitted.(28, 29) This includes incorporation of an age-correction factor permitting use in older adults.(30) Predictive equations depend on the assumption of a linear relationship between heart rate and $\dot{V}O_2$. Although this assumption fails at high workloads and can result in an underestimation of the predicted $\dot{V}O_2$max, it is a necessary assumption. Von Dobeln et al, describe an equation, based on the Astrand-Rhyming nonogram, which aimed to minimise the assumptions necessary to predict $\dot{V}O_2$max. They developed it using exercise data collected from men aged 30-70 years old, and included an age-correction factor permitting its use in older adults.(31) This equation was also developed for women.(30) Other examples of sub-maximal cycle ergometer protocols are: the YMCA bike test and the ACSM bike test.

Self-paced walking tests

Walking is a ubiquitous form of activity, appropriate for the majority of older adults, and is the principal requirement for performing daily activities independently. Therefore, it naturally provides a good basis for a submaximal exercise test. A single-stage, self-paced walking tests is commonly used. The 12 minute walk test (12-MWT) was first introduced by McGavin et al, in 1976 to assess exercise capacity in patients with pulmonary disease.(32) The test involves recording the distance that can be walked in 12 minutes. In this original protocol the test was carried out along a hospital corridor. 29 participants also undertook an incremental maximal cycle ergometer test including analysis of expired gases to

determine peak $\dot{V}O_2$. The authors report a significant positive correlation between distance travelled in the 2nd of three 12-MWTs (carried out to assess reproducibility of the test) and the peak $\dot{V}O_2$ achieved ($r=0.52$; $p<0.01$).(32)

The 6 minute walk test (6MWT) was subsequently developed along the same principals as the 12MWT but was designed for patients with severe respiratory disease.(33) The 6MWT has since been applied to a range of groups of people with severe limitations in exercise capacity,(34-36) including older adults aged 65-89 years old.(37) Strengths of the 6 and 12 MWT are that they are easy to administer, inexpensive and involve walking which is an activity typical for everyday life; this may seem achievable by most participants to at least some extent. Limitations in self-paced walking tests include: requirement of a corridor or space that is big enough (and quiet in terms of thoroughfare) for the test to be carried out. The corridor should be at least 30 meters long(38) in order to reduce turning points during the test which inevitably slow participants(39).

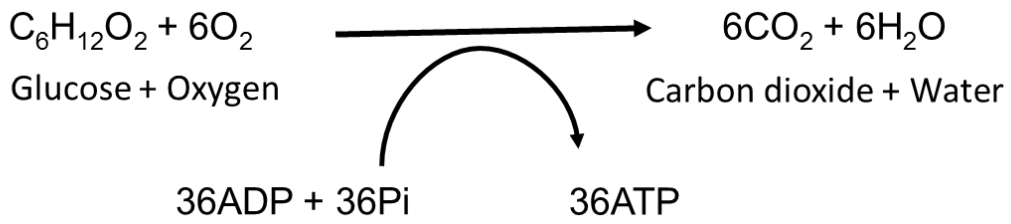
Step tests

Step tests offer a low cost and simple method for assessment of exercise capacity without need for expensive equipment. The Astrand-Rhyming step test was developed as a simple way of predicting $\dot{V}O_2$max from a submaximal exercise test that could be carried out in the home.(40) Other examples of submaximal free-standing step test protocols are: McCardles step test and the Canadian Aerobic fitness test.The low cost of step tests makes them particularly appropriate for studies that enrol a high number of participants. A disadvantage of using a free-standing step test is that it also requires the participant to have reasonable balance and stability.

1.2 Cardio-respiratory fitness

1.2.1 Definition

Cardio-respiratory fitness describes the overall ability to perform moderate-high intensity dynamic exercise for prolonged periods.(41) Energy for performing exercise for periods greater than a few minutes is supplied, largely, via oxidative metabolism. Therefore, cardio-respiratory fitness can be judged as the ability of the body to uptake and utilize O_2. Oxidative metabolism is the process by which oxygen (O_2) and a fuel source are used for production of Adenosine tri-phosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphates (Pi).(3, 42) Oxidative metabolism (using glucose as its fuel source) results in the production of the waste products carbon dioxide (CO_2) and water (H_2O) according to the following:



The processes involved in oxidative metabolism will be discussed in further detail the subsequent section; ‘Cellular oxygen consumption’.

Maximal cardio-respiratory function is measured using a cardio-pulmonary exercise test (CPET), of which, the primary outcome measure is maximal oxygen consumption ($\dot{V}O_2max$) at the whole body level.(3) Specific CPET protocols are described in detail in the subsequent section ‘Measuring cardio-respiratory function’. Briefly, $\dot{V}O_2max$ is measured by monitoring expired gases at the mouth during graded exercise; as exercise workload increases (via increase in speed or resistance) there is a parallel increase in oxygen consumption ($\dot{V}O_2$), if workload continues to increase it becomes impossible for the body to take-up enough oxygen to aerobically generate ATP, eventually, it is impossible to

generate enough ATP to sustain exercise. There are 2 other energy systems activated with initiation of exercise which provide ATP to allow contraction of muscle to be initiated in the absence of oxygen; the Phosphocreatine (PCr) system and Glycolysis.(3) These systems can rapidly provide ATP when oxygen is not available but are limited to a very short period of time. These systems will be discussed in the subsequent section; 'skeletal muscle metabolic function'.

The first experiments to describe $\dot{V}O_2max$ were carried out in the 1920s by Hill et al.(43) Much work has since been carried out to understand the individual components of $\dot{V}O_2max$ and to determine which are rate-limiting.(44) Components contributing to the whole body's capacity to uptake and utilize oxygen (O_2) can be described broadly in terms of four main systems: (I) pulmonary diffusion; (II) the capacity for oxygen carriage in the blood (III) cardiovascular function; and (IV) mitochondrial and cellular energy metabolic function (figure 1.2).(44)

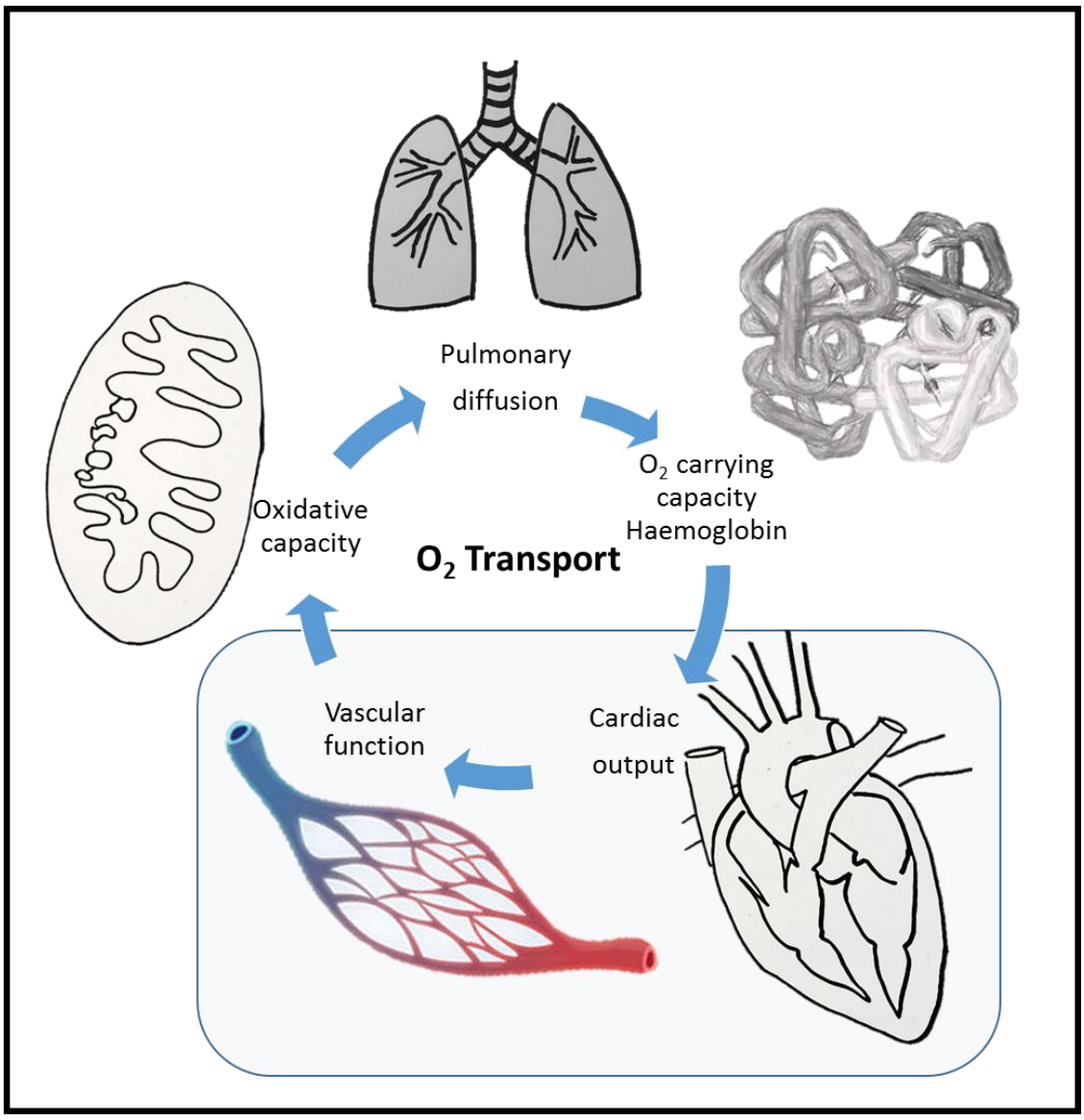


Figure 1.2 Components of oxygen (O_2) transport. A simplified overview of the components contributing to the whole body's capacity to uptake and utilize oxygen (O_2): pulmonary diffusion, the capacity for oxygen carriage in the blood which is determined by haemoglobin, cardiovascular function (encompassing cardiac function and vascular function) and mitochondria oxidative capacity. Adapted from McArdle et al.(3)

If there is an increase in oxygen consumption ($\dot{V}O_2$), O_2 transport must increase through the whole system; each component in the sequence (described above) has the potential to influence maximum throughput ($\dot{V}O_2max$) and therefore could become a rate-limiting factor.(45) Furthermore, each component can influence the performance of other components in the sequence.(45) The next 4 sections will discuss each of these systems (or components) in detail.

1.2.2 Pulmonary diffusion & the capacity for oxygen carriage in the blood

Breathing is under autonomic neural control, as the diaphragm moves downwards negative pressure is created inside the chest and air travels into the airway into the alveoli of the lungs. In healthy humans, in normal atmospheric conditions, a concentration gradient exists between the surface of the alveoli and the pulmonary capillaries, oxygen (O_2) diffuses across this surface and binds with deoxygenated haemoglobin. Haemoglobin then exists in its oxygenated form acting as a transporter for oxygen around the body. The blood in arteries of healthy humans at rest is generally accepted to be >95% oxygenated.(46)

In healthy, non-athletic humans exercising at sea level, pulmonary diffusion is not considered to be rate-limiting.(44) However, there are a couple of situations where this is not the case. Pulmonary diffusion is a limiting factor for maximal oxygen uptake ($\dot{V}O_2max$) in the presence of chronic obstructive pulmonary disease (COPD). This group of diseases, such as emphysema or chronic bronchitis, severely impair oxygen uptake by the alveoli as the lungs. Also, in elite athletes it has been shown that the capacity to greatly increase cardiac output results in rapid red blood cell transit through the pulmonary capillaries which reduces the ability of the lungs to perfuse in full. This is demonstrated by the increased likelihood of elite athletes for desaturation during exercise(47) and the restoration of arterial saturation in elite athletes when exercise is performed in a hyper-oxic (oxygen-rich) environment.(46)

1.2.3 Cardiovascular function

From the lungs, oxygenated blood to carried to the left side of the heart which is responsible for pumping blood around the body; cardiac output is the amount of blood which the heart ejects in 1 minute which depends on the heart rate (contractions per minute) and the stroke volume (usually cardiac output is expressed in L/min).

To initiate movement, central command provides feed-forward signalling which sets the pattern of motor activity in skeletal muscles and initiates cardiovascular activation in order to increase oxygen transport to the muscle. The cardiovascular response during exercise is co-ordinated via the autonomic nervous system (ANS).(48) This comprises withdrawal of parasympathetic activity (vagal tone) and an increase in sympathetic activity which results in an increase in heart rate and cardiac contractility (increase in cardiac output).(48, 49)

The 1960s landmark studies by Ekblom, et al(50) and Saltin, et al(51) demonstrated response of $\dot{V}O_2$max to a 50 days or 16 week training program, respectively. They showed that, in healthy, young men (age 19-27 years old) training-induced changes in $\dot{V}O_2$max were the result of small changes in arterial-venous difference (a-vO_2) but larger changes in cardiac output; estimated as a 3.6% improvement in a-vO_2 and 8% increase in cardiac output.(50) These studies used the dye-dilution method to determine cardiac output and concluded that, as the change in max heart rate was small, it was changes in stroke volume that primarily accounted for improvements in $\dot{V}O_2$max.

Blood travels from the heart through large blood vessels (arteries, arterioles) to very small blood vessels (capillaries) which are interspersed through skeletal muscle permitting diffusion O_2 from the high concentration in the vessel to the cell. Local vasodilation in the active skeletal muscle, coupled with vasoconstriction in non-exercising muscle, viscera and skin, guides the oxygenated blood flow to the exercising muscles to meet the metabolic demand.(52) This specificity in oxygen delivery occurs via afferent mechano-

and metabo(chemo)-reflex signalling in skeletal muscle which send signals to the control centre in the brainstem. Efferent nerve activation modulates the vascular response according to the muscle location and the exercise workload: this is termed the '*exercise pressor response'* (EPR). (Figure 1.3)

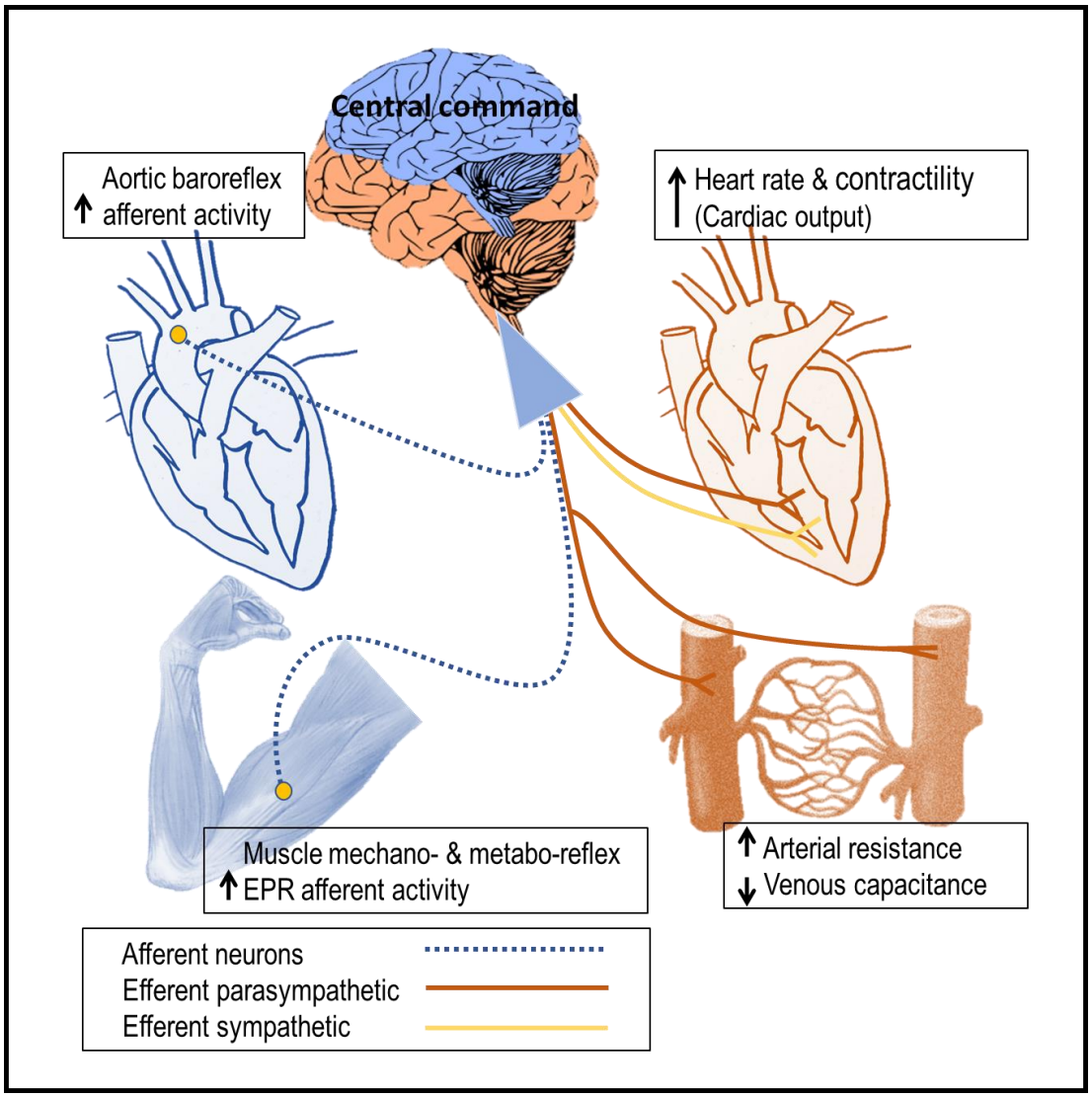


Figure 1.3 Autonomic cardiovascular regulation of the cardiovascular response during exercise. Shown are three neural systems which regulate the cardiovascular control of exercise; central command, the skeletal muscle exercise pressor reflex (EPR) and the arterial baroreflex. Central command recruits motor units for muscle contraction and stimulates cardiovascular control in the brainstem. Onset of muscle

contraction activates metabo- and mechanoreflex afferent neurons which stimulate the cardiovascular control centre. Efferent neural signalling co-ordinates the autonomic nervous system (sympathetic and parasympathetic branches) which controls heart rate/cardiac contractility/cardiac output appropriately for the exercise workload. Figure adapted from Spranger et al, 2015(49)

A further feedback system is the arterial baroreflex; under resting conditions, baro-receptors feedback blood pressure information to the control centre in order to maintain a normal resting blood pressure. During exercise blood pressure increases and the arterial baroreflex is reset in an exercise intensity-dependent manner but remains as sensitive in its regulatory response during exercise as it is at rest.(53) It is thought that the EPR and central command play a role in re-setting the arterial baroreflex (Figure 1.3).(53)

At rest cardiac output (CO) is ~5L/min, blood flow is distributed fairly evenly between the skeletal muscle (~15-20%; ~0.75L/min), brain (~15%; ~0.75L/min), liver & intestine (~20-25%; ~1L/min) and kidneys (~20%; ~1L/min). A smaller proportion is distributed to cardiac muscle (coronary arteries) (4-5%; ~0.2L/min) (figure 1.4) and skin, bone and other organs (not shown here). During heavy exercise, cardiac output increases to as much as 25L/min; between 80-85% (~20L/min) of this blood flow is distributed to skeletal muscle. The absolute blood flow to the coronary arteries als increases (from 0.2L/min to 1L/min), while absolute blood flow to brain, kidneys and intestine remains fairly constant (figure 1.4)

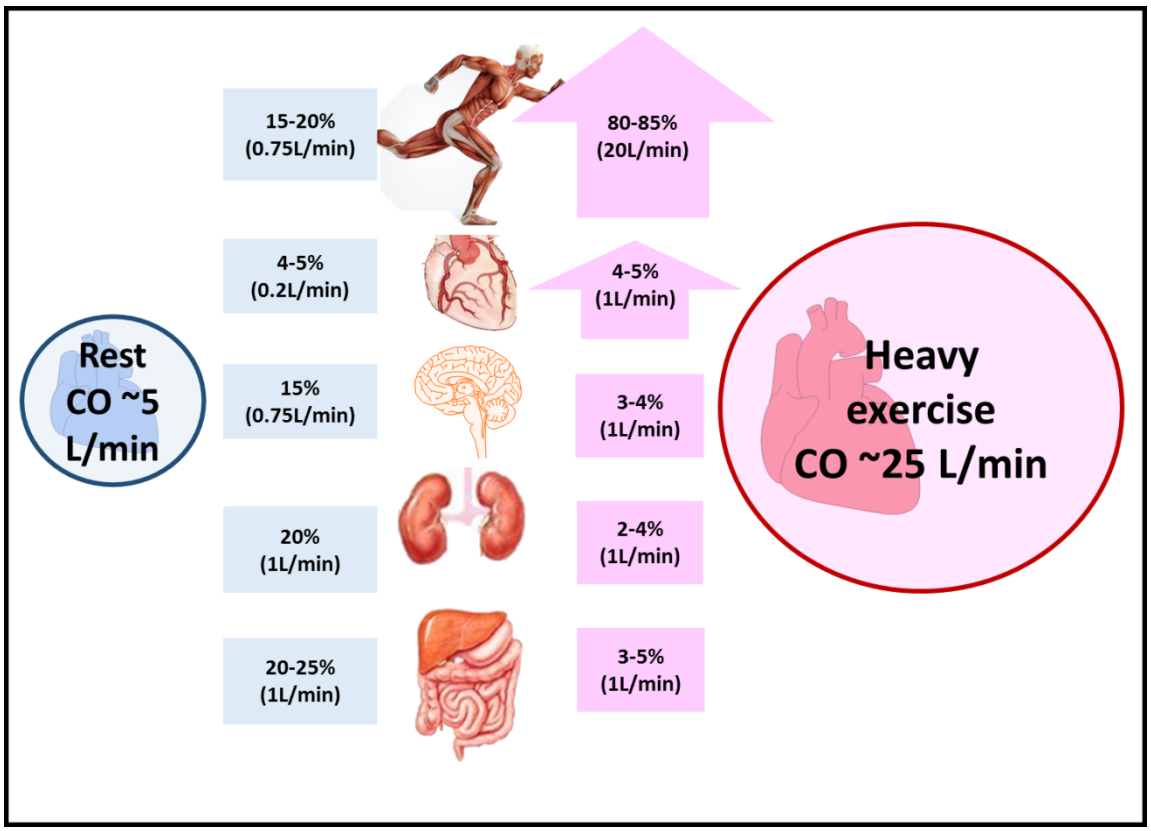


Figure 1.4 Blood flow redistribution during heavy exercise. Cardiac output (CO) is ~5L/min at rest, ~15-20% of this is distributed to the skeletal muscle, while blood flow is similar to the brain, gut and kidneys. A smaller proportion is distributed to coronary arteries (4-5%) and skin, bone and other organs (not shown here). During heavy exercise, cardiac output increases to as much as 25L/min; 80-85% of this output is distributed to skeletal muscle. Figure first described by Astrand.(52)

Venous return is increased during exercise via the skeletal muscle 'pump' and also via the respiratory 'pump'. This leads to an increase in end diastolic volume and, therefore, an increase in stroke volume. In humans, there is conflicting evidence regarding whether prolonged exercise is associated with a change in limb venous compliance.(54, 55)

1.2.4 Cellular respiration

In human skeletal muscle cells ATP is broken down to provide energy for actin-myosin cross-bridge formation and generation of force by contracting muscle.(56) ATP is produced from adenosine diphosphate (ADP) and inorganic phosphate (Pi). There are several processes through which ATP can be generated. Two anaerobic energy systems allowing rapid supply of ATP for cross-bridge cycling and oxidative ATP production.(3)

Initially, in the first few seconds of exercise, increased ATP demand is supplied by phosphocreatine (PCr) breakdown, this is followed by glycolytic ATP production and then complete activation of the oxidative phosphorylation pathway (1-2 minutes). Figure 1.5 is a schematic representation of these systems of ATP production during ~5minutes of exercise at a consistent, sub-maximal rate of exertion.(57)

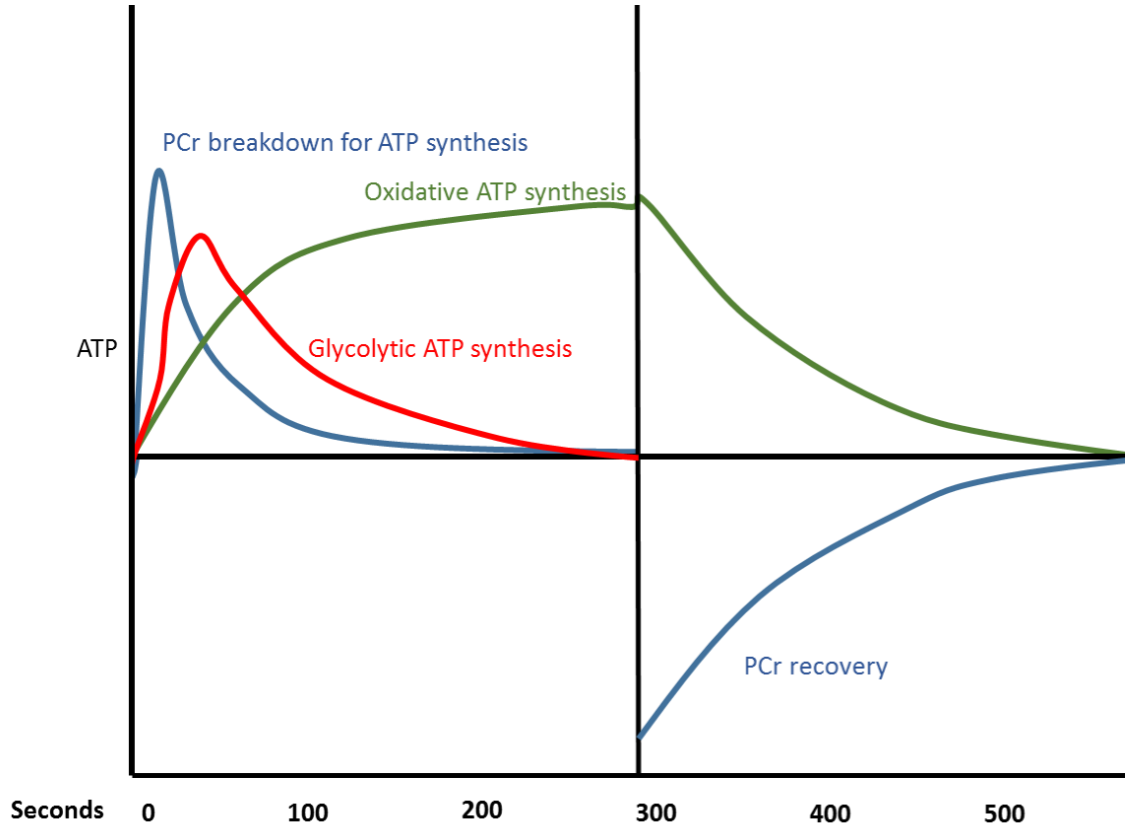


Figure 1.5 A schematic overview of the changes in energy systems required to generate adenosine triphosphate (ATP) during a short period of sub-maximal

constant-rate exercise. Rapid activation of ATP synthesis through phosphocreatine (PCr) breakdown is represented by the blue line, oxidative phosphorylation by the green line and glycolytic ATP production by the red line. During the recovery period, PCr is replenished through oxidative ATP synthesis only. The recovery can be fit by a mono-exponential curve from which a rate constant can be derived. This figure was adapted from Fiedler et al, 2016.(57)

Glycolysis occurs in the cytoplasm of the cell and produces pyruvate. Under aerobic conditions (when oxygen is present) pyruvate is converted to acetyl CoA and continues to the Kreb's cycle (figure 1.6). Energy is released from the acetyle groups as reduced coenzymes (NADH, FADH2). These are oxidized in the ETC to facilitate proton (H^+) transport across the inner mitochondrial membrane from the matrix to the inter-membrane space. The majority (32 molecules) of ATP is produced via ATP-synthase which is a protein spanning the inner membrane of the mitochondria (figure 1.6). Thus, mitochondria provide the site for oxidative ATP synthesis; this will be discussed in more detail in the subsequent section '*Mitochondria'*.

Glycolytic ATP synthesis is also activated for short periods at high intensities of exercise(57). Under anaerobic conditions, pyruvate is converted to lactic acid (lactate) and a small amount of ATP is produced. As exercise intensity increases the glycolytic pathway is activated more to compensate for the slow speed of ATP production via the ETC, thus, more lactic acid (lactate) is produced. Lactic acid is buffered by bicarbonate (HCO_3^-) which increases pulmonary CO_2, and the pulmonary system responds by increasing ventilation in an attempt to eliminate the excess CO_2.

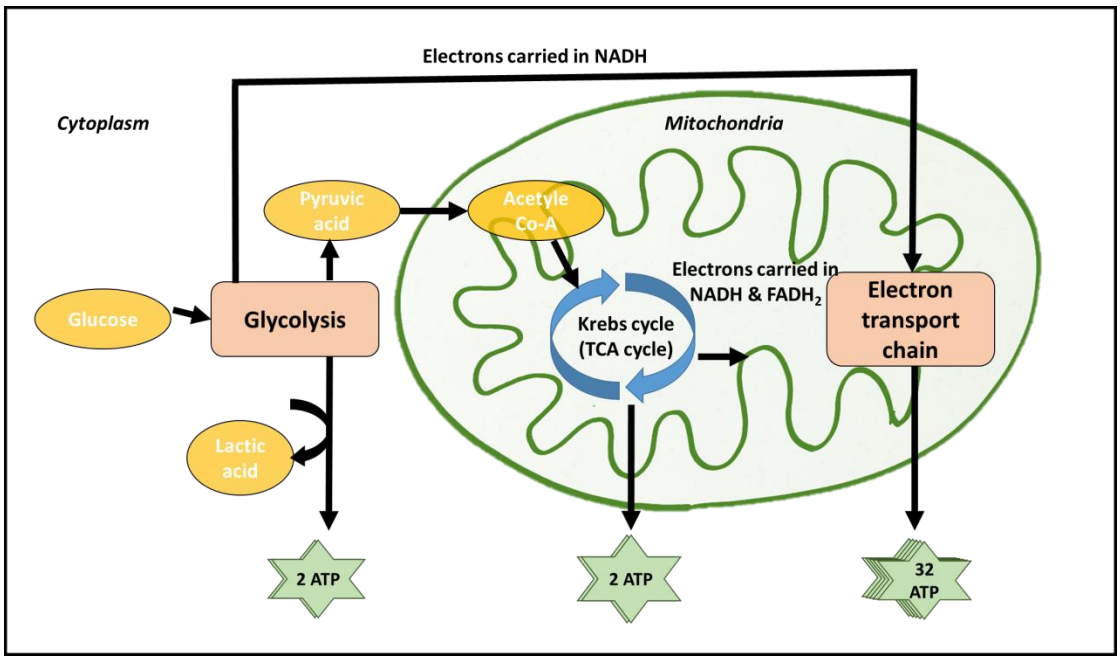


Figure 1.6 a simplified schematic representation of cellular generation of ATP. Two pathways for ATP generation are represented here. Glycolysis, which occurs in the cytoplasm, and, the Krebs cycle and electron transport chain (ETC), which occur in mitochondria in the presence of oxygen. ATP; adenosine triphosphate, NADH; Nicotinamide adenine dinucleotide, FADH; flavin adenine dinucleotide, TCA: tricarboxcylic acid cycle.

Human studies have previously shown what might appear to be a discordant response to training between oxidative capacity (quantified by the levels of mitochondrial enzymes present in biopsy samples of skeletal muscle) and measured $\dot{V}O_2$max. Mitochondrial enzymes can be doubled with training while $\dot{V}O_2$max increases by only 20-40%.(58) However, this can be explained by the improvement in exercise performance that is aligned with the increase in oxidative capacity, specifically an increase in exercise endurance performance.(59, 60) In addition, since these early studies, it has been shown that low-intensity training may improve mitochondrial function without improving $\dot{V}O_2$max.(61)

1.2.5 The importance of cardio-respiratory function

In a large meta-analysis improved cardio-respiratory fitness was associated with lower risk of all-cause mortality and incidence of cardiovascular disease (CVD).(62) This analysis included 33 studies, cumulating data from 102,980 individuals who were followed-up for all-cause mortality and 84,323 individuals followed-up for a combined risk of CVD and coronary heart disease (CHD). Cardio-respiratory fitness was described in metabolic equivalents (METs). One MET is equal to energy expenditure during seated rest; the meta-analysis concludes that having a maximum cardio-respiratory fitness above 7.9 METs (that is 8 times higher than resting) will substantially lower mortality risk (relative risk for all-cause mortality of low cardio-respiratory fitness compared to high was 1.70 (95%CI, 1.51-1.92; P< 0.001) and risk for CHD/CVD events was 1.56 (95%CI, 1.39-1.75; P<0.001)).(62) Follow-up years of the studies included ranged from 1.1 to 26 years. This was a comprehensive analysis, including an adjustment for publication bias (trim and fill).(62)

Interestingly, reduced muscle strength, captured by a simple hand-grip measurement, has also recently been described as a predictor of all-cause mortality and cardiovascular morbidity. This analysis was carried out using data from a large, international, longitudinal population study comprising 139 691 participants with a 4-year follow-up period; The Prospective Urban-Rural Epidemiology (PURE) study.(63) Further to this, Celis-Morales et al showed, in a large prospective UK-based study of 67, 702 participants, that strength and predicted $\dot{V}O_2$max, are moderators of the association between physical activity and mortality.(64)

1.2.6 Measuring cardio-respiratory fitness

Cardio-respiratory fitness is measured using a cardiopulmonary exercise test (CPET). This involves the individual undertaking a graded exercise protocol (as described in the previous section '*Measuring maximal exercise capacity*') while continuously monitoring

changes in oxygen and carbon dioxide being inspired and expired. This is typically done with a face mask that covers the nose and mouth completely. The gold standard exercise protocol involves continuous incremental (graded) loading on a selected exercise ergometer until exhaustion. Cardio-respiratory fitness is reported in terms of $\dot{V}O_2$max, often also adjusted for body weight (ml/min/kg); some studies report $\dot{V}O_2$max as a representative measure of exercise capacity. However, although over a wide range of values, cardio-respiratory fitness and exercise capacity are positively correlated, strictly speaking, they are not necessarily in line with one another, especially when the range of $\dot{V}O_2$max values is narrow.(65)

During a CPET the increase in exercise workload is linearly related to the increase in oxygen consumption throughout the test. Theoretically, a maximal CPET is defined by a plateau in the measured $\dot{V}O_2$ despite further increases in workload.(3) A plateau in $\dot{V}O_2$ is not always achieved, CPET protocols are generally terminated when the participant reports reaching exhaustion and an individual may terminate the test due to lack of motivation or fatigue. In this case, the $\dot{V}O_2$ achieved is referred to as the Peak $\dot{V}O_2$. Various physiological parameters, derived from the real-time gas analysis, allow investigators to decide if suitable effort has been made during the test to support attainment of a peak $\dot{V}O_2$. For example, if the respiratory exchange ratio (RER) is >1.1 or heart rate reaches within 10 beats of the age-predicted maximum. However, critical review suggests that a ‘true maximal’ cardio-respiratory effort is seldom reached at self-reported exhaustion and an extended rigorous protocol is necessary to ensure maximal $\dot{V}O_2$ has been achieved.(66) In this light, obtaining a ‘true’ measurement of $\dot{V}O_2$max becomes difficult even in the most motivated individuals. In older adults maximal effort is often not achieved during a CPET.(67) As described previously, sub-maximal exercise tests can be carried out in order to predict $\dot{V}O_2$max, this is a valuable alternative.

Typical exercise modalities employed for CPET are modalities which activate the large muscle groups, such as; treadmill running, stationary cycling (cycle ergometers) and bench stepping. Variation in the $\dot{V}O_2$max values exists when the tests are conducted using different modalities; this reflects the quantity of muscle enrolled in performing the exercise. For example, treadmill running usually produces a higher $\dot{V}O_2$max in the same person than they would achieve during a cycle ergometer test.(68, 69)

1.3 Skeletal muscle metabolic function

In this thesis skeletal muscle metabolic function is defined as the oxidative capacity of the mitochondria within skeletal muscle. Skeletal muscle metabolic dysfunction means '*impairment in the mitochondrial oxidation of substrates (lipid and carbohydrate) resulting from an impairment in oxidative phosphorylation*'.

1.3.1 Mitochondria

As described previously in the section 'Cellular oxygen consumption: Oxidative metabolism', mitochondria provide the site for ATP production via oxidation of nutrients (carbohydrate and lipid) and phosphorylation of ADP; oxidative phosphorylation. Mitochondrial are subcellular organelles that can vary in size, shape, number and location within the cell. They have a smooth outer membrane and a folded inner membrane. The protein complexes of the ETC span the inner membrane and the mitochondrial matrix (space inside the inner membrane) is the site for the Krebs cycle.(70) Mitochondria have their own autonomously replicating DNA (mtDNA) separate from the cells nuclear DNA and thought to be inherited only from the maternal line. mtDNA is arranged in structures called nucleoids which are composed of DNA-binding proteins involved in mtDNA maintenance and transcription as well as a range of peripheral signalling factors that regulate mitochondrial biogenesis. In humans, mtDNA is arranged in circular sets of 16,569 base pairs and codes predominantly for mitochondrial RNA. (71, 72)

It is worth recognising that, as well as being the site of cellular metabolism, mitochondria play a number of additional roles within the cell, these include: cell signalling, generation and clearance of reactive oxygen species (ROS), regulation of cell apoptosis (to some degree), transport of the mitochondria within the cell and regulation of intra-cellular calcium. As a result of their diverse functions a number of definitions of 'mitochondrial dysfunction' have been described.(73-75)

Mitochondrial dysfunction can be described in terms of; reduced mRNA levels of mitochondrial markers,(76) reduced protein levels (1, 5) or a reduction in enzymatic activity of key components of mitochondria driven oxidation (1, 6, 7, 8, 9), changes in mitochondrial size and shape (by electron microscopy),(5, 7, 10) impaired flexibility for substrate oxidation (6, 8) or propensity for production of ROS. The ability of mitochondrial to produce ATP through oxidative phosphorylation (oxidative capacity) is a component of mitochondrial function. Methods for the assessment of oxidative capacity will be discussed in subsequent sections.

1.3.2 In Vitro assessment of oxidative capacity

In vitro examination of skeletal muscle tissue biopsies allows oxidative capacity to be probed at the cellular and molecular levels. This is an important approach when investigating the underlying mechanisms involved in oxidative phosphorylation. Methods for *in vitro* and *in vivo* assessment of oxidative capacity are discussed in further detail in this and the section below. An overview of the main methods used to assess mitochondrial oxidative capacity of skeletal muscle is provided in table 1.1. This table is not exhaustive.

Aspect of oxidative capacity	Method of assessment
Mitochondrial morphology and size & position	Electron microscopy
Mitochondrial content	• ETC complex I-V protein content • Mitochondrial DNA content
Protein synthesis rate	Proteins are labelled in vivo and muscle biopsy samples analysed by gel electrophoresis & mass spectrometry to identify abundance
Mitochondrial biogenesis	Expression of genes involved in mitochondrial biogenesis or metabolic regulation (examples: PGC-1α and NRF)
Enzymatic activity	Activity of enzymes specific to oxidative phosphorylation (examples: citrate synthase or succinate dehydrogenase activity)
ATP synthesis	• Bioluminescence technique is used to visualise ATP production stimulated by step-wise addition of different substrate combinations , inhibitors and uncouplers to the medium • [^{31}P]MRS measured PCr recovery rate constant following depletion by exercise (oxidative ATP synthesis only)
Oxygen consumption (cellular respiration)	Polarographic measurement of oxygen consumption can be carried out using: isolated mitochondria, cell cultures or permeabilized muscle fibres

Table 1.1 An overview of some of the key aspects of mitochondrial oxidative capacity and example methods for assessment. ETC; electron transport chain, PGC1α; Peroxisome proliferator-activated receptor γ coactivator α, NRF; nuclear respiratory factor 1, MRS: magnetic resonance spectroscopy, PCr; phosphocreatine.

Mitochondrial size, morphology & position

A transmission electron microscope can be used to visualize size morphology, position and number of mitochondria within skeletal muscle cells. In the early 1970s Hoppeler et al, showed an increase in number and size of the mitochondria in the skeletal muscle in response to endurance training.(77) More recently Burkart et al, described increased mitochondrial numbers that were smaller in size in the pluripotent stem cells of individuals with diabetes.(78) The placement of mitochondria inside the myocytes (subsarcolemmal or intermyofibrillar) has also been shown to determine their oxidative capacity;

intermyofibrillar mitochondrial content was positively associated with metabolic fuel source flexibility.(79)

Enzyme activities

A commonly applied approach to assessing mitochondrial capacity for oxidative phosphorylation is to quantify concentration or maximal activity of selected complexes in the ETC, such as NADH-dehydrogenase (NADH:CoQ Oxidoreductase; complex I) or Cytochrome-C-Oxidase (complex IV), or Krebs cycle enzymes such as citrate synthase (figure 1.7).(74) Enzyme concentrations (protein content) can be assayed using antibody technology. To determine enzyme activity, skeletal muscle tissue samples can be frozen and analysed using spectrophotometric-based enzyme activity assays that are commercially available. This makes both methods simple and appealing. In addition, expression of these enzymes can be determined by assaying transcripts in microarrays.

The benefit of these measurements is that they do not require large amounts of sample tissue and the tissue can be frozen allowing analysis to be conducted at a later time, they are therefore a fairly simple and useful technique for mechanistic insight into human muscle tissue. However, there are a few important limitations. The assumption which these methods are based on is that alteration the protein(s) investigated directly alters rate of oxidative phosphorylation, thus, if the protein is not altered function is not impaired. In this scenario false positive, and false negative, results can be produced; single reactions are not likely to be representative of the entire complex process of oxidative phosphorylation. A further limitation of these measurements is that they require degradation of cell structure and, therefore, damage to the morphology of the mitochondrial reticulum (the way that the mitochondrial are arranged within the cell). The mitochondrial reticulum is thought to provide a conductive pathway for energy distribution throughout the skeletal muscle cell, thus allowing rapid communication between

mitochondria; destruction of this is likely to be detrimental to overall functional capacity.(80)

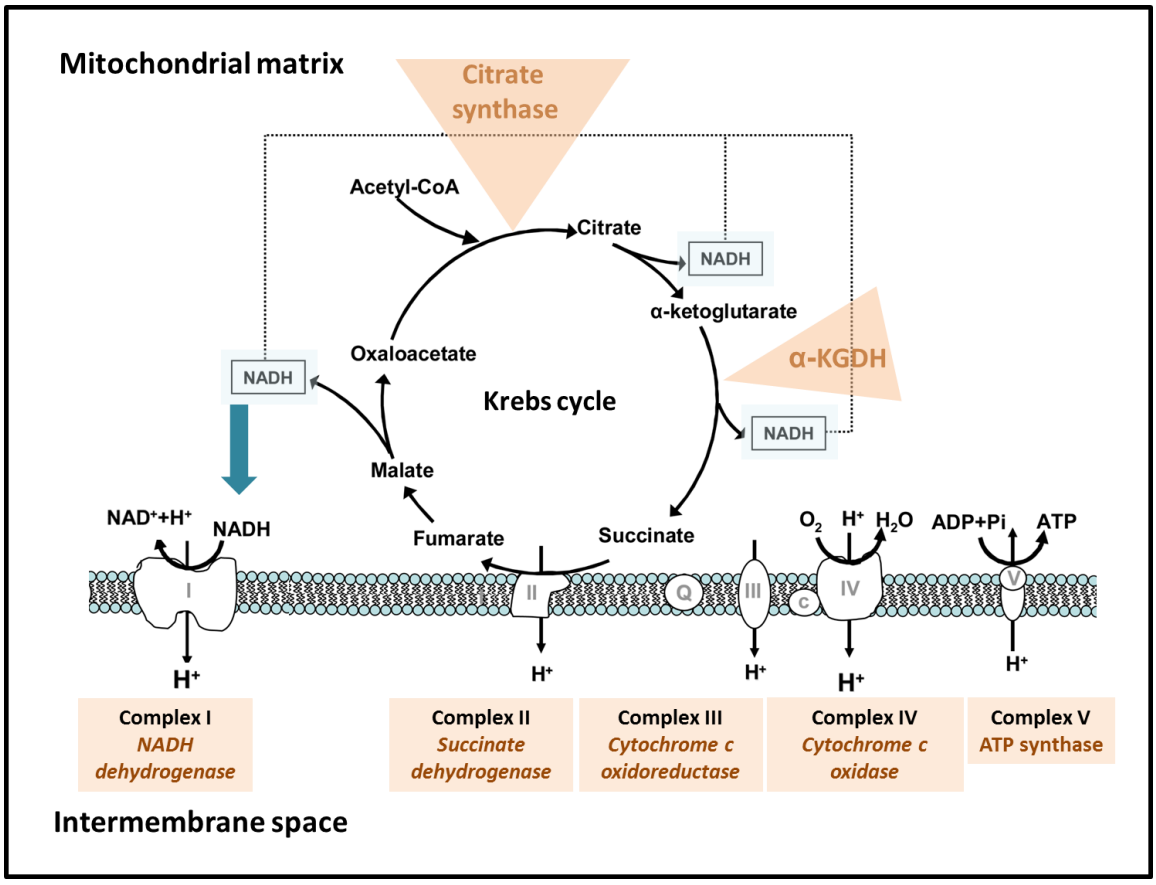


Figure 1.7 A simplified overview of mitochondrial oxidative phosphorylation and the enzymes typically measured. Oxidation of carbon substrates in the Krebs cycle (or tricarboxylic acid (TCA) cycle) within the mitochondrial matrix generates reducing equivalents (NADH) that subsequently provide electron flow to the electron transport chain (ETC). Electrons are transferred from NADH through NADH dehydrogenase and oxidation of succinate by complex II (succinate dehydrogenase). The proto gradient generated across the inner mitochondrial membrane drives phosphorylation of ADP to ATP by ATP synthase. Succinate dehydrogenase is also sometimes known as Succinate-Q oxidoreductase. Cytochrome c oxidoreductase is also sometimes known as cytochrome bc1 complex. α-KGDH; alpha ketoglutarate dehydrogenase, NADH;

Nicotinamide adenine dinucleotide. This figure was adapted from Lanza et al, 2009.(74)

ATP production

ATP synthesis rate can be quantified directly from isolated mitochondria. This involves exploitation of a photon-emitting reaction between luciferin, firefly luciferase and ATP.(74) Briefly, Isolated mitochondrial (in solution) are provided with the appropriate substrates to generate ATP. In the presence of ATP, Luciferin adenylate is generated from Luciferin, if oxygen is also present, luciferin adenylate becomes oxyluciferin and photons are emitted during this reaction. A microplate luminimeter with a photoncounting photomultiplier tube measures bioluminescence and, the concentration of ATP present is quantified by the light signal generated. This is performed several subsequent times, thus the rate of ATP synthesis is determined as the slope of the concentration over time. This technique has previously been described in detail.(74) The authors also describe methods for isolating mitochondria which extricate subsarcolemmal from intermyofibrillar placed mitochondria; an important distinction given the differences in functional capacity of these differentially placed mitochondria discussed above.(79)

On the surface, this appears to be a very simple method of assessing oxidative capacity, however, the combination of substrates and inhibitors included in the medium needs careful consideration.(74, 81) Different substrate combinations (conditions) allows investigation of specific ETC complex activities and, if an overall function is to be determined, then all pathways should be considered. For example, addition of glutamate and malate (GM) allows exclusive assessment of ATP production through NADH dehydrogenase (Complex I; figure 1.7). This is because addition of GM provides the substrates for dehydrogenase reactions within the Krebs cycle generating NADH which is oxidised by NADH dehydrogenase. Concurrently the increased malate concentration inhibits succinate dehydrogenase activity (complex II), therefore, specifically complex I

activity is measured. Assessment of ATP production is undertaken using a step-wise protocol involving addition of substrate, inhibitors and uncouplers. This includes non-mitochondrial ATP production via non-oxidative pathways; therefore, comprehensive assessment of oxidative ATP production capacity can be determined. Methodological details of the different substrate combinations typically used for specific complex activity has previously been described in detail.(74)

ATP synthesis rate determined during each condition can be compared by adjusting (or normalizing) the rate for the amount of mitochondria present in each individual medium. Typically normalization is for tissue weight or a marker of mitochondrial content such as citrate synthase activity, mitochondrial protein content or mitochondrial DNA copy number.(73, 74)

Oxygen consumption

As oxygen is reduced in the process of oxidative ATP production, quantifying the rate of oxygen consumption in mitochondria is also a way of examining oxidative capacity. The technique of measuring oxygen consumption is also referred to a mitochondrial (or cellular) respirometry. In skeletal muscle it can be carried out in isolated mitochondria, cultured cells and permeabilized muscle fibres. Previously this was not appropriate for human studies because a large amount of tissue was necessary; however, advances in technology have led to this technique of high resolution respirometry being possible with smaller sample volumes.(82, 83)

Oxygen consumption can be measured in a medium of respiring mitochondria using a Clark-type oxygen electrode. This is known as polarographic measurement of oxygen consumption and it can be carried out with small tissue samples. In the Clark-type system the anode and cathode are attached by a salt bridge covered in an oxygen permeable membrane. When O_2 diffuses over the membrane it is reduced creating a current which is proportional to the concentration of oxygen. Similarly to the techniques for assessing ATP

synthesis rates, a protocol is employed where substrates, inhibitors, and uncouplers are added to the medium in subsequent phases to provide comprehensive assessment of oxidative capacity across selected mitochondrial enzymes.(82)

Mitochondrial Biogenesis

Production of new mitochondria (mitochondrial biogenesis) is regulated by a co-ordination of multiple nuclear and mitochondrial genes. Peroxisome proliferator-activated receptor γ coactivator α (PGC1α) is a transcriptional coactivator (a protein or protein complex that increases the probability of a gene being transcribed by interacting with transcription factors but not itself binding to DNA) that plays a major role in regulating mitochondrial biogenesis.(84) PGC1α interacts with nuclear respiratory factor 1 (NRF1) to stimulate transcription of other mitochondrial genes. Transcription factor A (TFAM) is also stimulated by the PGC1α/NRF1 interaction which controls mitochondrial DNA replication and transcription. Because of its key role as a regulator of energy metabolism, expression of PGC1α indicates greater presence of mitochondrial and therefore, increased capacity for oxidative phosphorylation. Endurance exercise training is known to increase expression of PGC1α in human skeletal muscle(85) and expression of PGC1α has been reported to be down regulated in the presence of diabetes.(86, 87)

Limitations of in vitro assessment methods

Removing tissue from its physiological environment, either to isolate mitochondria or to perform measurements at the cellular level, exposes the it to non-physiological oxygen levels and temperatures.(73) Although tight control is usually employed to ensure minimal exposure, there still remains potential for this exposure to influence the measurements that are subsequently made and conclusions drawn from the experiment. Furthermore, and perhaps most importantly, muscle biopsies require a relatively large needle to extract the tissue sample; this may cause the participant some discomfort and potentially cause

psychological stress.(88) Many of the studies that closely examine the molecular detail of skeletal muscle enroll small sample sizes

To understand functional capacity of skeletal muscle it would be useful to develop non-invasive *in vivo* methods of assessment which can be made accessible for use in greater participant numbers and in a wider range of dynamic environments.

1.3.3 In Vivo assessment of oxidative capacity

Magnetic resonance spectroscopy (MRS)

Phosphorous magnetic resonance spectroscopy (^{31}P-MRS) can be used to measure any phosphorous-containing metabolites, therefore, ATP and phosphocreatine (PCr) concentrations can be quantified *in vivo*. MRS is based on the same principles as magnetic resonance imaging (MRI); instead of the hydrogen nuclei (H^+), a phosphorous nuclei is detected. The ^{31}P-magnetic resonance spectrum of skeletal muscle has 5 peaks: inorganic phosphate (Pi), PCr and the three phosphate moieties of ATP (α, β & γ). These peaks are due to differences in resonance frequency in the magnetic environment, which is shifted depending on the molecule or molecular site of the nuclei.

A saturation transfer experiment can be carried out to determine the effect of different nuclei that are associated i.e. in the case where one nuclei is consumed in order to produce another, for example, inorganic Pi and ATP. In this scenario, the resonance signal is sensitized to the rate of exchange (Pi – ATP) by selectively saturating the equilibrium magnetization of one of the nuclei and measuring the effect on the signal strength of its partner.(89)

The saturation transfer method has previously been used to determine resting rate ATP synthesis and interpreted as a measure of mitochondrial oxidative capacity.(90, 91) However, the problem with this interpretation is that, compared to other methods, this method overestimates resting oxidative ATP production in skeletal muscle as it cannot

differentiate between glycolytic, oxidative and PCr pathways; synthesis rates are not in line with rate of oxygen consumption measured directly from the tissue.(92, 93)

MRS also offers a valuable alternative to the saturation transfer method. This method depends on the rate of PCr recovery following exercise. It is useful to first describe the PCr shuttle system in order to fully understand this method.

1.3.4 The phosphocreatine (PCr) shuttle

The phosphocreatine (PCr) "shuttle" system provides a means for rapid ATP synthesis through the enzyme Creatine Kinase (CK). PCr is a smaller and less negatively charged molecule than ATP which allows it to diffuse into the cytoplasm more readily. CK is present in the mitochondria and in the cytosol. The mitochondrial isoform of CK catalyzes production of PCr using energy from ATP, synthesized through oxidative phosphorylation. The cytosolic isoform of CK facilitates the breakdown of PCr to provide the energy to generate ATP from ADP (equation 1.1) in the cytosol which facilitates cross-bridge formation.(94)

$$MgADP + PCr^{2-} + H^{+} \leftrightarrow MgATP^{2+} + Cr \qquad (1.1)$$

Figure 1.8 provides an overview of the phosphocreatine shuttle system. This system provides the basis for a further method that can be used to assess the functional capacity of the mitochondria in skeletal muscle.

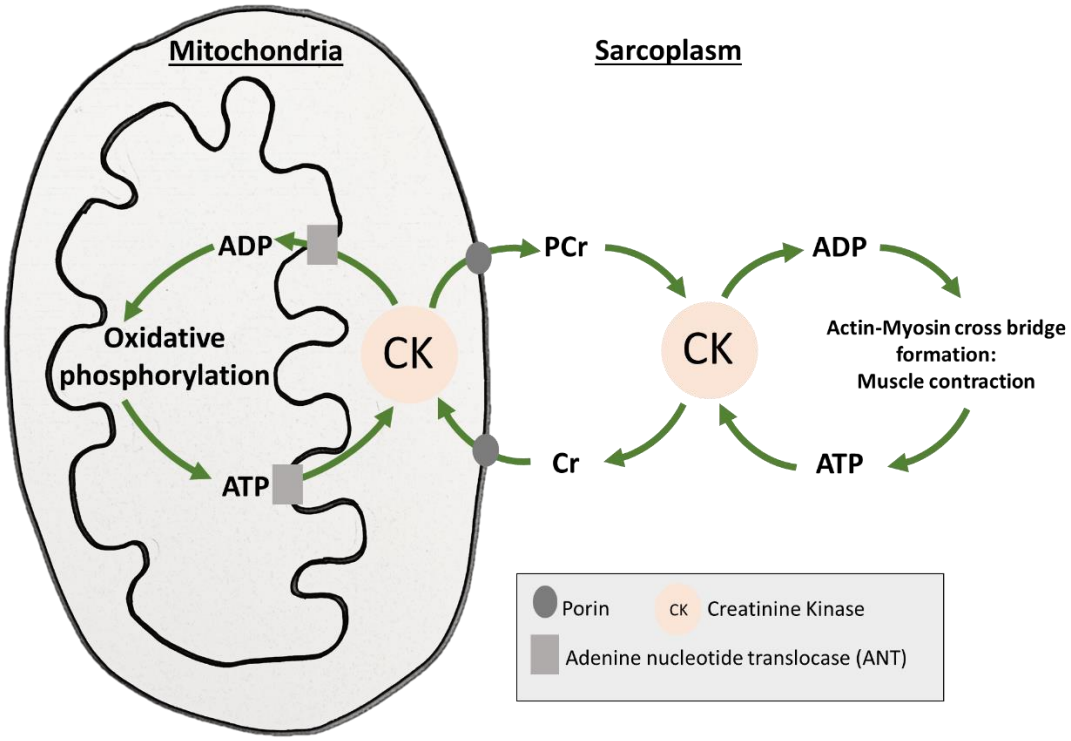


Figure 1.8 Schematic overview of the Phosphocreatine "shuttle" system. ADP: adenosine diphosphate; ATP: adenosine triphosphate; CK: creatinine kinase; Cr: free creatinine; PCr: phosphocreatine. Adapted from Guimarães-Ferreira, 2014.(94)

PCr is rapidly depleted within the first ~1minute of exercise. Immediately post-exercise PCr is regenerated and the ATP used for re-synthesis is exclusively derived from oxidative phosphorylation.(95) As described above, dynamic changes in ATP turnover can be assessed with high temporal resolution ^{31}P-MRS. If it is assumed that ATP is stable, that there is negligible glycolysis during recovery and that there is a mono-exponential recovery of PCr, the recovery rate of PCr can be used as an indicator of maximal oxidative capacity in the muscle.(73, 89, 93) Figure 1.9 demonstrates this technique by showing the change in resonance spectra with exercise (top panel) and the plot of recovery against time with a mono-exponential fit to the points.

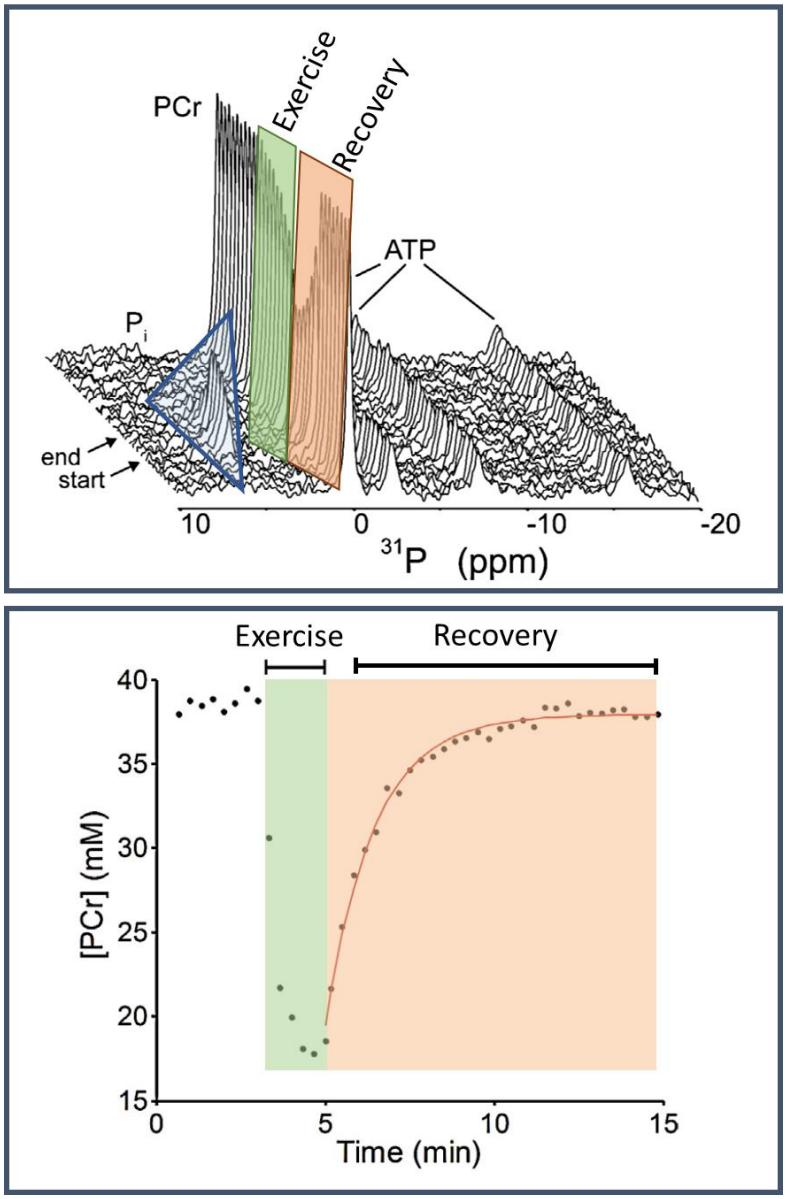


Figure 1.9 An example data set where MRS has been used to determine phosphocreatine (PCr) recovery following a short bout of exercise. The top panel is a stack plot showing the ^{31}P-MR spectra of skeletal muscle during rest (no colour), exercise (green) and recovery (orange). During the exercise PCr stores are depleted and there is a spike in the signal from inorganic phosphate (Pi; blue triangle). During recovery, PCr stores are re-synthesised. The rate of re-synthesis of PCr is derived as a time constant from a plot of PCr during recovery against time (example data is shown in the lower panel). Adapted from Prompers et al.(89)

The limitations of MRS are that it is expensive, some individuals cannot tolerate MRI scanning (largely due to claustrophobia) and contraindications to MRI, such as any metallic implants, mean that it is not suitable for everyone. In addition, there are limited dynamic activities which can be initiated inside the scanner due to the small environment and the common necessity that the participant is lying supine.

Faster recovery rates of PCr, determined in this way, represent better skeletal muscle oxidative capacity. Superior PCr recovery rates have previously been demonstrated in athletes versus non-athletes; including wrist flexor recovery rates in rowers(96) and gastrocnemius recovery in track athletes.(97) In competitive track athletes, training to compete over different distances imparted skeletal muscle benefits to varying extents. Endurance runners profited most greatly in terms of skeletal muscle PCr recovery.(98, 99)

1.3.5 Oxidative capacity and exercise capacity (performance)

Although, over a broad range of values, $\dot{V}O_2$max has an established relationship with exercise capacity, inside a narrow range of values, individuals with a similar $\dot{V}O_2$max may perform differently in terms of exercise capacity. For example, elite endurance runners who have similar $\dot{V}O_2$max may complete a 10-k race in different times.(100) In 1980 Conley et al described these differences in performance being due to differences in 'running economy'. Running economy is quantified as the $\dot{V}O_2$ required for running at a given pace.(100) Holloszy and Coyle proposed that improved endurance performance (a lower $\dot{V}O_2$ for a given pace that is maintained) is permitted via an increase in mitochondrial enzymes which results in fewer disturbances in mitochondrial homeostasis by : (I) increased fat oxidation (sparing valuable stores of glycogen) and (II) reduced lactate production.(60)

Typically, these kind of performance studies are carried out in young, healthy and usually, relatively fit individuals or athletes who are motivated to undertake maximal exercise tests and rigorous training regimes. The effect of minimal, unsupervised training, which is

typically undertaken in ‘real-world’ populations, has not previously been examined. Oxidative capacity, measured locally in the muscle, is not typically carried out in the context of a clinical exercise test.

In older adults oxidative capacity, characterized using ^{31}P-MRS to assess PCr recovery rate (the maximum rate of oxidative ATP production), has previously been shown to associate positively with peak$\dot{V}O_2$.(101) Some aspects of this study were repeated in a larger sample of even older adults (n=37, mean age 78 years) with the addition of analysis of permeabilized muscle fibers for maximum ATP synthesis rate. *In vitro* and *in vivo* measurements of oxidative ATP synthesis were positively correlated (r^2=0.47, p=0.004) Inclusion of these measured values in multiple linear regression models improved prediction of preferred walking speed (r^2=0.647,p<0.0001).(102) In another study, older men (74±3 years) showed favorable mitochondrial responses to aerobic exercise training, suggesting that the capacity to gain benefit from exercise is maintained into later life.(103) These are important findings which suggest that mitochondrial efficacy for oxidative phosphorylation is an important modifiable determinant of capacity of submaximal exercise capacity (walking speed) in older adults. Despite including a larger sample size than previously recruited, Coen et al(102) avoided the need to adjust for co-morbidities in their older adult population by applying strict inclusion criteria (*BMI 20–32 kg/m^2; ability to walk without the assistance; free of basic activities of daily living disability, no history of hip fracture; no cardiac disease or cardio-vascular disease within the past 3 months; no regular pain, aching, or stiffness in any joints when walking; no bilateral difficulty bending or straightening fully the knees; not regularly taking Coumadin, Plavix, Aggrenox, Ticlid, or Agrylin/Xagrid*). Thus, generalizability of these results to low-functioning participants is difficult to appreciate. They were also underpowered to examine gender differences and did not report the ethnicity of their participants.

The potential role for exercise in enhancing mitochondrial biogenesis has attracted a lot of interest as a therapeutic target for diseases such as T2D.(104) Exercise training is regarded as a substantial promoter of insulin sensitivity and has been associated with attenuation of lipid-induced insulin resistance.(105) Exercise training programs have delivered improved mitochondrial function in patients with type-2 diabetes.(106) As well as improvements in levels of oxidative stress and blood pressure.

1.4 Skeletal muscle metabolic function in the presence of disease

Understanding metabolic function of skeletal muscle is of value in a multitude of disease states. Recently, interest in skeletal muscle metabolic dysfunction associated with cardiometabolic diseases (e.g. obesity, diabetes, insulin resistance) and cardiovascular disease (CVD; e.g. myocardial infarction, stroke, heart failure, peripheral arterial disease)) has greatly increased. Type 2 diabetes and insulin resistance have been of particular focus with debate for and against a role of mitochondrial dysfunction in the disease development.(107)

Type 2 diabetes (T2D) was selected for investigation in this thesis because of its association with reduced exercise capacity/cardio-respiratory fitness well as its increased prevalence with age and the predisposition previously described in Asians.(108) Evidence from epidemiological studies in the UK suggests that higher levels of self-reported physical activity predict improved survival and reduced CVD mortality in people with diabetes.(109)

1.4.1 Diabetes mellitus (diabetes)

The global health burden of diabetes is high with mortality in 2012 estimated as much as 1.5 million according to the World Health Organization (WHO).(110) The number of people with diabetes globally is predicted to increase from 415 million (in 2015) to 642 million by 2040.(111) The global economic burden of diabetes on healthcare systems across the

world was placed at 12% per person in 2010 with a predicted rise in spending over the next decade.(112)

Diabetes consists of a group of metabolic diseases characterized by elevated blood glucose (hyperglycemia) due to defects in insulin secretion, insulin action, or both. Diabetes has been classified into the following general categories by the American Diabetes Association(113):

1. Type 1 diabetes (due to pancreatic β-cell destruction, usually leading to absolute insulin deficiency)
2. Type 2 diabetes (due to a progressive insulin secretory defect on the background of insulin resistance)
3. Gestational diabetes mellitus (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes)
4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation)

Diabetes is diagnosed by measuring fasting plasma glucose (FPG), monitoring plasma glucose following an oral glucose challenge or by measuring heamoglobin-A1C (HbA1C). Criteria for diagnosis are any one of the following;(113)

- FPG > 126 mg/dL (7.0 mmol/L)
- 2-h plasma glucose > 200 mg/dL (11.1 mmol/L) during an OGTT (glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water)
- HbA1C > 6.5% (48 mmol/mol)

- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose > 200 mg/dL (11.1 mmol/L)

The following background will focus on type 2 diabetes (T2D) as it is the most common form of diabetes and is most relevant to the population studied in this thesis.

People with T2D are at a higher risk of developing cardiovascular disease (CVD) than those without.(114) This can manifest as aggressive coronary artery disease (CAD) resulting in ischemia which can lead to myocardial infarction and subsequent heart failure or death. In addition, T2D also predisposes to heart failure in a pathway independent of CAD or hypertension.(115) Rates of CVD mortality in people with diabetes are thought to be as high as 50%.(116) Micro-vascular damage in the eye can lead to diabetes-associated blindness and, similarly, damage to the nephrons of the kidney can lead to kidney failure.(117) Accumulation of reactive oxygen species (ROS) and oxidative stress in the vascular smooth muscle and endothelial cells are characteristic in the development of diabetes-associated vascular disease. One of the sources of ROS in the endothelial cells lining blood vessels of people with diabetes is via the mitochondrial ETC.(114) The effect of skeletal muscle mitochondrial dysfunction in the vasculature in the presence of T2D remains unclear.

T2D is usually preceded by an asymptomatic period prior to diagnosis of diabetes. This period sometimes termed ‘pre-diabetes’,(118) is characterized by elevated insulin resistance, and offers a window of opportunity where insulin resistance can be targeted with lifestyle interventions such as diet or exercise, or medication such as metformin.(119, 120) The mechanisms underlying the positive effects of exercise on these pathways are not clearly understood.

1.4.2 T2D and skeletal muscle metabolic function

In healthy individuals, a post-prandial rise in blood sugar causes a rise in blood insulin. Insulin signals to cells to switch from the fasting fuel source, fatty acids (lipid), to carbohydrate. In the insulin-resistant state the capacity to oxidize fatty acids at rest and the ability to switch between fuel sources is impaired. This impairment has been termed *metabolic inflexibility*.(121, 122) Skeletal muscle is particularly susceptible to dysregulations of insulin signaling and the reduction in oxidation of fatty acids as a fuel source leads to accumulation of lipids in skeletal muscle.(123, 124)

Evidence supports an association between insulin resistance and impaired skeletal muscle oxidative capacity.(107, 125) This was first shown by Kelley and colleges, who demonstrated that skeletal muscle from individuals with T2D had smaller and fewer mitochondria with lower oxidative enzyme activity; specifically citrate synthase and NADH:O_2 oxidoreductase activity.(126, 127) Subsequent studies were carried out which supported this finding by demonstrating that T2D is associated with various other mitochondrial aberrations that lead to decreases substrate oxidation. Including reduced mitochondrial RNA content (128) and reduced protein content.(76)

Mootha et al, describe use of a technique called gene set enrichment analysis (GSEA) which uses DNA microarrays to demonstrate a reduced expression of a profile of genes that are important regulators of oxidative capacity in skeletal muscle.(87) This is an interesting approach which exploits the idea that, rather than an individual gene expression being vastly altered, alterations in gene expression might manifest in co-regulated gene sets. Using this technique they describe a coordinated reduction in expression of a group of genes involved in oxidative phosphorylation in men of a similar age (~60-70 years old) with T2D compared to men without.(87)

To try and understand the gene expression patterns associated with pathogenesis of T2D, Patti et al also examined skeletal muscle gene expression in 10 non-diabetic participants,

either with, or without ,a family history of T2D, and 5 participants with diabetes.(86) Fasting insulin was higher in the T2D group and also the participants with a family history of T2D compared to those without and insulin-sensitivity was reduced in people with T2D and those who were healthy but had a family history of T2D. They found that pre-diabetic (the study arm who had a family history of T2D) and diabetic skeletal muscle showed decreased expression of genes involved in oxidative phosphorylation and that expression of the regulator of expression of these genes (PGC1α) was also reduced in these groups compared to controls.(86) Participants in this study were fairly young (~30-50 years old) and were all American-Mexican ethnicity and, aside from T2D, were otherwise healthy. Therefore, the generalizability of these findings to the wider population of people with T2D is questionable.

Nevertheless, these studies both showed reduced PGC1α in the presence of pre-diabetic insulin resistance and T2D and, thus, suggested an intrinsic underlying mechanism for reduced mitochondrial function in T2D. They also provided evidence for a genetic predisposition to reduced oxidative capacity in T2D mitochondrial dysfunction, hinting at a causal effect of reduced oxidative capacity in T2D.

The existence of a causal relationship between mitochondrial dysfunction and insulin resistance remains unclear.(129) Plausible mechanisms related to the decrease in substrate oxidation are: (1) decreased substrate oxidation leads to lipid accumulation and deposition of metabolically active lipid mediators (diacylglycerols (DAG) and ceramides (CER)) which inhibit insulin signaling in the cell,(130) and (2) decreased substrate oxidation leading to electron leakage from the ETC which results in the formation of reactive oxygen species (ROS) that cause damage to mitochondrial components (figure 1.6).(107)

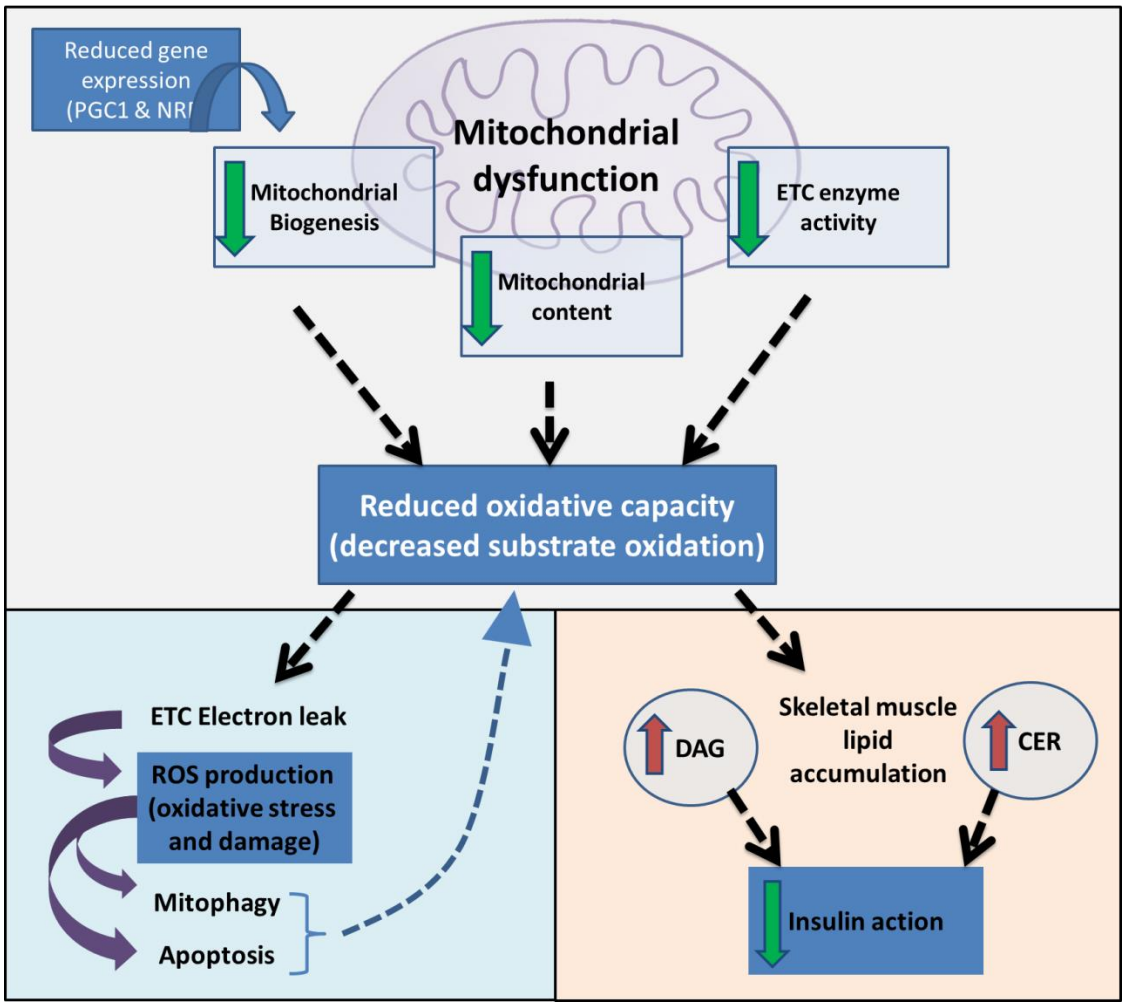


Figure 1.10 Aspects of mitochondrial dysfunction and potential causal effect on insulin resistance. Aspects of mitochondrial dysfunction that result in decreased substrate oxidation are shown in (A); reduced mitochondrial content, reduced protein content of proteins involved in the electron transport chain (ETC) and reduced mitochondrial biogenesis (through reduced expression of regulatory transcription factors such as Peroxisome proliferator-activated receptor-ɣ coactivator (PGC-1α). Potential mechanisms linking reduced substrate oxidation to insulin resistance are: ETC electron leakage leading to increased oxidative stress and thus a reduction in mitochondrial number and density (B). Cellular lipid accumulation resulting in increase in active lipid intermediates; diacylglycerols (DAG) and ceramide (CER) which interrupt insulin signaling (C). PKC; Protien kinase

C, TAG; triacylglycerols (triglycerides), IRS; insulin receptor substrate, Akt; protein kinase B. Figure first described by Montgomery & Turner, 2015.(107)

Impaired oxidative capacity in association with diabetes has also been captured *in vivo* as a lengthening of the half time (t½) of recovery of PCr immediately post-exercise.(131) This method circumvents some of the limitations of removing tissue from its physiological environment. However, it does not indicate the precise underlying mechanism of dysfunction. It remains unclear whether dysfunction in oxidative capacity is due to intrinsic differences in metabolism within mitochondria, or, due to a reduction in the number of mitochondrial or the content of mitochondria. Boushel et al suggest that the reduced O_2 flux observed in the presence of T2D can be attributed to a reduction in mitochondrial content (quantified in their study as DNA content or citrate synthase activity), not a reduction in the ability of mitochondria to perform oxidative phosphorylation (oxygen consumption measured in permeabilised muscle fibers as described above).(132)

Several studies report findings incongruent with the hypothesis that impairments in skeletal muscle mitochondrial function are directly caused by insulin resistance or obesity. Trenell et al, found no difference in PCr recovery between people with versus without T2D.(133) Although it was a small study (n=10 per group), participants were matched for age and physical activity level. The study also assessed participants' response to an 8-week walking intervention which they report improved lipid oxidation in the participants with T2D but did not improve PCr recovery half time. They suggest that the previous finding that max ATP turnover is reduced in T2D could be due to differences in physical activity or a deconditioning phenomenon associated with T2D.(134) Van Tienen et al, subsequently supported this finding by showing that only inactive patients with a long duration of exposure to T2D exhibit impaired oxidative capacity.(135)

One very neat study by Ritov et al, presented findings to suggest that mitochondria specifically located in the subsarcolemere had reduced ETC activity in the presence of T2D compared to lean controls, as well as, obese non-diabetic participants.(136)

1.4.3 Limitations of the current evidence

Previous studies investigating metabolic function in the presence/absence of T2D have employed small sample numbers; the number of participants recruited to comparison groups ranges from 8-12 participants in four commonly referenced sources of evidence.(126, 131, 136, 137) While these detailed studies provide important mechanistic insights, they do not allow multi-variable analysis and therefore adjustment for co-morbidities is not possible. Only in 3 small studies have the effects of either fitness, or physical activity level, been considered.(76, 133, 135) Often younger participants have been recruited for these studies (mean age range was 39-62 years old for the studies described above); this is perhaps because younger participants are less likely to have co-morbidities which complicate analysis and impair exercise capacity. However, with disease progress and the effects of aging, results from these studies may not necessarily be generalizable to older adults.

Muscle biopsy samples are frequently used to assess various aspects of mitochondrial function.(126, 136, 137) As discussed previously, the consequences of removing tissue from its physiological environment may is not fully understood. Furthermore, these techniques are based on the assumption that the maximum rate of ATP production, or oxygen consumption, achieved by tissue (or isolated mitochondria) *in vitro,* represents a limiting factor in the performance of skeletal muscle for either exercise (most likely elite athletes) or glucose clearance. *In vivo* assessment techniques are useful and, as mitochondrial activity is driven by ATP demand, it is additionally informative for techniques to permit functional assessments in a dynamic environment. As yet, dynamic in vivo assessments of mitochondrial function in people with diabetes have only been conducted

in the confines of an MRI scanner.(131) Therefore we do not know to what extent exercises, more associated with activities of daily living, are limited by skeletal muscle oxidative capacity.

1.5 Ethnic differences in T2D, cardiorespiratory fitness and skeletal muscle oxidative capacity

1.5.1 Prevalence of T2D

People originating from India, Pakistan and Bangladesh (referred to here as South Asian origin) have a higher prevalence of T2D than people of European origin.(138) This ethnic difference has been shown to be marked when South Asians migrate to countries where the host population is of European decent.(139, 140) In Western countries, compared to Europeans, T2D prevalence is also higher in men and women of African origin.(139, 141) Cross-sectional analysis of a tri-ethnic (South Asian, African Caribbean and European), population-based cohort of older adults (60-89 years old), living in west London, showed that both ethnic minority groups had increased incidence of T2D (34% in Indian Asians and 30% of African Caribbean's) compared to Europeans (14%).(142) In women from both groups this difference was attenuated by adjustment for insulin resistance and central obesity measured when the participants were middle aged (20 years previously) (adjusted sub-hazard ratio's were: Indian Asian women 0.77 (0.49–1.42), p=0.3 and African Caribbean women 1.48 (0.89–2.45), p=0.13). However, in men, the differences in incident diabetes could not be accounted for (adjusted sub-hazard ratios were: South Asians 1.98 (1.52–2.58), p=0.001 and African Caribbean's, 2.05 (1.46–2.89), p=0.001).(143) Thus, the excess risk of T2D is South Asian and African Caribbean men could not clearly be explained suggesting that investigation into novel risk factors is necessary.

Previous analysis showed that insulin levels remain higher in South Asians despite adjustment for BMI, waist-hip ratio, and adiposity.(144) BMI cut-off values for obesity

related diabetes and insulin resistant-associated metabolic dysfunction are lower in South Asians compared to people of European decent.(145) Therefore, it appears that risk factors for development of T2D are present at a lower BMI in South Asians than in Europeans.(139)

1.5.2 Cardiovascular disease (CVD)

South Asians are more susceptible to the adverse effects of T2D; they are at a higher risk of developing subsequent cardiovascular diseases (CVD) such as coronary heart disease (CHD) and stroke than Europeans. (146, 147) Overall, morbidity and mortality from stroke and CHD is higher in South Asians than in their European comparators.(146, 148) Although traditional risk factors, such as smoking and diet, are important in South Asians, they do not explain the excess risk of CVD in this group.

Despite the same elevated risk of T2D and stroke, African-Caribbean's do not appear to have the same elevated risk of coronary-heart disease seen in South Asians; in fact, coronary disease rates were ~50% lower in African Caribbean's compared to Europeans.(142) The mechanisms for this difference are not entirely clear. However, Park et al describe poorer markers of arterial function in South Asians compared to Europeans and a stronger association of these with chronic hyperglycaemia (HbA1c).(149) While, in contrast, African-Caribbean's in this study had improved arterial function compared to Europeans.(149)

CVD is a risk factor for subsequent development of heart failure. This is especially important in older adults (>75 years old).(150) In addition to the T2D-CVD pathway that increases risk of heart failure, T2D also directly increases the risk of heart failure independently of CVD; known as diabetic cardiomyopathy. Interestingly, this pathway is thought to be driven by metabolic disturbances.(151) Heart failure is recognised as a multi-system disorder with a key feature being impaired exercise capacity; the extent to which exercise capacity (or VO_2max) is impaired in heart failure is not always reflected in in an

impairment in cardiac (left ventricular) function.(152, 153) There is increasing interest in a cause-effect relationship between skeletal muscle metabolic disruptions and heart failure.

The ethnic differences in T2D prevalence and the cardiovascular response to hyperglycaemia provide an interesting context in which to study the mechanisms of development of T2D and its negative cardiovascular consequences. In addition, it has been suggested that, with age, patterns of CVD development appear to be different from patterns seen in middle-aged populations.(154) Therefore, describing ethnic differences in T2D, cardiovascular function and risk factors for its development in aging population is constructive towards developing understanding and, perhaps, ethnicity-specific therapy.

1.5.3 Exercise capacity, cardio-respiratory fitness and physical activity

Lower cardiorespiratory fitness ($\dot{V}O_2max$) has been reported in South Asians by several study populations, including; college-aged South Asian men,(155) middle-aged, BMI-matched men and women(156) and healthy age- and BMI-matched men.(157) Lower physical activity levels are also described in South Asians.(158) In the UK-based study by Ghouri et al, lower habitual physical activity levels did not, however, explain the ethnic difference in cardio-respiratory fitness.(157) No data were available for women in this study.

Data from the National Health and Nutrition Examination Survey (NHANES) also suggests ethnic differences in cardiorespiratory fitness exist independently of physical activity levels.(159) This study characterized participants, living in North America, into self-identified ethnic groups; *non-Hispanic White, non-Hispanic Black, Mexican American, and other*. Results showed lower cardiorespiratory fitness (estimated $\dot{V}O_2max$) in non-Hispanic Black men and men from other racial/ethnic groups compared to non-Hispanic White and Mexican American men.(159) As this sample did not include a distinct South Asian group, direct comparison between South Asians and African-Americans could not be made. There is a known hereditary component to $\dot{V}O_2max$,(160) therefore, it is possible that

factors exist, common to ethnic origin, which may be associated with innate differences in $\dot{V}O_2$max.

Hall et al, furthered insight into impaired exercise capacity in south Asians by showing that, as well as reduced cardiorespiratory fitness, age and BMI-matched South Asian men have reduced capacity to utilize lipid as fuel source during exercise.(161) In the same study they also showed that the association between reduced fat oxidation in exercise and lower insulin sensitivity is independent of total body adiposity.

Prevention trials for T2D generally focus on increasing aerobic exercise or physical activity levels.(162) It has previously been suggested that guidelines should include higher physical activity recommendations for South Asians than Europeans.(163) If the underlying components responsible for reduced cardiorespiratory fitness in South Asians were clearly understood, guidelines could also include more targeted recommendations for physical activity such as specific resistance or cardiovascular exercise programs.

1.5.4 Skeletal muscle metabolic function

Forouhi et al observed elevated intra-myocellular lipid (IMCL) in non-diabetic South Asians men compared to Europeans also suggesting a deficiency in skeletal muscle lipid metabolism.(144) As described above, lipid accumulation has previously been linked to a vicious cycle of impaired skeletal muscle oxidative capacity and consequent insulin resistance.(107, 126) Observations have been made that the metabolic profile in people with and without T2D differs depending on their ethnicity. For example, South Asians are dyslipidaemic and have greater central adiposity than Europeans versus the African-Caribbean's who are less centrally obese and do not show the same pattern of dyslipidaemia.(108, 164, 165)

However, Nair et al, found that although non-diabetic South Asians (Indian Asians) were more insulin resistant, they showed greater mitochondrial DNA abundance, greater

maximal ATP production rate and increased citrate synthase activity above rates that the found in age, sex and BMI matched Americans of European decent.(166) The study by Hall et al, described above, also did not find reduced expression of oxidative metabolism genes in the young South Asian men who participated.(161)

The studies described above all enrolled young or middle-aged, mostly male, participants. Ethnic differences in cardiorespiratory fitness and skeletal muscle oxidative capacity have not previously been reported together in older adults. Previous studies have enrolled fairly small sample sizes, with the exception of Ghouri et al who compared 87 South Asian and 99 European men. However, their study focused on differences in cardio-respiratory fitness between the ethnic groups and its influence on insulin resistance. The determinants of reduced cardiorespiratory fitness, or impaired exercise capacity, in older adults from different ethnic origins have not previously been described.

Skeletal muscle measurements have previously all been done from biopsy sample analysis (mitochondrial ATP production rate, mtDNA content, citrate synthase activity); an overall, *in vivo* inspection of oxidative capacity by ethic group has not previously been described.

1.6 Near-infrared spectroscopy (NIRS) for skeletal muscle measurements

1.6.1 NIRS technology

Beer-Lamberts Law

Near-infrared light can penetrate biological tissues with less scattering and absorption than visible light offering a useful property allowing imaging and quantitative measurements in tissue.(167) In its simplest form, a NIRS device consists of a light-source emitting 2 or more wavelengths of light in the near-infrared range (650–1000nm) and a detector placed

at a known distance from the source(s). NIRS uses the principal of Beer-Lambert's law to derive concentrations of chromophores in tissue from changes in absorbance of NIR light at specific wavelengths.(168)

The Beer-Lambert law states that, in a non-scattering medium, for a given substance dissolved in a solute, molar absorptivity is constant (i.e. the absorption of photons is constant if the concentration of the solute in solution is constant).(168) Thus, the absorbance (*A*) is proportional to concentration (equation 1.2, Beer-Lambert Law). In Beer-Lambert law; A= absorbance (amount of light absorbed, optical density (OD)), ε= absorptivity (absorption coefficient characteristic of the solute) (Mol/cm), l= path-length of light traveling through the solution (cm), c= concentration of the solute in solution (Mol).

The chromophores, Hb and myoglobin (Mb), are oxygen carriers in blood and skeletal

$$A\ (Absorbance, OD) = \ \varepsilon * \ l * c \qquad (1.2)$$

myocytes, respectively. Their absorbance of near infra-red light differs depending on whether they are in an oxygenated or deoxygenated state. Figure 1.7 shows the absorptivity (absorption spectrum) for oxygenated and deoxygenated hemoglobin/myoglobin.(169) The relative contribution of the two chromophores, haemoglobin and myoglobin, to the NIRS signal is discussed later.

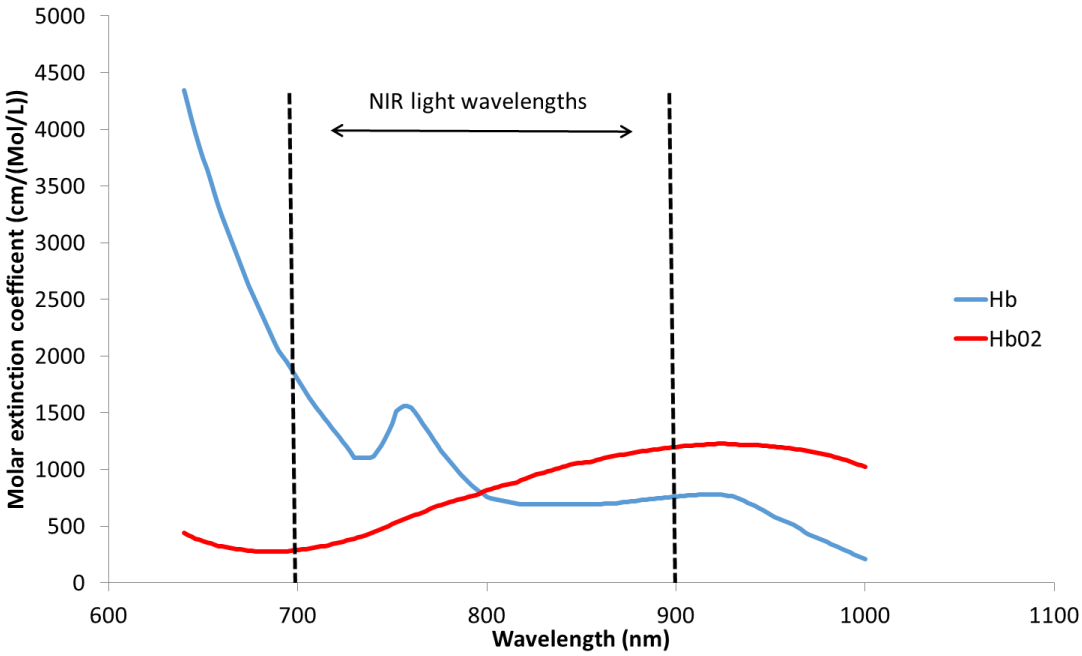


Figure 1.11 Absorption spectra for oxygenated haemoglobin (HbO2, red) and deoxygenated haemoglobin (Hb, blue). This figure was generated from online open source data (169).

The modified Beer-Lamberts law

The Beer-Lambert law is only valid in non-scattering medium. Biological tissue is not homogenous and is therefore considered a scattering medium.(168) A non-homogenous medium does not have uniform properties throughout its volume, that is to say, it may contain irregular parts within it or layers which scatter light. When light is shone into biological tissue it can be either; scattered, absorbed or reflected. A schematic representation of light scattering, absorbance and reflection in shown in figure 1.12.

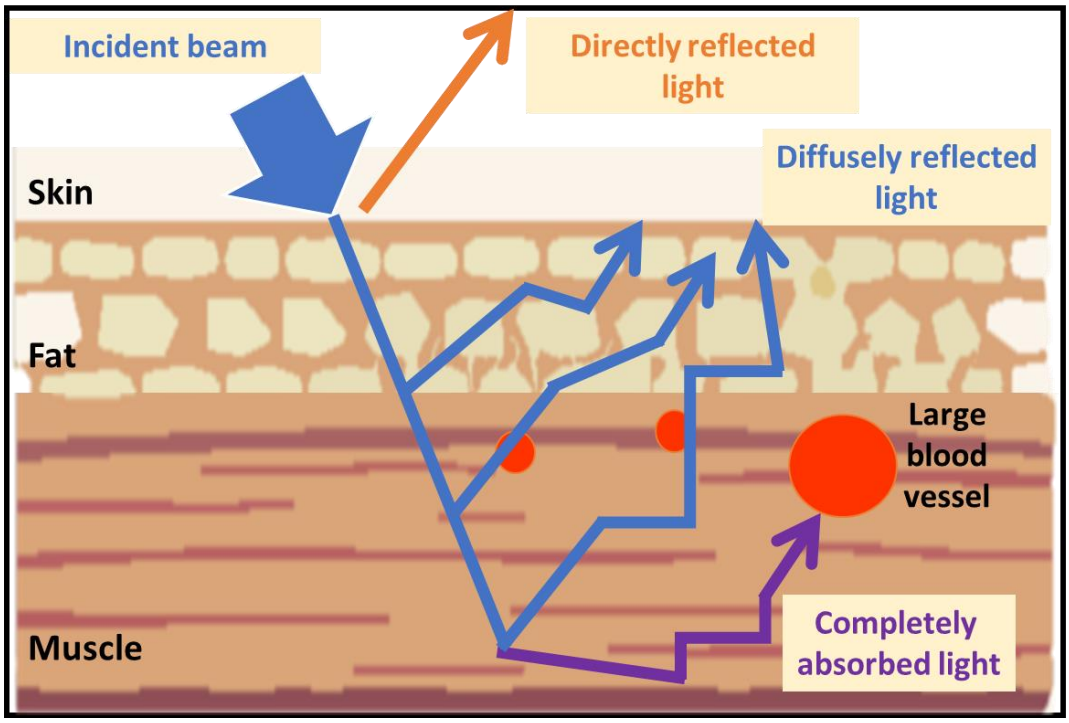


Figure 1.12 schematic representation of light transport through biological tissue. **Light passes into the tissue (incident beam) and can be directly reflected (orange arrow), absorbed completely (purple arrow) or diffusely reflected (scattered) where its intensity is detected.**

In 1988 Delpy et al, described a modification of the Beer-Lambert Law which accounted for the scattering in biological tissue.(170) This was called the modified Beer-Lambert law, it permits relative, but not absolute, concentrations of oxy- and deoxy Hb/Mb to be estimated.(168, 171)

The modified Beer-Lambert law is as follows;

Where *Δc* is the relative concentration of the chromophore (mM), *ODλ* is the optical density of the medium, *ελ* is the extinction coefficient of the chromophore (mM/cm), *L* is the

$$\Delta c = \frac{\Delta OD\lambda}{\varepsilon\lambda * L * B} \quad (1.2)$$

distance between the light source and the detector (4cm) and *B* is the differential path-length factor (DPF) to account for the scattering.

The original NIRS devices were all continuous wave devices. This means the device measures only changes in light intensity i.e. near-infrared light is shone into the tissue and the intensity of the scattered and diffusely reflected light is measured. Detailed discussion of NIRS instrumentation has been reviewed previously.(168) In brief, other than continuous wave, devices can be either time- or frequency-resolved. These NIRS techniques include a measurement of the absolute optical path-length and the intensity of light at the detector. This allows absolute concentration to be measured;(172) however scattering (and hence optical path-length) may change in skeletal muscle as a result of some typical interventions (e.g. exercise) complicating the interpretation of NIRS data.(173, 174) CW NIRS allows higher sampling frequencies than other NIRS devices permit. In this light, CW NIRS devices could be considered more robust when used during exercise. In this thesis, 'NIRS' should be considered as a reference to continuous wave NIRS.

1.6.2 NIRS signal components

Light passing into blood vessels >1mm diameter should contribute little to NIRS signals as it will be almost completely absorbed.(175) Oxy- and deoxy Hb signals therefore represent Hb concentrations in blood vessels smaller than this (i.e. small arteries, arterioles, capillaries and venules)(137, 176). It is generally assumed that the major part of the Hb-related NIRS signal arises from capillaries since these micro-vessels compose the largest portion of vascular volume in skeletal muscle. However, the synchronous cardiac pulsatile nature of the signal observed at rest in both oxy- and deoxy Hb signals from skeletal muscle (figure 1.8) suggests that at least some of signal arises from small arteries and larger arterioles where cardiac pulsatility has not dissipated.(177) Since arteriolar and venular pulsatility are not usually synchronous(177, 178) the presence of simultaneous

oscillations in the oxy- and deoxy Hb signal in this example suggests some cross-talk between the signals, presumably due to limitations in the homogeneous medium assumption and with the use of a mean optical path length.(171)

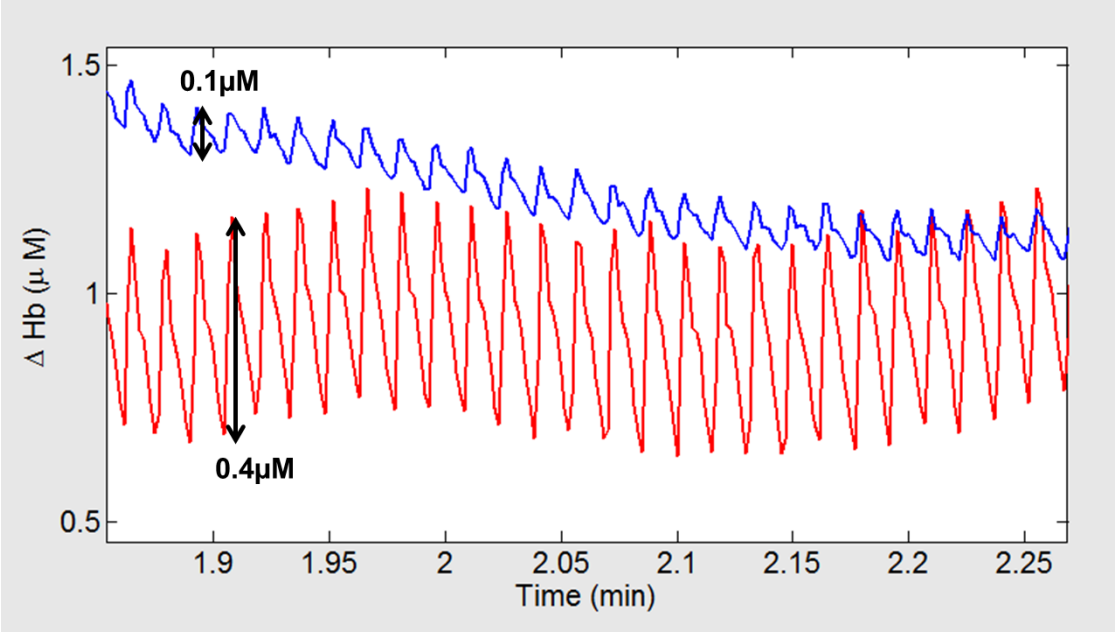


Figure 1.13 Example data showing pulsatility in the NIRS measured oxygenated (HbO_2) in red and deoxygenated (HHb) in blue haemoglobin signals.

Due to the stability of arterial O_2 saturation under most conditions, changes in tissue saturation index (TSI%) that are unrelated to cardiac pulsatility or muscle contraction, are thought to predominantly represent changes in the venous compartment and have been used to examine recovery of muscle oxygenation post-exercise.(137, 179)

1.7 Limitations of NIRS technology

The central limitation of CW NIRS measurements is that only relative, and not absolute, concentrations are possible, as discussed above. To allow comparisons of results to be made between subjects, a physiological calibration can be applied; this involves calibration of the relative concentration values to a normalized scale: a baseline is achieved by application of a total ischaemic occlusion until the oxy-Hb signal plateaus.

The hyperaemic response, following cuff release, provides a functional maximum that can be used to scale responses.(98, 180) The hyperaemic response to 5 minutes occlusion of the brachial artery has recently been reported to show good intra-subject reproducibility.(181) The technique may however be limited by poor subject acceptance of total occlusion,(182) especially in older adults.

1.7.1 Adipose tissue thickness

Adipose tissue thickness at the site of measurement influences NIRS measurements through its effect on the scattering properties of the tissue.(183) This has implications for the choice of measurement site. For example, thigh muscle (e.g. Vastus Lateralis) is a major working muscle for locomotion but the thickness of adipose tissue in the thigh may exceed 3cm in people with diabetes.(184) Although not directly related to locomotion, muscle in the forearm is often examined because adipose tissue is less thick here and occlusive cuffs can be easily applied above the elbow. For NIRS measurements in the lower limb of older individuals, the gastrocnemius may be a useful compromise since subcutaneous adipose thickness rarely exceeds 1cm at this location.(185)

1.7.2 The effect of myoglobin

The spectral absorbance of the oxy- and deoxy-Hb and -Mb is almost indistinguishable,(186) therefore, attenuation by skeletal muscle is attributable to both chromophores. Estimates of relative contribution from Hb and Mb to the NIRS signal are conflicting: Hb has been proposed to contribute as much as ~90%(179) (187) or as little as ~10-20%(188-190) to the signal. A recent paper by Davis and Barstow(191) critically reviewed the topic and estimated the likely contributions of Hb and Mb to NIRS signals based on anatomical and experimental data. They suggested that Mb is likely to contribute ~50-70% of the NIRS signals at rest in many, but not all, mammalian skeletal muscles. They also estimated that the relative contribution from Mb was likely to increase during exercise.(191) Lai et al. used a mathematical model of O_2 transport, metabolism and

distribution of blood volume in muscle and suggested that the Mb contribution is dynamic and varies in relation to blood flow.(192) On the basis of simulations of muscle response under hypoxic and normoxic conditions Spires et al.,(193) suggested the Mb signal would be more affected by reductions in blood flow than the Hb signal and proposed that the Mb contribution to NIRS signals may therefore differ in disease.(193)

1.7.3 Skin perfusion

During exercise skin perfusion may change significantly in response to the rise in body temperature(194) and both oxy- and deoxy Hb signals from skin may therefore confound the muscle signal. The contribution of skin to the NIRS signal has been considered minimal;(179) however, more recent studies indicate a more substantial contribution from skin blood flow.(195) Use of spatially resolved techniques may reduce the impact of the cutaneous layer.(196)

1.7.4 Melanin contribution

Melanin in the skin and Cytochrome C also absorb light in the near infrared range. Wassenaar et al, 2005 described attenuation of light reflectance in direct relation to increased melanin in a small study.(197) Technological developments addressing signal loss, such as increased signal intensity or improved detection, could be useful for reducing attenuation. Applying a physiological calibration (described above) to the signal allows inter-subject comparisons to be made with different levels of skin pigmentation.

1.7.5 Heterogeneity of blood flow in the muscle

The heterogeneity of blood flow and O_2 utilisation within the muscle can only be examined if multiple source-detector pairs are used.(198, 199) There has been a recent increase in the number of skeletal muscle studies exploiting multi-channel instruments.(69, 200, 201) Studies assessing the perfusion/utilisation relationship (matching of O_2 delivery to requirement) provide a comprehensive assessment of skeletal muscle function but as the number of source-detector pairs increases data collection becomes more complex, and

less portable. With development of improved analysis techniques and more portable complex devices, parameters for assessing 'matching' of O_2 delivery and utilisation can be studied more readily. These studies also highlight the importance of consistent probe position within a study where inter-subject comparisons are made using a simple device with fewer source-detector pairs.

Despite these technical limitations, the non-invasive and cost effectiveness of CW NIRS makes it attractive. The technology has been developed in small, wireless instruments showing good intra- and inter-subject reproducibility.(202, 203)

1.8 NIRS applications in clinical and research settings

NIRS has previously been used in various clinical populations where O_2 delivery and/or utilization of O_2 are implicated in the disease process.(204) Examples include muscle myopathies, (205) diseases causing muscle atrophy,(206) heart failure(207, 208) and peripheral arterial (or vascular) disease(209) (PAD). Parameters described in studies using NIRS are based on response to exercise and response to simple physiological interventions (arterial and venous occlusion). These include: post-exercise recovery time constants and recovery half time of tissue saturation, post-occlusion recovery time and recovery half-time of tissue saturation, hyperaemic response post-occlusion and recovery time constant for rate of $\dot{V}O_2$.

As described previously, oxidative capacity within skeletal muscle can be measured using techniques involving muscle biopsies or magnetic resonance spectroscopy (MRS).(73) However, repeated biopsies are uncomfortable for participants and MRS is an expensive tool.

1.8.1 Resting muscle oxygen consumption ($\dot{V}O_2$) measured with NIRS

In the absence of changes in blood volume, NIRS signals represent the balance between O_2 delivery and consumption. Applying an arterial or venous occlusion above the site of measurement can be used to provide a measure of local muscle $\dot{V}O_2$.(210) An arterial occlusion creates a closed circuit system with no blood flow in or out; therefore, in the absence of volume change between vascular compartments within the occluded tissue, the rate of decrease in oxy-Hb (or deoxy-Hb increase) is a measure of muscle $\dot{V}O_2$. During venous occlusion, arterial blood flow is maintained but venous outflow is obstructed until venous pressure exceeds the pressure in the occluding cuff. In the early quasi-linear phase following inflation of the venous occlusion cuff, the rate of increase in Hb is therefore proportional to inflow; if it is assumed that blood flow matches $\dot{V}O_2$, the rate of increase in deoxy-Hb represents $\dot{V}O_2$.(211)

Studies comparing $\dot{V}O_2$ measured by venous and arterial occlusion have reported moderate correlations (Pearson's r = 0.647(210) and Spearman's rho = 0.41(211)) but in both studies venous occlusion yielded estimates of resting $\dot{V}O_2$ that were ~14-25% higher than values calculated following arterial occlusion. The lower values measured by arterial occlusion may be explained by continued inflow of arterial blood during venous occlusion. Venous occlusion derived $\dot{V}O_2$ values show higher variability(211) and poorer reproducibility.(212) Therefore, muscle $\dot{V}O_2$ was derived from arterial occlusions only in this thesis.

1.8.2 Exercise muscle $\dot{V}O_2$ measured with NIRS

Exercise induces a large increase in muscle blood flow(213). During rhythmic exercise the muscle pump action acts on blood vessels to elicit volumetric shifts,(214) these can be seen as cyclic changes in the oxy- and deoxy-Hb signals, corresponding to stepping action. Performing arterial occlusions to estimate $\dot{V}O_2$ throughout exercise is not practical and unlikely to yield useful information. A simple alternative is to surmise that immediately

post-exercise the $\dot{V}O_2$ determined via arterial occlusion is equivalent to the $\dot{V}O_2$ during the final stages of the exercising protocol.(215)

1.8.3 Skeletal muscle oxidative capacity

A process of transient arterial occlusions in the immediate post-exercise period, originally described by Motobe et al in 2004,(216) can be used to generate a time constant, τ, for recovery of muscle $\dot{V}O_2$. As discussed above this represents skeletal muscle oxidative capacity. Good agreement has been demonstrated between NIRS derived recovery time constants and phosphocreatine (PCr) recovery time constants found with MRS(217) as well as good correlation with in vitro assessed oxidative capacity via muscle biopsy analysis.(218) The technique has shown good reproducibility that is uninfluenced by the type of exercise in healthy individuals (215, 219) and in patients with COPD.(220)

Sensitivity of the time constant to athletic capacity has been demonstrated by several groups.(99, 221) Brizendine et al. reported muscle $\dot{V}O_2$ recovery time constants of the *Vastus lateralis* were nearly twice as fast in endurance athletes as non-athletic, age-matched controls.(99) The effects of training and de-training on the recovery time constant of muscle $\dot{V}O_2$ measured by NIRS have also been shown in wrist flexor muscles.(222)

1.9 Research questions

The focus of this thesis is to investigate skeletal-muscle metabolic function, specifically oxidative capacity, and cardio-respiratory fitness and how they influence exercise capacity. The main questions to be addressed in this thesis are:

1. How reproducible is the 6MST for assessment of sub-maximal exercise capacity and prediction of cardiorespiratory fitness in older adults (>65 years old)?
2. How reproducible are NIRS-derived assessments of skeletal muscle oxidative capacity in the context of the 6MST?

3. What are the reasons for early 6MST termination? And are they different between people with versus without T2D?
4. Are there differences in sub-maximal exercise capacity and skeletal muscle oxidative capacity between older adults with and without T2D? Is the relationship between submaximal exercise capacity and skeletal muscle oxidative capacity different between people with and without T2D?
5. Are there ethnic differences in sub-maximal exercise capacity (6MST and grip-strength) in older adults originating from South Asia, the Caribbean and Europe? And is oxygen uptake or predicted maximum oxygen uptake different by ethnic group?
6. Is skeletal muscle oxidative capacity different between ethnic groups? And can this explain differences in exercise capacity/cardiorespiratory fitness?
7. Does endurance exercise training improve skeletal muscle metabolic function in sedentary adults undertaking their first marathon without supervised training?
8. Is skeletal muscle oxidative capacity and cardio-respiratory fitness independently associated with exercise capacity?

Chapter-specific objectives are laid out in more detail at the end of each introduction section.

1.10 Hypotheses

The main hypotheses are:

1. The 6MST will assess sub-maximal exercise capacity in older adults reproducibly and NIRS-derived assessments of skeletal muscle oxidative capacity will be reproducibly measured.
2. Sub-maximal exercise capacity and skeletal muscle oxidative capacity will be impaired in older adults with T2D

3. Sub-maximal exercise capacity and cardio-respiratory fitness will be lower in older adults originating from South Asia compared to Europe.
4. Is skeletal muscle oxidative capacity will be lower in older adults originating from South Asia compared to Europe and this will explain some of the differences in exercise capacity/cardiorespiratory fitness
5. Endurance exercise training will improve cardiorespiratory fitness and skeletal muscle metabolic function in young, healthy sedentary participants.

Chapter 2: General methods

2.1 Participants

2.1.1 Older adult participants

The older adults described in this thesis belong to a longitudinal, population-based cohort study called the Southall And BRent REvisited (SABRE) study. SABRE is a tri-ethnic cohort study comprised of first generation South Asian and African-Caribbean migrants and European individuals who were resident in West London, UK, at the time of initial recruitment in 1988. Tillin et al provided a full cohort profile in 2012.(223) SABRE originated as a combination of 2 studies; The Southall study and The Brent study.(224)

The Southall study was designed in response to the observation that Indian Asians migrating to the UK (and other countries) were at greater risk of cardiovascular disease (coronary heart disease and stroke) and had higher rates of diabetes that their Caucasian counterparts.(224) The Brent study focused on health differences between white Europeans and African-Caribbean migrants to the UK. The latter group having high rates of diabetes and hypertension but paradoxically lower rates of coronary heart disease(225)

Between 2008 and 2011 a 20-year follow-up was carried out of the original SABRE study participants. The objective of this follow-up was to further elucidate ethnic differences and to understand mechanisms underlying these differences in cardiometabolic risk. Information on survival and cardiometabolic morbidity was collected via questionnaires and healthcare record review. Participants were invited to attend a follow-up clinic day that included detailed cardiovascular phenotyping to determine the presence of pre-clinical cardiovascular disease or diabetes. Full details of attendance and measurements carried out can be found in the cohort profile.(223)

In this thesis the older adult population described in subsequent chapters were SABRE study participants who agreed to undertake a third follow-up clinic visit (2014-2018). In addition to the index participants (original SABRE study members) spouses or partners of participants were invited to participate. The (2008-2011) 20-year follow-up clinic attendees comprised 522 Indian Asians, 232 African Caribbean's and 684 Europeans (total: 1438), this included 451 participants (31%) undergoing treatment for diabetes and 365 participants (25%) with an ejection fraction <55%, indicating some level of systolic dysfunction. Therefore, the 2014-2018 third clinic visit provides apposite opportunity to investigate differences in exercise capacity and skeletal muscle function between ethnic groups with, or without, diabetes.

2.1.2 Young adult participants undertaking Marathon training

Young adult participants included in this thesis had been enrolled in a prospective observational study investigating the effect of training for a marathon for the first time on cardiac remodeling: The Marathon Study.

The Marathon Study recruited young healthy men and women who had never previously run a Marathon and self-reported little or no current participation in running. Inclusion criteria were: age 18-35 years old at recruitment, no past significant medical history, no previous marathon-running experience and less than 2 hours of running participation per week at recruitment. As the majority of people who enter to run a marathon are not elite athletes and race entry requires no prior marathon running experience or qualifying time, the study inclusion criteria were set in order to allow a representative sample of the novice runners to be recruited. A Consort flow diagram describes the recruitment process and attrition to the study for all participants enrolled in The Marathon Study (figure 2.1). Details of the sub-group of participants who underwent skeletal muscle measurements are provided in the results section of Chapter 6.

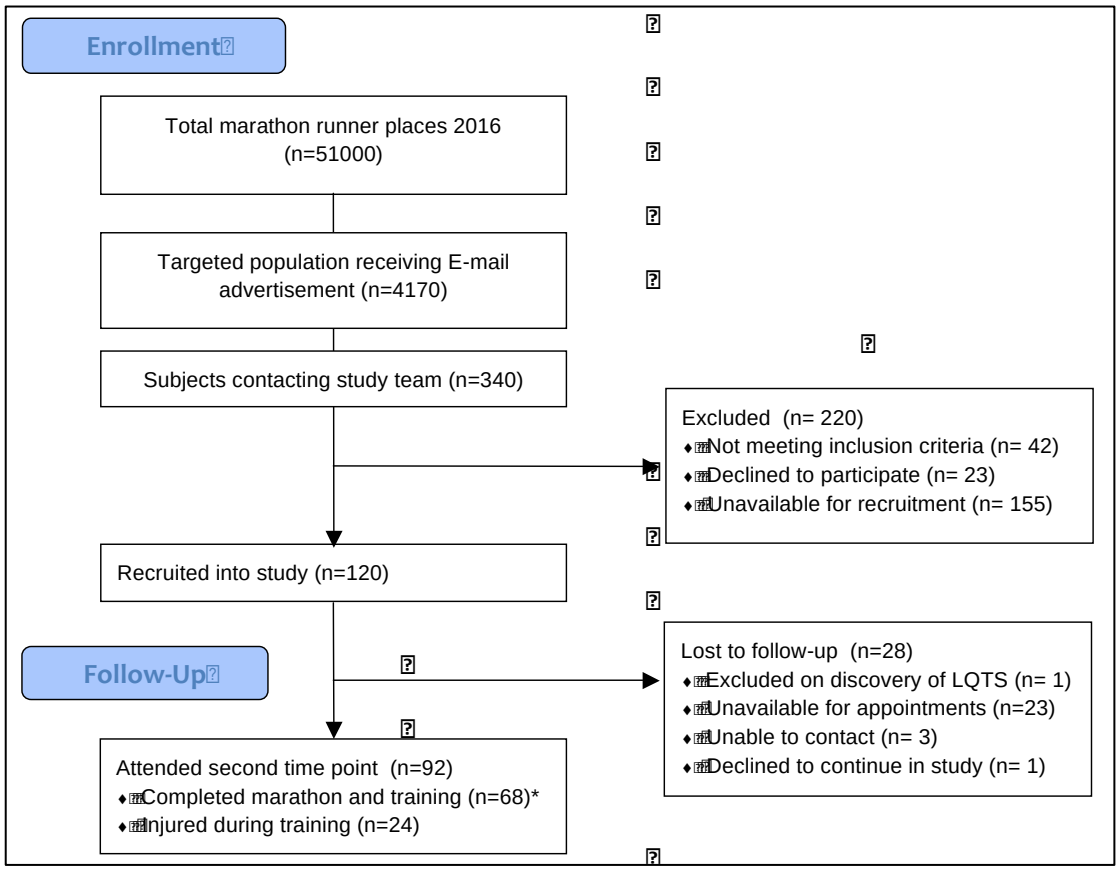


Figure 2.1 Consort flow diagram describing the recruitment, attendance and drop-out for The Marathon Study in full. Unpublished data with permission from the authors.

Participant characteristics and basic results from the CPET for all participants enrolled in The Marathon Study pre-training and post-training are provided in table 2.1.

Mean±SD or Median(IQR)	Pre-training (n=27)	Post-training (n=27)	P-value
Weight (kg)	72.0±13.0	72.0±12.0	0.98
BMI	22.9±2.9	23.0±2.7	0.84
Body fat mass (kg)	15.0±5.5	14.4±6.2	0.30
Resting HR (bpm)	66±13	64±14	0.63
Peak HR (bpm)	167(161-176)	165(157-171)	0.29
Peak VO_2 (ml/min/kg)	39.2±5.7	39.2±6.7	0.97
Resting muscleVO_2 (mmol/s)	0.25(0.19-0.32)	0.21(0.16-0.32)	0.35
Post-Ex muscle VO_2 (mmol/s)	1.04(0.85-1.29)	1.48(1.04-2.16)	0.004
ΔmusVO_2 (mmol/s)	0.88±0.56	1.45±0.94	<0.001

Table 2.1 Participant characteristics and cardiopulmonary exercise test results for all participants enrolled in The Marathon Study. Data expressed as mean±SD if normally distributed and median [IQR] if non-normally distributed. BMI, body mass index; BP, blood pressure; VO_2 oxygen consumption.

2.2 Exercise testing in older adults

2.2.1 The 6-minute stepper test

The exercise test used to test exercise capacity in the older adult population was a self-paced, 6MST (Homcom, miniStepper), as pictured (figure 2.2). Exclusion criteria were based on the ATS/ACCP guidelines(17) and included; angina or a recent cardiovascular event (MI, stroke, TIA), an uncontrolled arrhythmia, uncontrolled arterial hypertension, severe aortic stenosis, severe symptoms of COPD and any orthopedic impairment severely compromising exercise performance. Reason for exclusion was recorded if the participant met criteria.

The testing protocol is described to participants using the following instructions:

- *The objective of this test is to complete as many steps as possible within 6 minutes*
- *Start at a pace you feel you could continue at for 6 minutes and try to maintain this pace*
- *If you become exhausted or experience dizziness or chest pain please stop immediately*

Prior to undertaking the test participants were permitted to familiarize themselves with the stepping procedure, all participants were permitted to use the custom-built wall support for balance during the test if necessary. Termination of the test prior to 6 minutes was made based on ATS/ACCP guidelines(17) or because the participant reported intolerable dyspnea or muscle fatigue. Participants were not permitted to re-start stepping if they stopped before 6 minutes was completed. Duration was not limited by heart rate in line with previous research (226). The number of steps completed and duration was recorded; if 6 minutes was not completed, the reason for early termination of the test was recorded. Number of steps completed was divided by duration (minutes) to determine step-rate (steps/min). At the end of the test, participants were asked to grade their perceived level of exertion on a 0-10 Borg scale where 0 is resting and 10 is maximal exertion.(227)

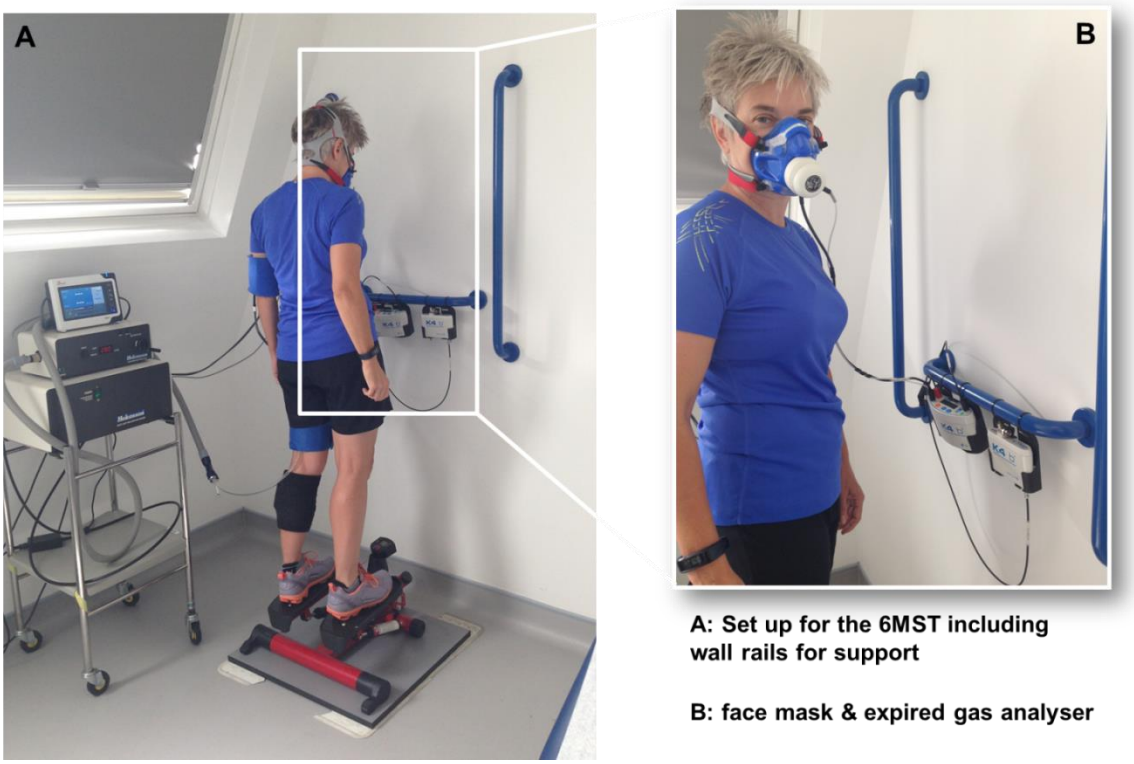


Figure 2.2 A & B. Example set-up for the 6-minute stepper test (6MST). Rails are fixed on the wall for support (A). A face mask covers the mouth and nose (B) and portable gas analyzer allow gas exchange to be measured. Other physiological measurements; blood pressure and muscle oxygenation are also demonstrated here (A).

2.2.2 Calibration of the stepper

Calibration of the stepper was carried out using weights and a tape measure. The tape measure is used to measure the distance from the underside of the pedal to the red floor bar. Height can be adjusted via a dial at the front of the stepper; clockwise raises the height of the pedals. At maximum height there was a distance of 24 cm (figure 2.3), this allowed the distance that the foot travelled with one step to be 15cm.

The weights were used to check that, with time, the resistance, from the hydraulic jacks, did not drift up or down i.e. pedals do not become easier/harder to depress. It should require no less or no more than 5Kg + the weight of the bar (4X1.25kg weights on the bar)

to initiate a downward motion of either pedal from a starting point of its highest (figure 2.3). To check this, the following steps were taken:

- *Using the right foot pedal, place one 1.25kg weight on the right of the bar and screw it to the bar, add the 3 other 1.25kg weights sequentially to the left side of the bar*
- *The pedal should not start to move downwards until the third 1.25kg weight has been added to the left side of the bar*
- *Repeat the above steps for the left pedal*

Calibration of the stepper was performed several times in the initial few weeks of the study. The settings were found to be stable across these initial calibrations; therefore, the calibration interval was increased to 3-4 months.

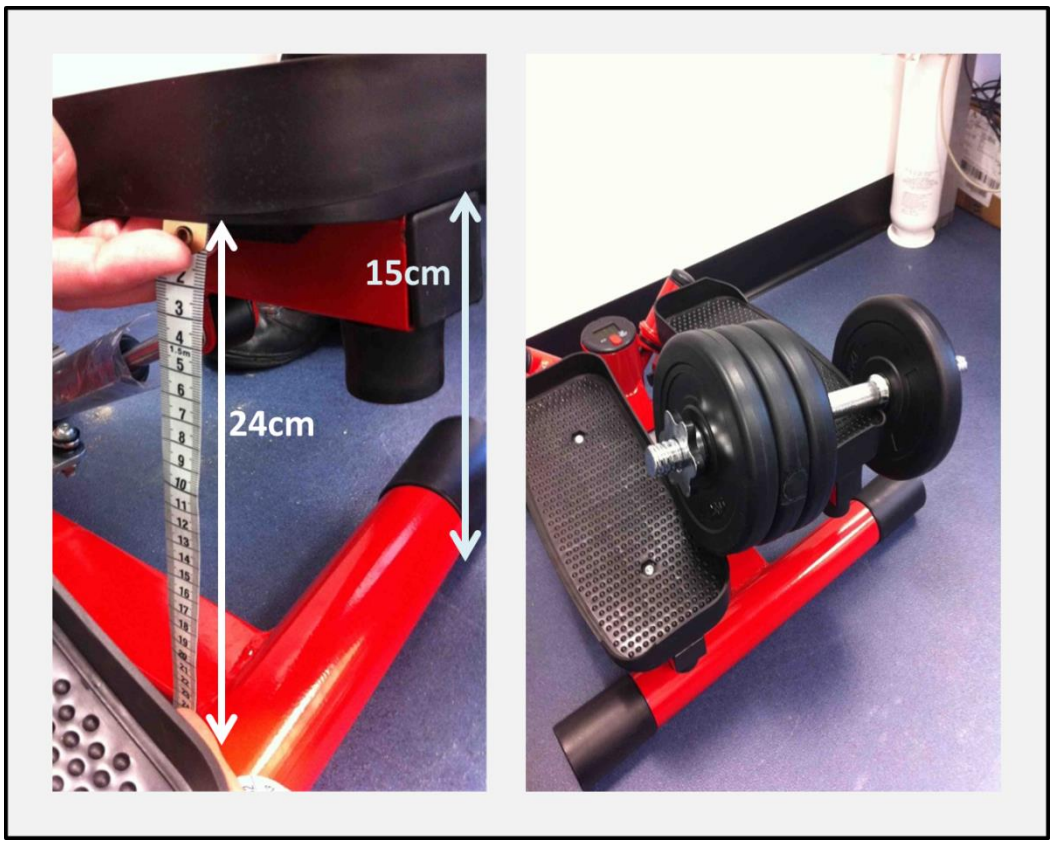


Figure 2.3 Process for calibration of the stepper for the 6-minute stepper test. The height is calibrated to ensure the pedal travels a vertical distance of 15cm; the distance from the bottom of the pedal to the floor is 24cm (left panel). The hydraulic jacks are calibrated using four 1.25 weights (right panel).

2.2.3 Heart rate and $\dot{V}O_2$ measurement during the 6MST

Heart rate and expired gas variables were measured continuously throughout the 6MST using a heart rate monitor strap and portable gas analyzer, belonging to the same system (K4b^2, CosMed, Rome). The gas analyzer was warmed-up prior to calibration which was carried out between tests according to manufacturer guidelines.(228) Three stages of the calibration are; (1) room air calibration which determined temperatures, pressures and humidity of the air in the testing room as well as % O_2 and CO_2 in the room air (2) reference gas calibration using a cylinder of known mixed gas concentrations (16% O_2, 5%

CO_2; BAL. N_2) and (3) turbine calibration using a 3L syringe. Gas delay calibration was performed every 2 weeks which involved timed breathing through the turbine.

One of 3 masks (small, medium or large) was selected for the participant and fitted securely over the participant's nose and mouth and the straps adjusted over the head; care was taken to ensure there were no air was leaking through the mask by asking the participant to breath out for a very short period against a blocked flow turbine.

The portable gas analyzer was not validated to a larger CPET platform for the purpose of this study. A comparison of portable gas analyzers previously found $\dot{V}O_2$max differences of between 0.01-0.29L/min during submaximal exercise between devices.(229) Direct comparison of the K4 device with a large platform stationary gas exchange system (Medgraphics D-Series) has also been independently carried out, funded by the National Institute for Health (NIH). This study reported excellent agreement between the 2 systems when measuring during steady-state, self-paced exercise on a treadmill; ICC ranged from 0.93 to 0.97 for weight indexed $\dot{V}O_2$max (ml/kg/min), total $\dot{V}O_2$max (ml/min), and VCO_2 (ml/min).(230) The O_2 cell in the K4 device was replaced 3 times across the whole of the data collection phase as recommended by the manufacturer.

2.2.4 Post-processing

Breath-by-breath values for $\dot{V}O_2$ and heart rate were collated using manufacturer software (OMNIA, CosMed, Rome) and, for each participant, values were exported to excel. Post-processing of expired gas analysis and heart rate was carried out using open source software (Python Software Foundation, version 2.7. http://www.python.org) and custom written scripts. Measured oxygen consumption ($\dot{V}O_2$, ml/min/kg) is reported as the highest value determined from a rolling 60-second average calculated across the duration of exercise. Peak heart rate is reported as the highest measured value determined from a rolling 60-second average calculated across the duration of exercise.

Predicted maximal heart rate ($_{max}$HR) was estimated using two equations (1 & 2) commonly used in the literature(231, 232) :

$$maxHR(Fox) = 220 - age\ (yr) \quad (2.1)$$

$$maxHR(Tanaka) = 208 - (0.7 \times age\ (yr)) \quad (2.2)$$

The percentage of predicted maximum heart rate achieved was calculated from the achieved heart rate (measured) and the predicted max heart rate for both methods described above.

2.2.5 Exercise blood pressure

Blood pressure was measured using a motion-insensitive validated device (Tango M2, SunTech) at rest, 2, 4 and 6 minutes following the start of exercise, immediately post-exercise termination and at 3 minutes recovery. Measurements were carried out on the participant's non-dominant arm (the arm belonging to their hand which they do not use for writing). Participants were asked to relax their non-dominant arm grip on the rails during the measurement.

2.3 Exercise testing in young adults pre- and post-marathon

2.3.1 Semi-supine ergometer

The CPET in the Marathon Study was carried out on a semi-supine ergometer (Ergoselect1200, Ergoline, Germany) (figure 2.4) paired to software that allowed an incremental workload protocol to be programmed (OMNIA, Quark CPET, CosMed, Rome). A semi-supine ergometer was used to allow concurrent echocardiography to be performed during exercise; echocardiographic results are not presented in this thesis.

The participant was invited to sit on the recumbent cycle ergometer and place their feet on the pedals. The seat height, arm rest and head rest were adjusted to ensure safety and comfort. Feet were then strapped securely into the pedals using Velcro and the waistband was placed around the participant to hold the torso securely on the ergometer. The backwards tilt of the ergometer was kept the same for each test.

Figure 2.4 Example experimental set up of the supine cycle ergometer (Ergoselect1200) used for cardio-pulmonary exercise testing in young adult participants of The Marathon Study.

2.3.2 Analysis of expired gas and heart rate during exercise

Expired gases were analyzed throughout using a metabolic cart system (Quark CPET, CosMed, Rome). The Quark CPET includes a fast-response O_2 sensor to measure

changes in total body oxygen consumption and rapid infrared for changes in CO_2 as well as a bidirectional flow-meter for respiratory changes.

The machine was warmed-up for 15 minutes prior to first test of the day. Calibration of the flow meter was carried out using a 3L syringe. The gas sensors were calibrated using a cylinder of known concentrations of mixed gas (16% O_2, 5% CO_2; BAL. N_2). Calibration of room air was also performed. All calibrations were carried out according to the manufacturer's instructions at the start of the day and after, at most, 3 tests; or more frequently at the discretion of the lead technician.

ECG electrodes were attached across the participant's chest; these were not placed in the standard position in order to allow access to echocardiographic windows. The ECG leads were visualized on a PC using software (Quark T12x, CosMed, Rome) to ensure the QRS complex could be visualised; the R-wave is used by the software to continuously measure heart rate. The EGC is monitored throughout the test for presence of abnormal rhythms or ECG changes that indicate ischemia.

A blood pressure cuff was placed around the participants arm above the elbow. Measurements were made at rest, at 2 minute intervals throughout exercise and at the end of the recovery phase.

A mask was fitted and adjusted securely across the participant's nose and mouth. Care was taken to ensure there were no leaks around the seal between the mask and the face. The participant's height, weight, age and unique ID number were entered into the gas analysis software. An incremental testing protocol was used for all participants; for men workload increments were 25 Watts per minute (W/min) and for women 20W/min. This could be adjusted at the discretion of the lead technician according to the participant's body size: If participants are shorter than average (<160 cm women; <170 cm men) deduct 5 W/min and if participants are taller than average (>180 women; >190 cm men) add 5 W/min. All technicians were trained and experienced in exercise testing and had

discussed the process of protocol allocation in previous study meetings. The decision to allocate different protocols was made with the objective of each participant exercising for roughly the same amount of time (despite reaching different workloads).

Participants were given a 2 minute resting phase while wearing the face mask followed by a 3-minute unloaded pedalling phase as a warm-up before the incremental protocol began.

2.3.3 Terminating the test

All participants were required to achieve a respiratory exchange ratio (RER) value above 1.1 during the CPET, however, the test was not terminated until the participant reported exhaustion or could no longer maintain the cycling cadence (~60-70rpm). Encouragement was given by the technician for the participant to continue pedalling at a cadence >60rpm as long as possible.

2.3.4 Post-processing

The peak$\dot{V}O_2$ was determined as the rolling 30-second average value between the final 15-seconds of exercise and first 15-seconds of recovery. Maximal predicted $\dot{V}O_2$ was calculated according to the equation by Wasserman et al,(188, 189) and the percentage of this predicted value reached during the exercise test was calculated. Resting heart rate was measured using a 12-lead ECG conducted in the semi-recumbent position, 45 degrees to the horizontal, following a 2-5 minute resting period. Peak heart rate was the highest rolling 60-second average value during the final 30-seconds of exercise.

2.4 skeletal muscle measurements

2.4.1 NIRS device

All skeletal muscle measurements included in this thesis were made using the same commercially available continuous wave NIRS device (Portamon, Artinis Medical Systems, Netherlands). This device is a portable wireless system with 3 light sources each emitting

2 wavelengths of light (730nm and 830nm) and one detector. Changes in oxy-Hb, deoxy-Hb, total-Hb and the difference between oxy- and deoxy-Hb (diff-Hb) were measured from the lateral gastrocnemius.

Position and orientation of the device was standardized between individuals. The device was attached using tape and covered completely using a neoprene sleeve. Measurements were acquired at a frequency of 10Hz throughout all protocols (figure 2.5).

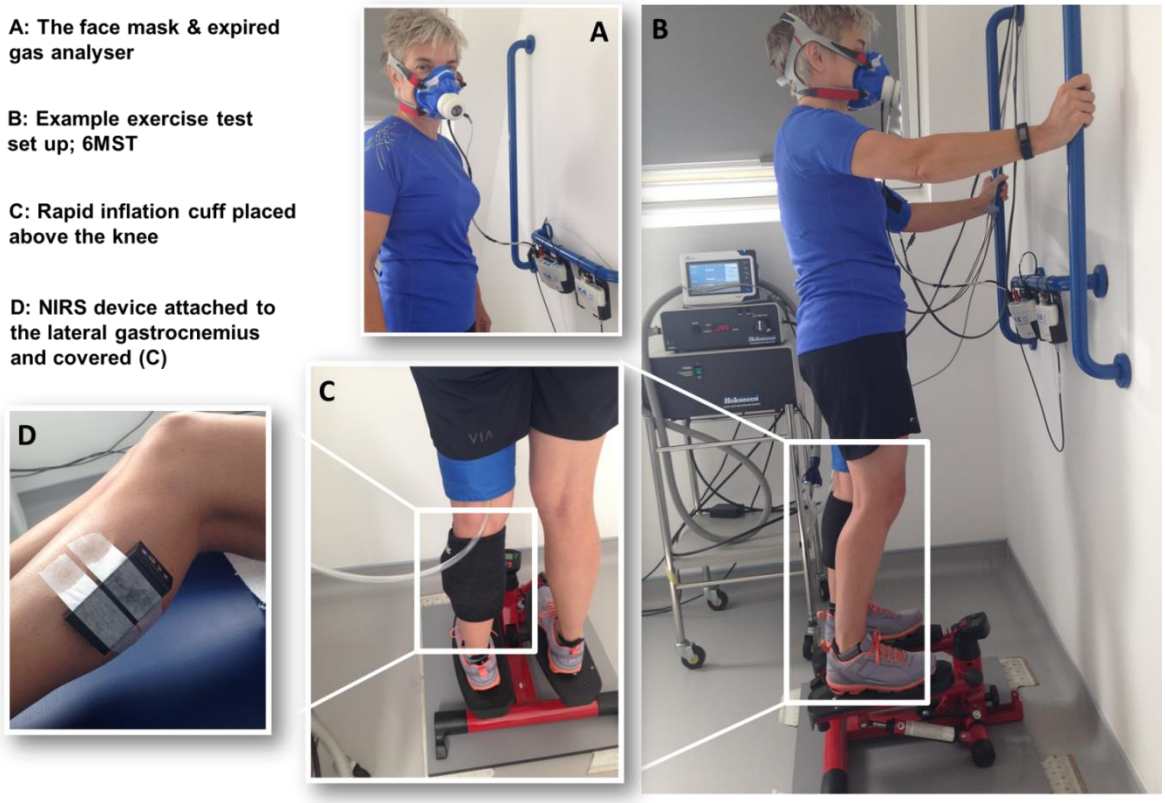


Figure 2.5 A, B, C & D. Example set up of the 6-minute stepper test (6MST) including skeletal muscle monitoring with near-infrared spectroscopy (NIRS) and the rapid inflation cuff for arterial occlusions. The 6MST set up is shown (B) including the face mask for analysis of expired gases (A). The NIRS device (Portamon) is attached to the lateral gastrocnemius of the left leg (C & D) and covered entirely by a neoprene sleeve (C). The occlusion cuff is wrapped around the upper leg directly above the patellofemoral articulation (C).

The gastrocnemius was selected for measurement because it is likely to be covered by less adipose tissue than the thigh or larger locomotive muscles (233) therefore the influence of adipose tissue thickness (ATT) on NIRS measurements is less.

2.4.2 Measuring ATT

Prior to attaching the NIRS device, ATT was measured at the site of NIRS measurement using B-mode ultrasound (vivid I, GE healthcare) equipped with a 3.5-10MHz linear array transducer. Higher frequency ultrasound produces better resolution but is limited in depth while lower frequencies allow greater depths but have poorer resolution.(234) The ultrasound transducer was placed on the measurement site over the skin and was orientated longitudinally along the direction of the muscle fibres, a thick layer of ultrasound gel was applied under the transducer so that minimal force could be applied to avoid compression of the adipose layer. Analysis was made offline using GE software; fat was measured using onscreen electronic callipers and defined as the perpendicular distance between the upper border of the dermal/adipose interface and the upper border of the adipose/muscle interface (Figure 2.6). Three measurements were made and the final ATT calculated as the average.

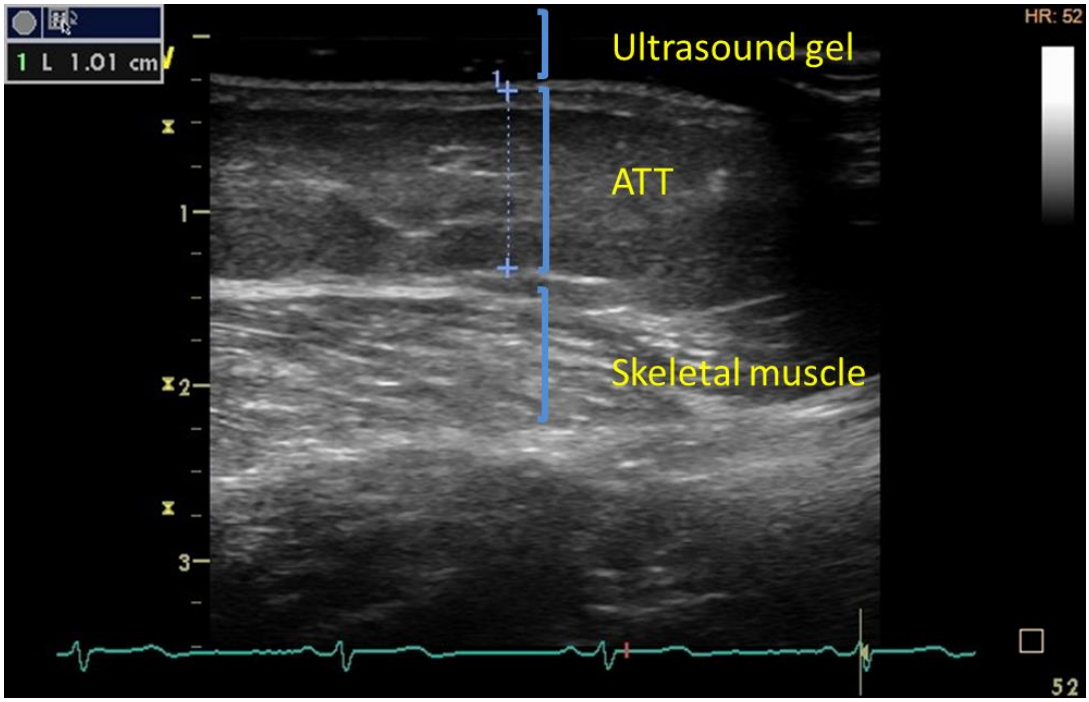


Figure 2.6 An example measurement of adipose tissue thickness (ATT) above the gastrocnemius. Measurements were conducted using B-mode ultrasound (vivid I, GE healthcare) equipped with a 3.5-10MHz linear array transducer. A thick layer of ultrasound gel is applied above the skin to allow propagation of ultrasound waves with minimal pressure to the area of interest.

2.4.3 Arterial occlusions

An arterial occlusion prevents blood from flowing into, or out of, the tissue of interest. In a limb, it is applied proximal (closer to the heart) to the tissue of interest and is usually performed using an inflatable cuff and a sphygmanomometer which allows measurement of the pressure which the cuff is applying around the limb. This allows the pressure to be adjusted appropriately; in the case of an arterial occlusion a pressure above systolic blood pressure is necessary to illicit a total arterial occlusion (figure 2.7).

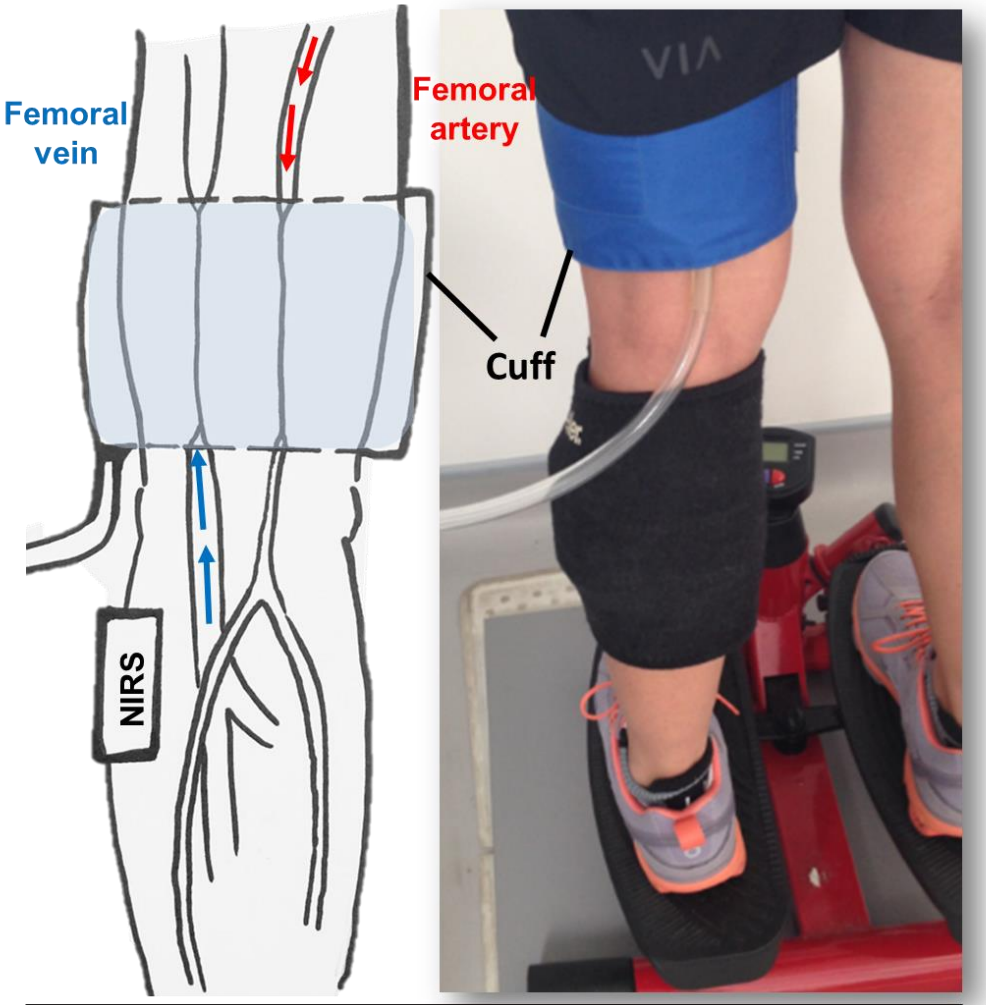


Figure 2.7 schematic representation of a total arterial occlusion. A picture of the cuff position and near infrared spectroscopy (NIRS) device position (right side) and a schematic diagram of the effect of cuff occlusion on the underlying blood vessels (left side) is shown.

Arterial occlusions were performed at rest and immediately following the exercise test using a rapid inflation cuff (Hokanson, SC10D/E20, PMS Instruments, UK) placed on the thigh directly above the patellofemoral articulation (figure 2.7). The cuff was inflated to a

pressure above 250mmHg and inflation or deflation was complete in 0.3 seconds. At rest the cuff was inflated twice for 30 seconds each time, post-exercise the occlusion duration was 5-8 seconds. Care was taken to minimize the time from the end of exercise to the initial occlusion post-exercise by leaving the cuff in place throughout exercise, however, one limitation of this method is that, despite care, the time from the end of exercise to the initial occlusion of the recovery phase may vary between participants.

2.4.4 Post processing NIRS signals; resting and post-exercise muscle$\dot{V}O_2$

Analysis of NIRS data was conducted using custom written programs in MATLAB R2014a (MathWorks Inc.). Signals were rejected if visual inspection showed movement artifact or if there was evidence that complete arterial occlusion had not been achieved. Complete arterial occlusion was judged on (a) absence of a pulsatile signal in the oxy-Hb signal and (b) the direction of the oxy-Hb and deoxy-Hb signals which, under complete arterial occlusion, are expected to move in opposite directions. Presence of movement artifact was assessed by the person analyzing the signals and was assigned if there was so much artifact in the signal that the appearance of the downward slope of oxy-Hb during the occlusion could not be identified.

Example data from one participant is shown in Figure 2.8 A. Resting and post-exercise muscle$\dot{V}O_2$ measurements were estimated by fitting the slope of the difference between oxy-Hb and deoxy-Hb signal during each occlusion (Figure 2.8 B & C).(235) Using the difference signal is in line with previous research suggesting a greater signal-noise-ratio and that this method corrects for blood volume shifts that may accompany arterial occlusion. (236) More negative values represent higher muscle oxygen consumption (oxygen declines with consumption); therefore, in order to align our values with the cardio-pulmonary peak $\dot{V}O_2$ and simplify statistical interpretation, we inverted the values to provide a positive indices of muscle oxygen consumption (Figure 2.8 D). Exercise-related

increases in muscle$\dot{V}O_2$ (Δmus$\dot{V}O_2$) were calculated as the absolute difference between resting muscle$\dot{V}O_2$ and muscle$\dot{V}O_2$ measured immediately post-exercise (Figure 2.8 D).

Therefore, three indices of muscle oxygen consumption were calculated for each participant; resting muscle$\dot{V}O_2$, post-exercise muscle$\dot{V}O_2$ and the difference Δmus$\dot{V}O_2$. The units for these indices are changes in the difference between oxy-Hb and deoxy-Hb in micro-molars per second ($\Delta Hb_{diff}\mu M/s$).

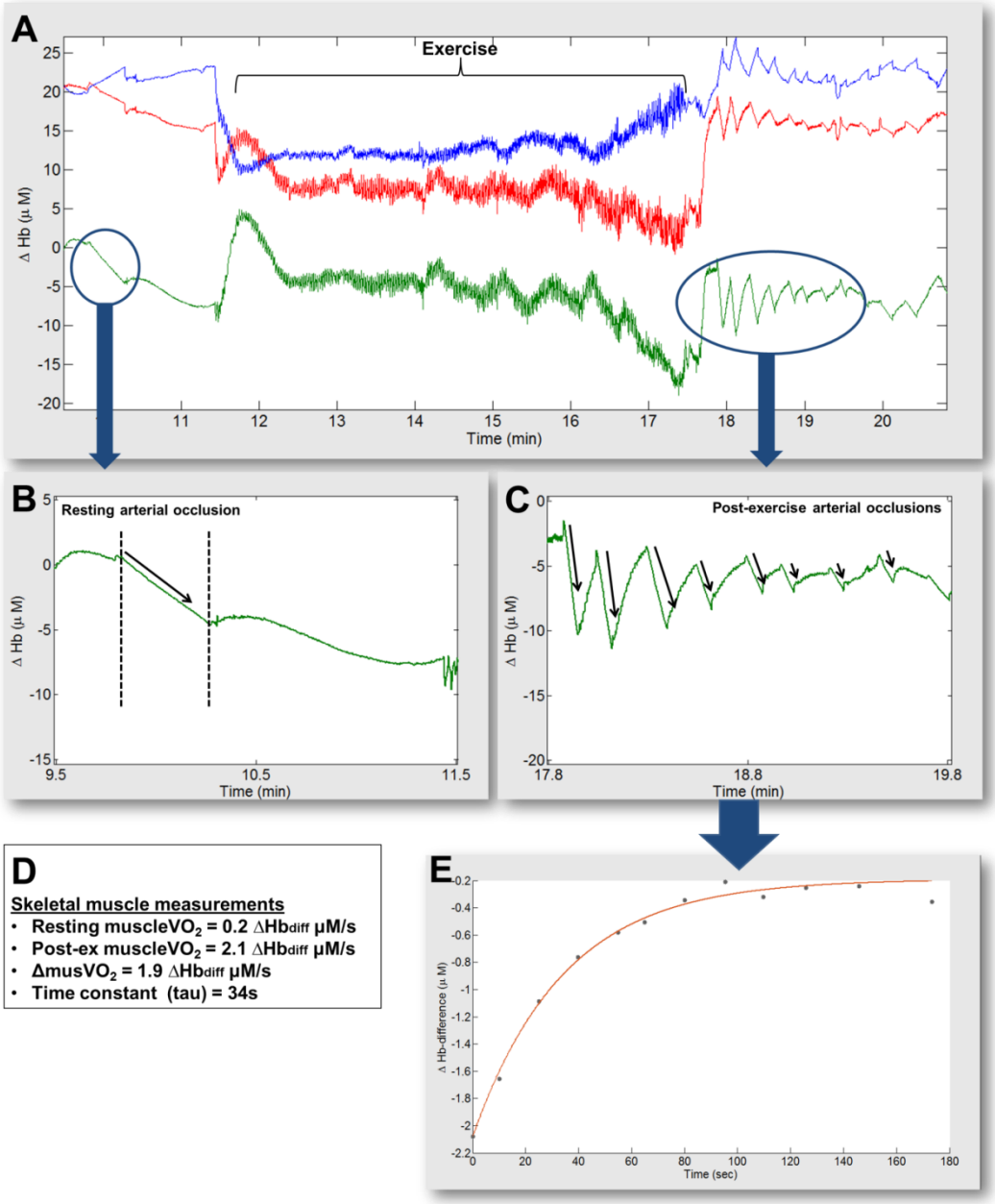


Figure 2.8 A, B, C, D & E. Example data from one participant for the process of data collection and post-processing for generation of skeletal muscle measurements using NIRS. Example NIRS data collected before, during and after a period of exercise; changes in oxy-Hb (red), deoxy-Hb (blue) and the difference between them (diff-Hb; green) are shown (A). A 30-second arterial occlusion is performed at rest in order to derive resting muscle $\dot{V}O_2$ from the slope of the difference between oxy-Hb and deoxy-Hb. Vertical dashed lines show the onset of occlusion and release of cuff (B). Immediately post-exercise transient 5-8 second arterial occlusions are performed throughout a 3-minute recovery period; the first 2 minutes of recovery is shown here. The initial occlusion is used to derive post-exercise muscle $\dot{V}O_2$ (C). The time constant for $\dot{V}O_2$ recovery (τ) is generated using the subsequent transient occlusions carried out through the recovery period (E). The example values for each measurement are given (D).

2.4.5 Correction for ATT

Variance in ATT affects NIRS signals (237, 238) and the influence of ATT on measurements of muscle oxygen consumption rate should not be ignored.(239) During an arterial occlusion, oxygen consumption rate measured by NIRS is underestimated with increasing ATT.(235) Various methods for correcting for ATT during occlusions have been described(237, 239, 240) based on phantom studies and Monte Carlo simulations.(241)

Niwayama et al, provided a simple equation for generating an ATT correction factor which can be used to correct the values derived from the slope of decline in oxy-Hb during ischemic occlusions.(240) It was later reported that these values compared well with ^{31}P-MRS (previously named ^{31}P-NMR) measurements of muscle $\dot{V}O_2$.(242)

Resting and peak muscle $\dot{V}O_2$ were corrected for ATT using a correction factor calculated from the measured ATT according to an equation previously determined using model data and simulations,(240, 243) the equation is as follows:

$$S_{muscle} = \exp\left[-\left(\frac{\mathrm{h}}{A}\right)^2\right] \qquad (3.1)$$

Where S_{muscle} is the correction factor to be applied to the NIRS-measured oxygen consumption, h is the measured adipose tissue thickness and A is a constant depending on the separation of the light source and detector in the NIRs device (4cm in the Portamon which was used here).

2.4.6 The time constant for muscle $\dot{V}O_2$ recovery

The time constant (τ) was derived by fitting a slope to the changing diff-Hb signal during each of the occlusions throughout the recovery period. Each slope was then plotted against time (figure 2.8 E) and these data were fit to a mono-exponential curve to derive τ. Goodness of fit for the curve was defined as the r^2 of the time constant values were rejected if the r^2 for the curve fit to the data was <0.6. The specific cut-off value of 0.6 was selected arbitrarily based on the reasonable assumption that if less than 60% of the variation in the data points could not be explained by a mono-exponential curve, then there was likely to be excessive noise in the signal. When the distribution of the r^2 values was examined <5% of the curves have an r^2 <0.6.

2.4.7 ATT corrected muscle $\dot{V}O2$ versus cardio-respiratory peak measured $\dot{V}O_2$

ATT-corrected values of muscle $\dot{V}O_2$ at rest and immediately post-exercise were higher than the uncorrected values; because correction factors were always < 1 (table 2.2).

Median(IQR)	Uncorrected for ATT	Corrected for ATT
Resting muscle $\dot{V}O_2$ (ΔHb_{diff} µM/s) (n=180)	0.28(0.16-0.41)	0.44(0.30-0.61)
Post-Ex muscle $\dot{V}O_2$ (ΔHb_{diff} µM/s) (n=151)	1.14(0.70-2.02)	1.85(1.22-3.04)

Table 2.2 Median (IQR) values of skeletal muscle oxygen consumption (muscle $\dot{V}O2$) at rest and immediately post-exercise with and without a correction for adipose tissue thickness (ATT)

Corrected and uncorrected values of muscle $\dot{V}O_2$ at rest are presented against ATT measurements in figure 2.9. ATT-corrected values of resting and post-exercise muscle $\dot{V}O_2$ were higher and had a greater scatter around the mean than the uncorrected values. The median uncorrected values for resting and exercise muscle $\dot{V}O_2$ are ~40% smaller than the corrected values; this is similar to other groups who have reported mean values ~50% lower.(240) In figure 2.9 corrected and uncorrected values are plotted against ATT. One previous study also made this comparison, although they present their values in different units and fewer participants were enrolled, the relationship between corrected and uncorrected tracks the changes in ATT with a similar trend.(242)

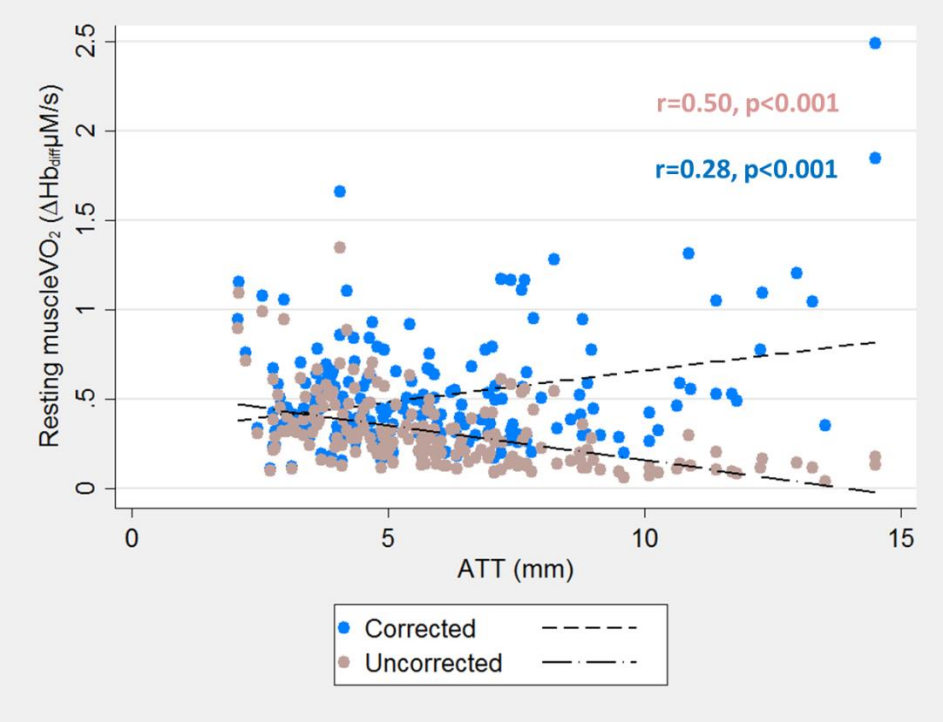


Figure 2.9 Corrected and uncorrected muscle oxygen consumption (muscle$\dot{V}O_2$). Resting values are plotted versus adipose tissue thickness measured locally at the $\dot{V}O_2$ measurement site. ATT-corrected values (blue points) and uncorrected (pink points) are shown with the line of best fit plotted for each relationship to ATT.

Chapter 3: reproducibility of assessment of exercise capacity using the 6MST and skeletal muscle oxidative capacity using NIRS

3.1 Introduction

3.1.1 Overview

Exercise capacity is an importance facet of physical function, as described above. It is valuable to develop methods for determining exercise capacity in older adults because of the known decline in both physical function with age and in disease processes associated with age.

Cardio-respiratory fitness is measured as $\dot{V}O_2$max which is the maximum capacity for oxygen uptake measured at the whole body level. $\dot{V}O_2$max is the gold standard marker of cardio-respiratory health and it has previously been shown to predict cardiovascular morbidity, cardiovascular mortality and all-cause mortality.(62, 64)

Skeletal muscle metabolic dysfunction is associated with a number of diseases (126, 208) and is also thought to decline with healthy ageing (244, 245) (as described in Chapter 1). It is therefore valuable to develop and understand methods which assess skeletal muscle metabolic function and are suitable for use in age-diverse populations. It is also valuable to develop methods that can be paired with exercise testing to allow the physiological limits of the muscle to be assessed.

3.1.2 Assessing exercise capacity

An exhaustive cardio-pulmonary exercise test (CPET) is the most well established test for determining $\dot{V}O_2$max. However, this may not be acceptable to older individuals, in whom

up to 50% may be unable or unwilling to undertake maximal exercise testing.(15, 246) Moreover, in older adults achievement on these testing protocols may not represent real-life functionality (247, 248) and exercise to exhaustion is often not achieved (67). Exhaustive CPETs are time-consuming, require specialist exercise laboratory testing facilities and, as they carry risk of adverse events, should be carried out under trained medical supervision. Submaximal exercise testing is a valuable alternative(21) and numerous ways of predicting $\dot{V}O_2$max from submaximal test results have been described.(22)

Incremental protocols, regardless of modality, also pose the difficulty of selecting an initial work rate. An initial work rate needs to be achievable and also provide adequate exertion from which increments in power can be added resulting in either maximal or submaximal exertion being reached at a similar time across the individuals being compared. The initial workload that can be tolerated by older adults, which still allows higher workloads to be achieved, will be considerably different between individuals as, while absolute exercise capacity declines with age, heterogeneity in achievable capacity increases. Allowing participants to exercise at a continuous, self-selected pace circumvents these difficulties and has been shown to provide reproducible outcomes.(249) It is also likely to improve acceptability by older adults who become anxious performing even the lowest programmable workloads on treadmills.

In older adults barriers to performing well on exercise testing protocols are often related to poor balance and frailty(13). Self-paced walking tests, such as the 6 minute walk test, have previously been well tolerated in older adult population studies (250, 251) but are limited by the need for a long corridor (20) and are not conducive to accurately measuring physiological changes during the exercise period such as observing skeletal muscle changes.

To address some of these limitations a 6-minute step test (6MST) was developed for patients with chronic obstructive pulmonary disease (COPD) (252). Borel et al, 2010(253) used the test in a group comprising older people with COPD and healthy young participants (COPD patients mean age: 61.4 years, healthy subjects mean age: 29.0 years), and reported that the step test was sensitive to ability, secure, reproducible and well tolerated.

A 6-minute stepper test (6MST), conducted on an upright stepper ergometer, was previously developed for patients with chronic obstructive pulmonary disease (COPD) (252). There are several benefits of this test: first, the stepper test does not require a corridor or similar space which is often limited in clinical and research settings. Second, the stepper test can be used to assess exercise capacity in frail elderly subjects or those with poor balance by using support rails. And third, the stepper allows a wider range of physiological responses to be measured during exercise; importantly, the assessment of skeletal muscle changes prior to and following the test is markedly easier with the testing being carried out in the static environment.

Borel et al, 2010 concluded that the step test was reproducible and well tolerated in COPD patients and distinguished between healthy individuals and patients with COPD (253). This was a small but comprehensive assessment of the reproducibility of the 6MST (n=16 patients with COPD and n=15 healthy controls) in which the 6MST was carried out twice on 3 days within a week (Monday, Wednesday and Friday). The authors suggest that the test is reproducible because there was no significant difference between the numbers of steps completed on the second test of each day; a two-way repeated measures ANOVA (‘test number’ versus ‘day’ was used across the tests. This detected a greater number of steps completed on the second test of each day which the authors attribute to ‘warming-up’ of the hydraulic jacks on the stepper. It could be argued that, if the hydraulic arms warm up with use, then, as the test progresses it should become easier and therefore

more steps would be completed in the final minutes than the first. It is more likely that, the first task of the day acts as a 'warm-up' for the participant and they are either unable to perform as well or they consciously conserve some effort for the second test. The test has since been shown to correlate with the 6 minute walk test (6MWT) and maximal exertion CPET in a larger study sample of COPD patients.(249, 254)

The feasibility of this test for a population-based sample of older adults, with no upper limit for age, has never previously been assessed. Furthermore, although free-standing step tests have been advocated for the prediction of maximal aerobic capacity,(255) the stepper test has not previously been used for this purpose. Previously reasons for exclusion or poor uptake of exercise testing protocols in older adults have not been described in detail. It is therefore, difficult to fully appreciate the determinants of acceptability of exercise tests in this population.

3.1.3 Assessing skeletal muscle oxidative capacity

Human skeletal muscle metabolic function can be evaluated *in vitro* using muscle biopsy samples to assess mitochondrial content, measure enzymatic activity and to sequence mitochondrial DNA(74) (as described in Chapter 1). This can provide valuable mechanistic insight into the systems necessary for oxidative phosphorylation but does not provide a measure of overall oxidative capacity within a physiological environment. The gold standard *in vivo* techniques to examine muscle metabolic function involve ^{31}P-MRS(73) which is expensive and limits the type and intensity of exercise/muscle activation that can be carried out during the test.

Continuous wave near infrared spectroscopy (NIRS) is a non-invasive, optical technology that measures relative changes in oxy- and deoxy-Hb up to a depth of ~1.5cm.(256) Measurements can be acquired using wireless NIRS devices and are therefore appropriate for use in a dynamic environment. The technology is cheap compared to MRS and requires less skill in its application. Although NIRS circumvents some of the limitations

that accompany tissue biopsies or MRS, it is not without limitations (these were described in detail in Chapter 1).

Two important limitations in application of NIRS to skeletal muscle are: (I) changes in the Oxy- and deoxy-Hb signals represent a composite of blood flow changes and changes in oxygen consumption rate; these cannot be distinguished from each other using NIRS alone, and (II) the variability in adipose tissue thickness (ATT) at the site of measurement alters the scattering of photons which influences the signal returning to the device.

Oxy- and deoxy-Hb signals derived using NIRS are not absolute values and represent relative concentration changes. During exercise changes in oxygen consumption rate and blood flow occur concurrently, therefore, it is impossible to differentiate these effects using NIRS alone. Applying an arterial occlusion above the site of NIRS measurement temporarily nulls the influence of blood flow allowing rate of oxygen consumption to be measured.(257) Capacity to utilize O_2 in the muscle measured during an arterial occlusion, has previously demonstrated good reproducibility.(171, 235) Applying transient arterial occlusions in the post-exercise period can also be used to determine the kinetics of muscle$\dot{V}O_2$ recovery(216, 236, 258) these measurements have also shown good reproducibility(215) and results compare well with results from established ^{31}P-MRS measurements of muscle oxidative capacity(259) and results from in vitro assessments using tissue samples.(260)

3.1.4 Objectives

The objectives of this chapter were:

(1) To investigate uptake and performance of the 6MST in terms of (a) the number of participants who are eligible and the number who decline to start the test and (b) the number of participants who do not complete 6 minutes of stepping and the reasons for not completing the test.

(2) To determine if the 6MST is sensitive enough to detect the expected sex differences in performance and predicted maximal oxygen uptake. Further to this to determine if differences in workload of the exercise (stepping-rate) result in differences in measured $\dot{V}O_2max$

(3) To compare 2 methods of predicting $\dot{V}O_2max$ from the submaximal workload and heart rate achieved during the 6MST: a method using predictive equations only with a method that uses an extrapolated maximum workload with a predictive equation

(4) To evaluate intra-test reproducibility for the 6MST and determine if improvement of performance due to a 'learning effect' is in line with previously reported learning effects in exercise testing

(5) Establish if the measurement of performance (number of steps completed) agrees with performance measured on the, well established, self-paced exercise test, the 6-minute walk test (6MWT)

(6) Determine the reproducibility of NIRS measured skeletal muscle oxidative capacity (the time constant) when carried out during the recovery from the 6MST

3.2 Methods

3.2.1 Participants

Participants in this study were recruited from the Southall And Brent REvisited study (SABRE) which is described in detail in Chapter 2.(223) Participants and spouses or partners of participants, attending the 3rd SABRE follow-up clinic visit, were invited to undertake the exercise test unless exclusion criteria were met.

All procedures were in accordance with the principles of the Helsinki declaration, all participants gave written informed consent and the study was approved by the National Research Ethics Service (NRES) Committee London – North Fulham.

A sub-set of 20 participants completed a second 6MST within one week of the first test, including physiological measurements, to assess test-retest reproducibility. Number of steps completed, perceived exertion, heart rate, systolic blood pressure in the final minute of exercise, measured $\dot{V}O_2$ and predicted $\dot{V}O_2$max were compared between the first and second 6MST. A further sub-set of 10 participants also completed a standard 6MWT (20). Number of steps or distance walked, perceived exertion, measured heart rate and highest measured $\dot{V}O_2$ were compared between the 6MST and the 6MWT.

Inclusion criteria for skeletal muscle NIRS measurements were; the participant was wearing loose fitting clothing which facilitated attachment of the NIRS device to the gastrocnemius and cuff to the lower part of the thigh, trained staff available to carry out the measurement and the participant was able to tolerate inflation of the cuff above systolic blood pressure.

A sub-set of participants (n=9) were enrolled to undergo the exercise test and muscle measurements twice, on different days, to determine reproducibility of the skeletal muscle measurements.

3.2.2 The 6-minute stepper test

The participants undertook a 6MST as described in Chapter 2 including analysis of expired gases and heart rate monitoring. A repeat test was carried out on a separate day within 1 week of the initial test. Participants were asked to abstain from caffeine and alcohol on the day of the test. Participants were not asked to fast prior to either test, this was for practical reasons relating to the clinic visit design, however, participants were asked not to eat a large meal within 2 hours before the exercise.

As described in detail in Chapter 2, heart rate and expired gas variables were measured using a heart rate monitor and portable gas analyser (K4b^2). Measured peak oxygen consumption ($\dot{V}O_2$, ml/min/kg) is given as the highest value determined from a rolling 60-

second average calculated during the 30 seconds prior to and following the transition from exercise to recovery.

3.2.3 Six minute walk test (6MWT)

Participants were directed to walk up and down a 40 meter corridor (this was the longest available corridor within the department) at a pace as fast as could be maintained without running. Their objective was to walk as many lengths of the corridor a possible. The total number of lengths and the distance covered from the start to the stop position was recorded. Thus, the total number of meters covered could be calculated.(231, 232)

3.2.4 Predicting maximal oxygen consumption

Predicted maximal heart rate ($_{max}$HR) was estimated using two equations (Fox 3.1 & Tanaka 3.2) commonly used in the literature:(231, 232)

$$maxHR(Fox) = 220 - age\ (yr) \quad (3.1)$$

$$maxHR(Tanaka) = 208 - (0.7 \times age\ (yr)) \quad (3.2)$$

The percentage of predicted maximum heart rate achieved was calculated from the achieved heart rate (measured) and the predicted max heart rate for both methods described above.

Maximal oxygen consumption ($\dot{V}O_2max$) was estimated in 2 ways: (1) using predictive equations previously described for men(31) and women.(30) and (2) using an extrapolation method.

Predictive equations

$$(Men)\ VO_2max = 1.29 \cdot \sqrt{\frac{Load[kg.m.min^{-1}]}{Peak\ heart\ rate[bpm] - 60}} \cdot e^{-0.0088 \cdot age} \quad (3.3)$$

$$(Women)\ VO_2max = 1.18 \cdot \sqrt{\frac{Load[kg.m.min^{-1}]}{Peak\ heart\ rate[bpm] - 60}} \cdot e^{-0.0090 \cdot age} \quad (3.4)$$

The workload (or power) was calculated from stepping rate (steps/min), step height (meters), which was constant at 15cm for all tests, and participant weight (Kg), equation 3.5.

$$Workload(kg.m.min^{-1}) = (step\ rate \cdot stepHt \cdot Wt + 5) + (\frac{step\ rate \cdot stepHt \cdot Wt + 5}{3}) \quad (3.5)$$

Participants were excluded from this analysis who were medicated with a β-blocker (n = 79) or who did not achieve a heart rate of at least 95bpm during exercise (n=25). The percentage of predicted $\dot{V}O_2$max during the exercise was calculated using the highest $\dot{V}O_2$ measured during the test.

Extrapolation method

The work rate (Watts) at maximal exertion was estimated by extrapolation of the linear relationship between workload and heart rate from resting and measured sub-maximal to the age predicted maximal heart rate (using Tanaka method for prediction of maximum heart rate).(10, 261) Maximal oxygen uptake was then estimated using the equation previously employed by Celis-Morales et al.(64) as follows:

$$VO_2max(ml.min.kg) = 7 + 1.8(\frac{Watts}{weight}) \quad (3.7)$$

3.2.5 Statistical analysis

Categorical data are presented as n (%). Continuous data were examined for normality and participant characteristics are presented as means ±standard deviation or median (interquartile range) if skewed; other results are presented as means (95% confidence

interval) after log transformation of skewed data. Reproducibility data were assessed using Bland-Altman plots and are presented as mean differences [limits of agreement (LOA), i.e. ±1.96 x standard deviation of differences]. Correlations were assessed using Pearson's correlation coefficients, or concordance correlation coefficients for reproducibility data, and the latter are presented as plots including the line of equality for reference. Student's *t*-tests or analysis of covariance was used for statistical comparisons. Statistical significance was assigned if $p<0.05$.

3.3 Results

3.3.1 Participants undertaking the 6MST

635 participants underwent screening for exercise testing, of these 518 (82%) undertook the 6MST. 117 participants (18%) did not undertake the exercise test. Only 4 participants who were invited to undertake the test declined because they did not want to do the 6MST. The remaining 113 participants were not eligible for inclusion for the following reasons: uncontrolled hypertension (n=40), severe arthritis (n=17), non-arthritic mobility-related limitations (n=20), recent episode of angina or cardiovascular event including TIA, stroke or MI in the past 6 weeks (n=18), uncontrolled arrhythmia (n=10), no trained staff present to conduct the test or lack of time during the visit (n=5), severe COPD (n=1), recent aortic root repair (n=1) and visual impairment (n=1).

Expired gas analysis was possible in 476 (92%) participants who performed the exercise test. Gas analysis was not possible in 8% of participants because of a technical problem with the equipment or because the participant declined to wear the face mask. Participant characteristics and exercise performance, stratified by gender, are shown in Table 2.1.

363 participants (70%) completed 6 minutes of stepping. Reasons for terminating the test before 6 minutes were: excessive diastolic or systolic blood pressure (>115 & >230mmHg, respectively) (n = 43), a sudden blood pressure drop (n=1), instability (n=3), excessive

dyspnea (n = 18), muscle fatigue (n =29), general fatigue or arthritic joint/back pain (n = 61).

Mean±SD Characteristic	Total (518)	Men (299)	Women (219)	P value
Age (Years)	71.2±6.4	73±5.4	68.8±6.8	<0.001
Height (cm)	165.6±8.9	170.7±7	158.6±6.2	<0.001
Weight (Kg)	75.8±12.8	79±12	71.1±12.6	<0.001
Steps completed	197±78	213±77	175±72	<0.001
Steps/minute	38±10	40±10	35±10	<0.001
Peak Borg	5.3±2.2	5.1±2.2	5.5±2.1	0.083
	(n=476)	*(n=278)*	*(n=198)*	
Measured VO_2 (ml/min/kg)	15.8±4.1	16.89±4.1	14.33±3.59	<0.001
Peak HR (bpm)	123±24	120±23	127±25	<0.001
Percent maxHR achieved (%)	83±16	82±16	84±16	0.064

Table 3.1 Participant characteristics, exercise performance and physiological response (mean±SD) for 518 participants who undertook the 6 minute stepper test and the 476 who had expired gasses analysed. The p value refers to the unadjusted comparison of men and women.

3.3.2 Participants undergoing skeletal muscle measurements

180 participants underwent 2 measurements of resting muscle$\dot{V}O_2$. Participant characteristics for this sub-group are given in table 3.2. 9 participants underwent resting skeletal muscle measurements on different days and undertook a second exercise test. Post-exercise muscle$\dot{V}O_2$ was also measured in these participants and the time constant

representing skeletal muscle oxidative capacity was determined. Participant demographics for this further sub-group are given in Table 3.2.

Characteristic	Mean±SD or n(%) (n=180)	Mean±SD or n(%) Sub-group (n=9)
Gender male (%)	132(73)	8(89)
Age (years)	72.2±6.1	70.0±2.3
Height (cm)	167.0±8.4	171.7±9.3
Weight (kg)	73.1±12.0	75.2±11.6
BMI (kg/m^2)	26.2±3.4	25.5±3.1
$ATT_{gastroc}$ (cm)	0.60±0.26	0.61±0.28

Table 3.2 Participant characteristics for 180 participants who underwent 2 resting skeletal muscle$\dot{V}O2$ measurements and 9 participants who underwent skeletal muscle measurements on 2 separate days. Data are mean±SD for continuous variables and n(%) for categorical variables.

3.3.3 Gender differences

Men outperformed women in terms of steps completed (213±77 versus 175±72 steps, $p<0.001$) and measured $\dot{V}O_2$ (16.9±4.1 versus 14.3±3.6 steps, $p<0.001$) (table 3.1). For both men and women, the measured $\dot{V}O_2max$ showed the expected correlation with the self-selected workload (stepping rate); Pearson's r was 0.74 ($p<0.001$) and 0.72 ($p<0.001$), for men and women, respectively.

$\dot{V}O_2max$ was predicted for 372 participants (men, n=208 (56%)); both methods predicted higher values for men compared to women (Astrand-Von Dobeln(AVD) equations: 28.4±4.2 versus 25.2±5.0 ml/min/kg; extrapolation: 24.7±3.0 versus 23.0±4.8 ml/min/kg)

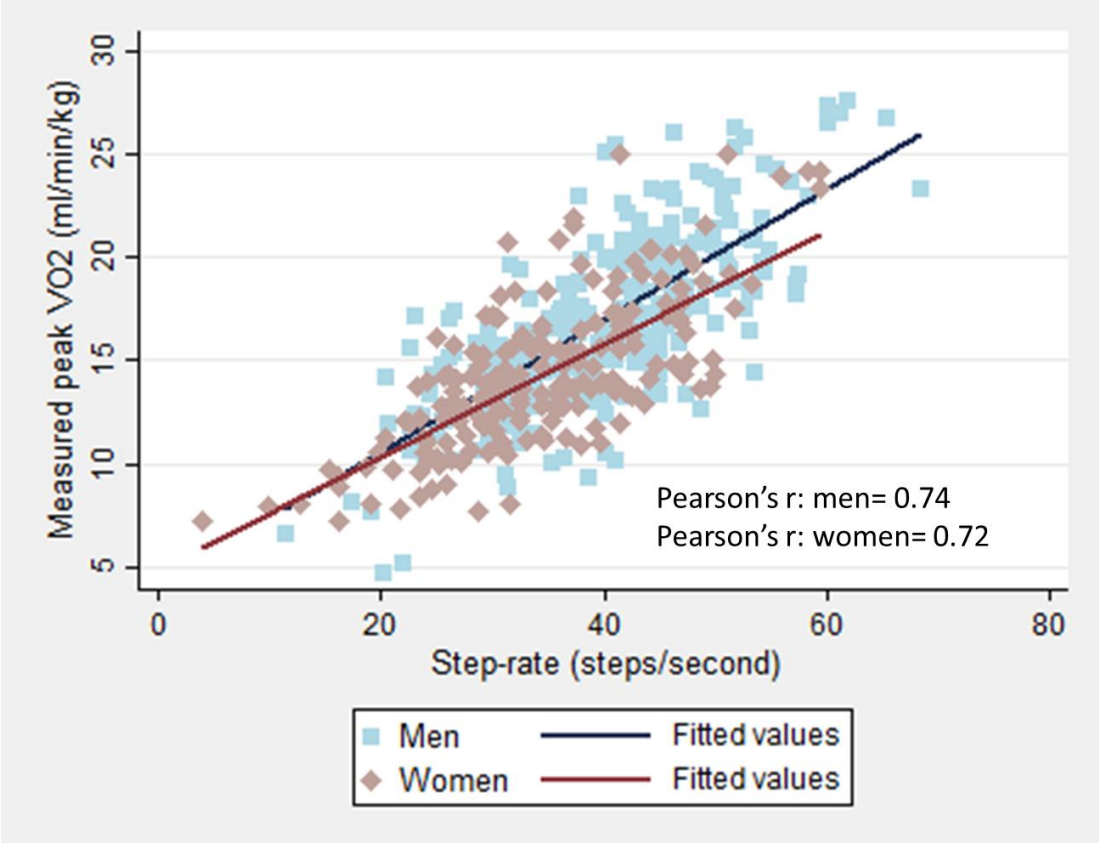


Figure 3.1 Correlation between step rate and the highest measured oxygen consumption during exercise (peak $\dot{V}O_2$) stratified by gender. Squares (blue) and diamonds (red) are individuals' data for men and women respectively. Blue and red lines are lines of best fit with correlation coefficients of 0.74 (p<0.001) and 0.72 (p<0.001), for men and women, respectively.

3.3.4 Predicting max heart rate and $\dot{V}O_2$max

Maximum heart rate predicted using the Tanaka method was 9.4bpm higher than that predicted using the method described by Fox et al (158±5 versus 149±6 bpm, p<0.0001). Percent maxHR(Fox) or maxHR(Tanaka) and percent predicted $\dot{V}O_2$max showed strong positive correlations (r = 0.74; p < 0.001 and r =0.74; p < 0.001 respectively). 61 participants achieved a higher heart rate than their predicted maximum HR, as determined using the Fox method; 28 participants exceeded predicted maximum HR using the Tanaka method.

$\dot{V}O_2$max was predicted for 372 participants using both methods (equation and extrapolation). The Tanaka method of predicting maximum HR was used in the extrapolation method. For the 28 participants who achieved a heart rate above the predicted maximum, the workload achieved in the test was assumed to be the maximal workload in equation 3.7.

$\dot{V}O_2$max values predicted using the AVD were 2.8ml/min/kg higher than values predicted via extrapolation (mean±SD: AVD 27.0±4.8ml/min/kg; extrapolation 24.2±3.2ml/min/kg). Values determined via the 2 methods were strongly correlated (Pearson's r = 0.71, p<0.0001; figure 3.2)

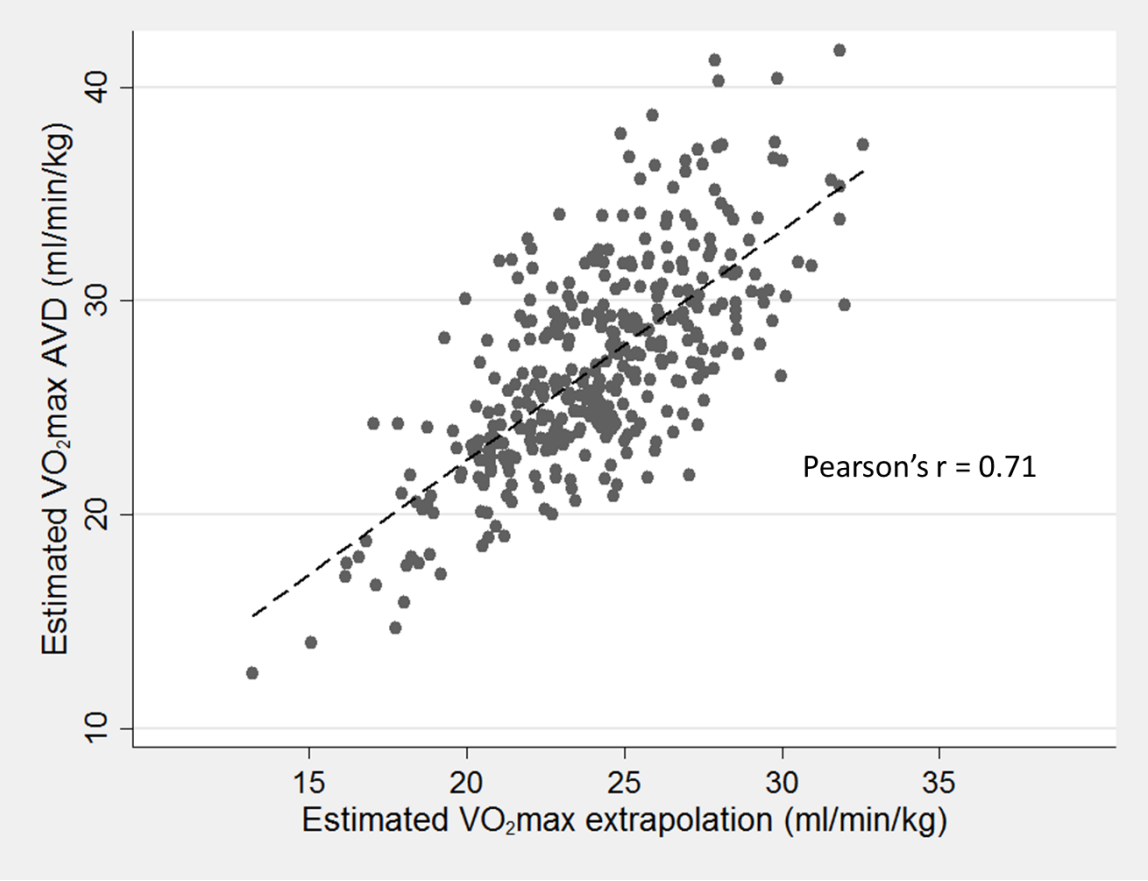


Figure 3.2 shows correlation between values for maximal oxygen consumption ($\dot{V}O_2$max) predicted using equations described by Astrand-Von Dobeln (AVD) (y-axis) and values predicted using the extrapolation method.

3.3.5 Reproducibility of the 6MST

20 participants completed a repeat 6MST. On average participants completed 34 steps more in the second test compared to the first (table 3.2). Figure 3.3a shows a Bland-Altman plot of agreement between the number of steps completed in the first and second step tests (Mean difference=34 steps, LoA = -62, 130).

Perceived exertion, measured $\dot{V}O_2$ and measured peak heart rate were all higher on the second test compared to the first. The difference between the highest measured $\dot{V}O_2$ between tests did not reach significance (table 3.2). The mean predicted $\dot{V}O_2$max did not differ between the tests and values show good agreement between the first and second stepper tests (table 3.2 and Figure 3.3b). Blood pressure measurements were possible during the final 3 minutes of exercise in all participants; the average systolic blood pressure in the final 3 minutes of exercise was not significantly different between stepper tests (table 3.2).

Mean±SD	6MST 1	6MST 2	p value (ttest)	r
Steps completed	242±80	276±79	0.005	0.82
Step rate (steps/min)	43±10	47±12	0.001	0.89
Perceived exertion (Borg)	6.2±2.2	6.9±1.8	0.03	0.82
Systolic Blood Pressure (mmHg)	183±7	183±9	0.99	0.66
Measured peak heart rate (bpm)	124±19	130±20	0.02	0.82
Measured VO_2 (ml/min/kg)	16.9±4.9	18.1±4.6	0.07	0.82
Est VO_2max (ml/min/kg) (AVD)	30.1±5.0	30.0±4.9	0.80	0.97
Est VO_2max (ml/min/kg) (extrap')	25.7±3.2	26.8±3.8	0.01	0.89

Table 3.2 shows differences in performance and physiological measures and predictions from stepper test 1 to stepper test 2. The p value given in column 4 was

calculated using a paired t-test and r is Pearson's for the correlation between each variable at test 1 versus test 2, p for the significance of the correlation was <0.01 for all the correlations. AVD; Astrand-VonDoubln, Est; estimated.

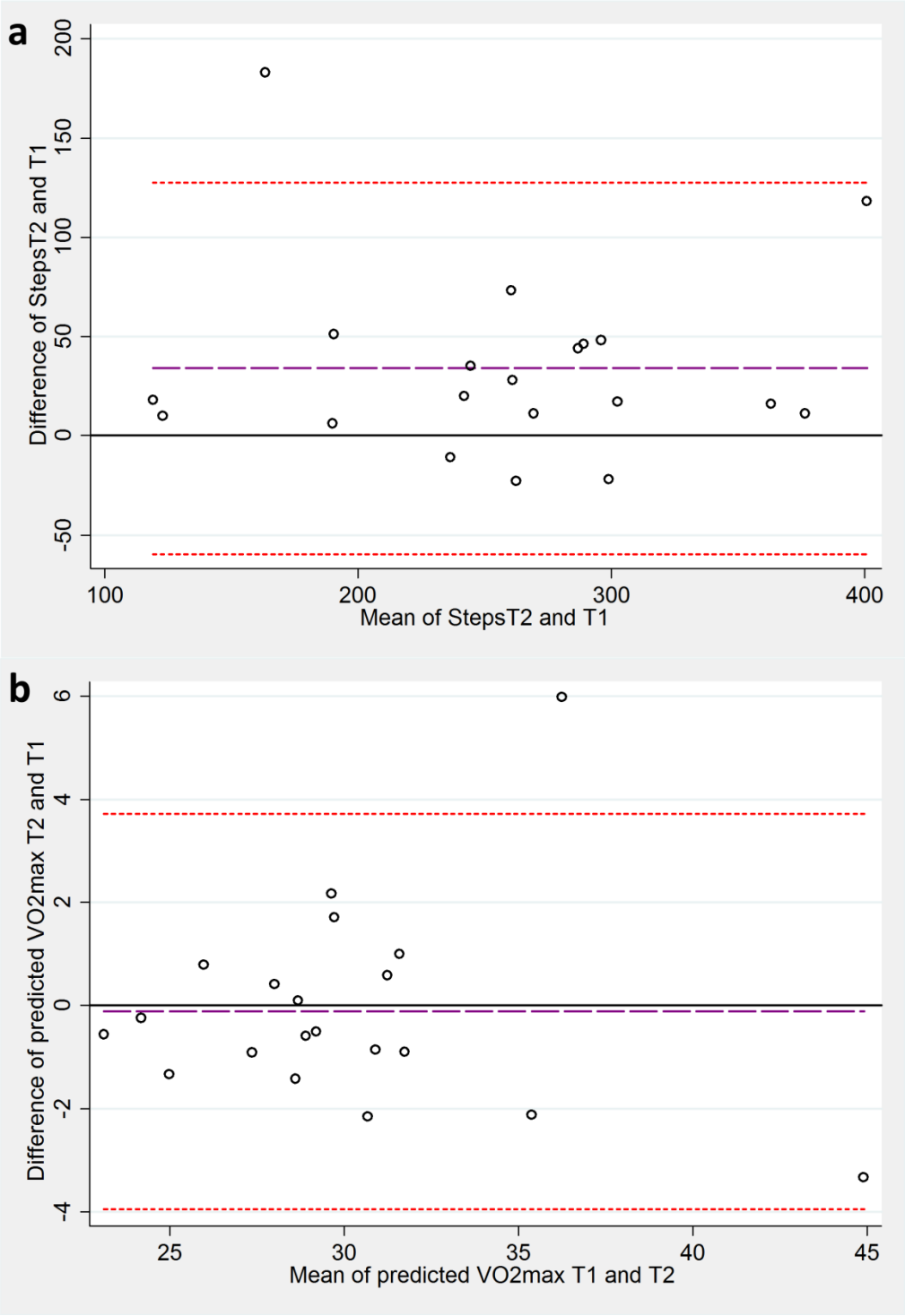


Figure 3.3 a & b Bland-Altman plots demonstrating levels of agreement between stepper test 1 (T1) and stepper test 2 (T2) for (a) steps completed, and (b) predicted maximum oxygen consumption ($\dot{V}O_2$max). Mean difference is plotted as a purple dashed line, upper and lower limits of agreement are plotted as red-dashed lines. Mean difference [limits of agreement] were (a) 34[-59.8, 127.7] steps and (b), 0.11 [-3.95, 3.72] ml/min/kg.

3.3.6 Correlation of the 6MST with the 6MWT

10 participants completed a 6MST and a 6MWT. Walking rate and step rate were positively correlated (r=0.77; p<0.001) as were walking distance and total number of steps (r = 0.61). Average values for peak heart rate, measured $\dot{V}O_2$ and perceived exertion (modified Borg) were similar and measured $\dot{V}O_2$ (ml/min/kg), peak heart rate and measured $\dot{V}O_2$ also showed positive correlations between the 6MST and the 6MWT; however, there was no relationship between perceived exertion on the two tests (table 2.3).

Mean±SD	Stepper test	Walk test	r	p value
Steps completed/meters walked	211±89	518±105	0.61	0.06
Step rate (steps/min)/walk rate(m/min)	40±10	86±18	0.77	<0.01
Perceived exertion (Borg)	6.7±1.7	5.8±1.4	-0.14	0.7
Resting HR (bpm)	63±9	70±9	0.83	0.004
Measured pk HR (bpm)	116±21	114±21	0.5	0.1
Measured pk $\dot{V}O_2$ (ml/min/kg)	16.5±6.4	17.8±5.2	0.43	0.2

Table 3.3 Results of the stepper test and walk test and Pearson's correlation coefficient (r) for the two tests with a p value for the correlation. Data are means±SD of 10 observations. Pk HR; peak heart rate, BPM; beats per minute.

3.3.7 Reproducibility of resting muscle $\dot{V}O_2$

Agreement between 2 resting muscle$\dot{V}O_2$ measurements made on the same day without removing the NIRS device was good. Mean difference[LoA] was 0.00[-0.14, 0.14] (Bland-Altman plot; Figure 3.4 A). Figure 3.4 B is a plot of the first muscle$\dot{V}O_2$ (R1) versus the second (R2); concordance correlation coefficient was 0.93.

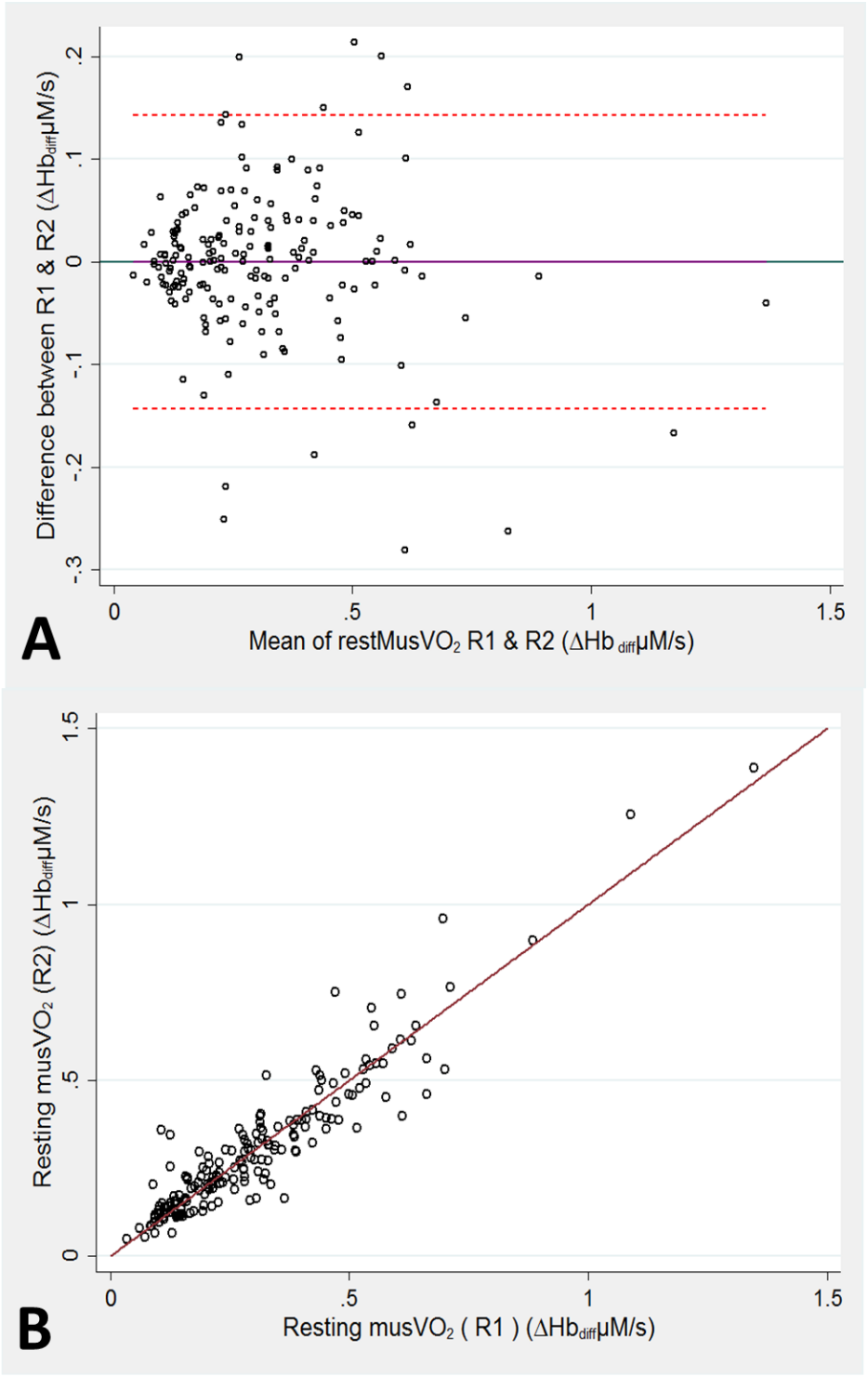


Figure 3.4 A & B Agreement between 2 measurements of resting muscle V̇O2. (A) A Bland-Altman plot demonstrating levels of agreement between resting muscleV̇O2 measurements 1 (R1) and 2 (R2). Mean difference is plotted as a purple line, upper and lower limits of agreement are plotted as red-dashed lines; mean difference

(limits of agreement) were 0.00(-0.143, 0.143) ΔHb_{diff}µM/s. (B) resting muscle$\dot{V}O_2$ measured R1 versus R2 are plotted; concordance correlation coefficient(SE) were 0.931(0.010). The line of equality (x=y) is plotted in red.

Agreement between measurements of resting muscle $\dot{V}O_2$, made on 2 separate days was excellent. Mean difference(LoA) was 0.05(0.16, 0.25) (Bland-Altman plot; Figure 3.5 A;). Figure 3.5 B is a plot of the first resting muscle$\dot{V}O_2$ (D1) versus the second (D2) (concordance correlation coefficient(SE)=0.90(0.07)).

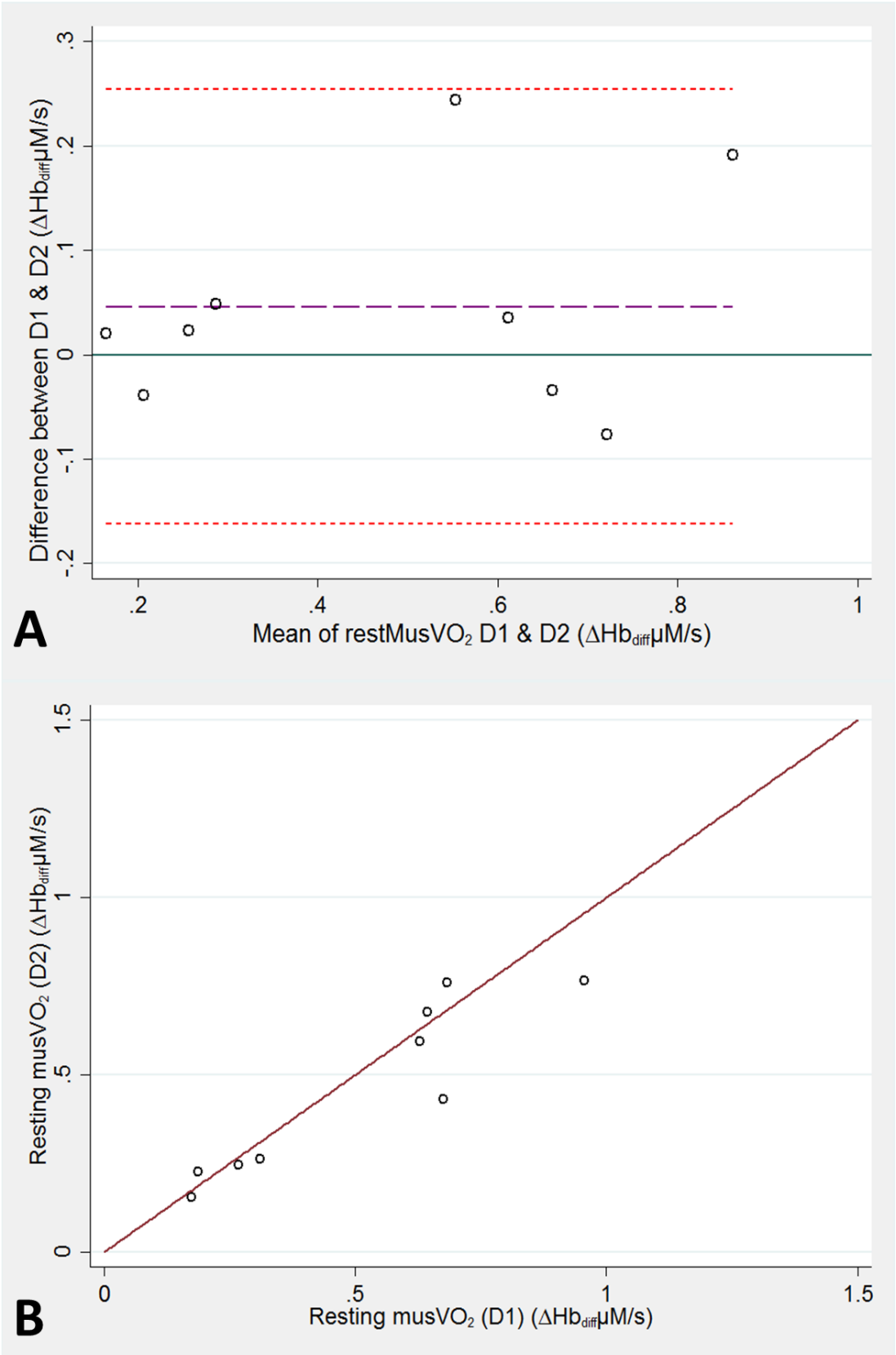

A
Difference between D1 & D2 (ΔHbdiffμM/s)
-.2
-.1
0
.1
.2
.3
.2
.4
.6
.8
1
Mean of restMusVO2 D1 & D2 (ΔHbdiffμM/s)
B
Resting musVO2 (D2) (ΔHbdiffμM/s)
0
.5
1
1.5
0
.5
1
1.5
Resting musVO2 (D1) (ΔHbdiffμM/s)

Figure 3.5 A & B Agreement between 2 measurements of resting muscle V̇O2 made on separate days. **(A) Bland-Altman plot demonstrating levels of agreement between resting muscle$\dot{V}O_2$ (rest Mus$\dot{V}O_2$) carried out on day 1 (D1) and day 2 (D2). Mean difference is plotted as a purple line, upper and lower limits of agreement are plotted as red-dashed lines; mean difference(limits of agreement) were 0.05(-0.162, 0.254) ΔHb_{diff}µM/s. (B) resting muscle$\dot{V}O_2$ measured on D1 versus D2 is plotted; concordance correlation coefficient(SE) were 0.901(0.067) ΔHb_{diff}µM/s. The line of equality (x=y) is plotted in red.**

3.3.8 Exercise capacity in the sub-group

In the sub-group of 9 participants who underwent 2 exercise tests there was good agreement between the number of steps achieved on each test. Mean difference(LoA) was 14.8(-37.6, 67.2) steps (Bland-Altman plot; Figure 3.6 A). The concordance correlation coefficient(SE) was 0.84(0.11) (Figure 3.6 B).

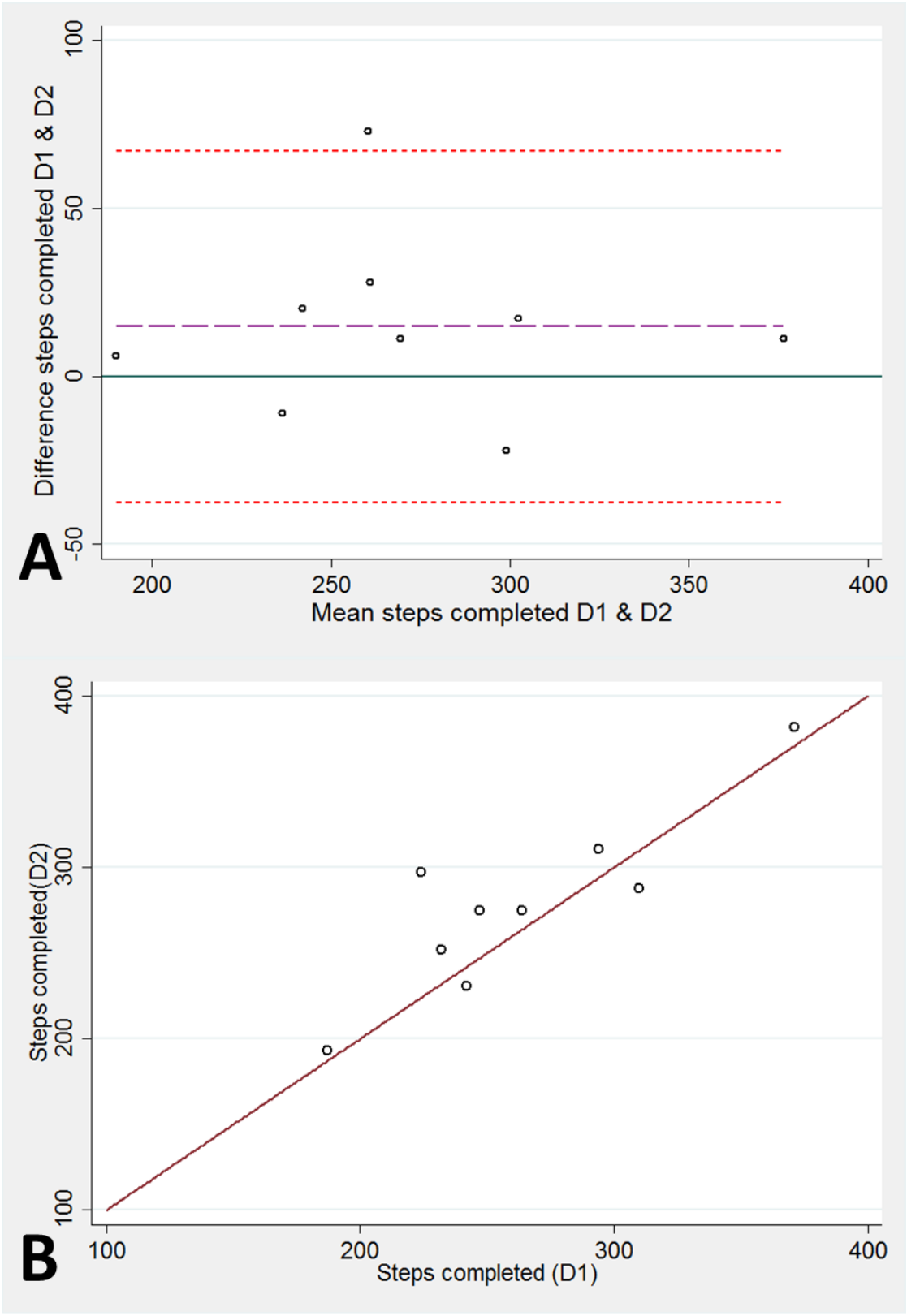

A
Difference steps completed D1 & D2
100
50
0
-50
200
250
300
350
400
Mean steps completed D1 & D2
B
Steps completed(D2)
400
300
200
100
100
200
300
400
Steps completed (D1)

Figure 3.6 A & B. Agreement between exercise capacity measured by the 6MST completed on 2 different days. (A) Bland-Altman plot demonstrating levels of agreement between the steps completed on day 1 (D1) and 2 (D2). Mean difference is plotted as a purple line, upper and lower limits of agreement are plotted as red-dashed lines; mean difference (limits of agreement) were 14.778(-37.629, 67.184) steps. (B) steps completed on D1 and D2 are plotted; concordance correlation coefficient(SE) were 0.841(0.106) steps. The line of equality (x=y) is plotted in red.

3.3.9 Reproducibility post-exercise muscle $\dot{V}O_2$

Agreement between post-exercise muscle $\dot{V}O_2$ (Post-Ex Mus $\dot{V}O_2$) measurements made on 2 separate days was good. Mean difference(LoA) was 0.60 (-1.01, 2.21) (Bland-Altman plot; Figure 3.7 A). Figure 3.7 B is a plot of the first exercise muscle $\dot{V}O_2$ (D1) versus the second (D2) (concordance correlation coefficient(SE)=0.73(0.14)).

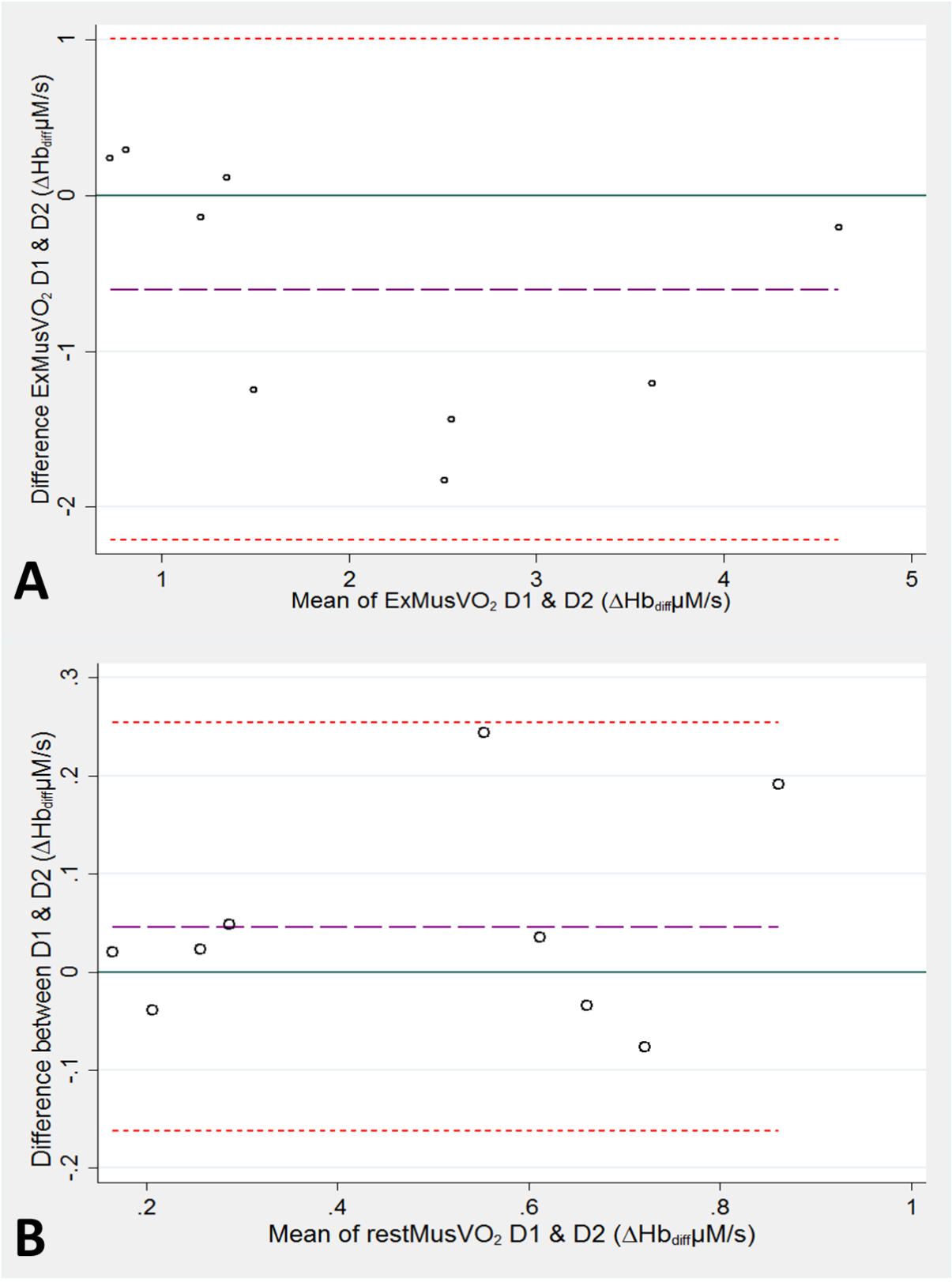

A
Difference ExMusVO2 D1 & D2 (ΔHbdiffμM/s)
1
0
-1
-2
1
2
3
4
5
Mean of ExMusVO2 D1 & D2 (ΔHbdiffμM/s)
B
Difference between D1 & D2 (ΔHbdiffμM/s)
.3
.2
.1
0
-.1
-.2
.2
.4
.6
.8
1
Mean of restMusVO2 D1 & D2 (ΔHbdiffμM/s)

Figure 3.7 Agreement between 2 measurements of post-exercise muscle$\dot{V}O_2$ (ExMus$\dot{V}O_2$) made on separate days. (A) Bland-Altman plot demonstrating levels of agreement between ExMus$\dot{V}O_2$ carried out on day 1 (D1) and day 2 (D2). Mean difference is plotted as a purple line, upper and lower limits of agreement are plotted as red-dashed lines; mean difference(limits of agreement) were 0.604(-1.006, 2.213) ΔHb_{diff}µM/s. (B) ExMus$\dot{V}O_2$ measured on D1 versus D2 is plotted; concordance correlation coefficient(SE) was 0.749 (0.140) ΔHb_{diff}µM/s. The line of equality (x=y) is plotted in red.

3.3.10 Reproducibility of the time constant for muscle$\dot{V}O_2$ recovery

Data from one participant was excluded from the time constant analysis because too few recovery arterial occlusions were achieved to allow curve fitting. Agreement between the recovery time constant for muscle$\dot{V}O_2$ made on 2 separate days was good. Mean difference(LoA) was 1.74(-41.24, 44.73) (Bland-Altman plot; Figure 3.8 A). Figure 3.8 B is a plot of the first time constant (D1) versus the second (D2) (concordance correlation coefficient(SE)=0.89(0.09)).

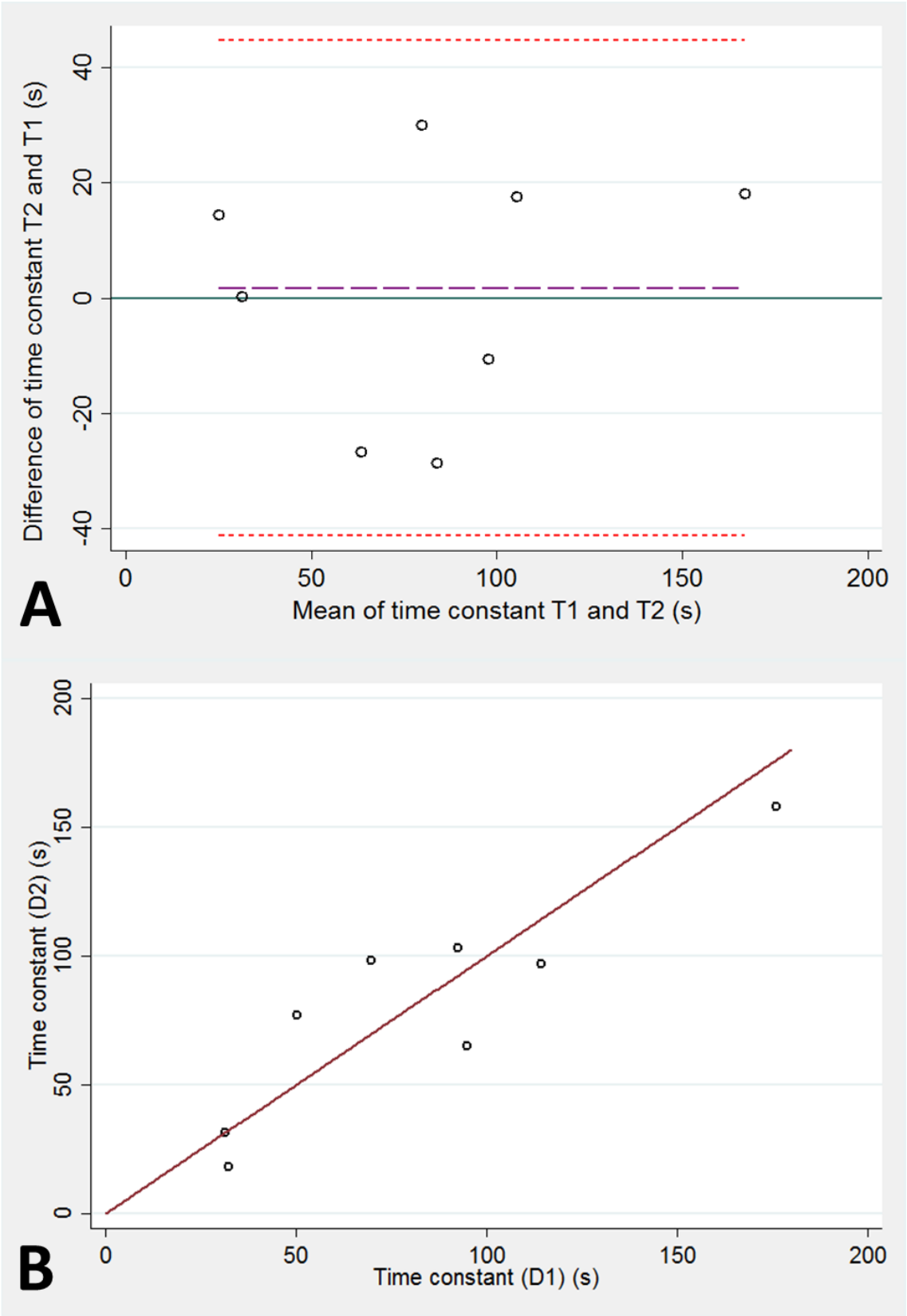

A
Difference of time constant T2 and T1 (s)
40
20
0
-20
-40
0
50
100
150
200
Mean of time constant T1 and T2 (s)
B
Time constant (D2) (s)
200
150
100
50
0
0
50
100
150
200
Time constant (D1) (s)

Figure 3.8 A & B. Agreement between 2 measurements of time constant. (A) Bland-Altman plot demonstrating level of agreement between time constant of recovery carried out on day 1 (D1) and day 2 (D2). Mean difference is plotted as a purple line, upper and lower limits of agreement are plotted as red-dashed lines; mean difference(limits of agreement) were 1.743(-41.239, 44.725) s. (B) Time constant measured on D1 versus D2 is plotted; concordance correlation coefficient(SE) were 0.887(0.085) s. The line of equality (x=y) is plotted in red.

3.4 Discussion

3.4.1 Key findings

This study demonstrates that the 6MST is a reproducible method for assessing exercise capacity in a population-based sample of adults over the age of 65 and that the expected sex-differences in exercise capacity can be detected. Performance on the 6MST, in terms of either steps completed or stepping rate, was positively correlated with performance on the 6MWT ($p=0.06$ & $p<0.01$, respectively). Although only <1% of the study population declined to start the test, 30% of participants did not manage to complete 6 minutes of stepping.

The 6MST provided an adequate sub-maximal heart rate allowing aerobic capacity ($\dot{V}O_2max$) to be predicted via 2 different approaches, a valuable marker of physical function that is difficult to measure directly in this population (248). However, 25 (5%) participants were excluded from this analysis because they did not achieve a heart rate of >95bpm and 79 (15%) were excluded because of medication with β-blockers.

This study also describes application and reproducibility of NIRS for assessment of resting muscle$\dot{V}O_2$, post exercise muscle$\dot{V}O_2$ and a recovery time constant for muscle$\dot{V}O_2$ representing skeletal muscle oxidative capacity. Good agreement was demonstrated between two resting measurements of muscle$\dot{V}O_2$, performed using NIRS, conducted on

the same day in the same older adult cohort and two resting muscle$\dot{V}O_2$ measurements conducted on different days. Good agreement was also demonstrated between two post-exercise measurements of muscle$\dot{V}O_2$ and two measurements of the time constant for muscle$\dot{V}O_2$ recovery.

3.4.2 Uptake and acceptability of the 6MST in older adults

Free standing step tests are widely used to assess exercise capacity and predict $\dot{V}O_2$max(255) but stepper tests are a less commonly described method of exercise testing. Previous studies verifying the 6MST were carried out in COPD patient populations with a younger mean age than the sample in this study (253, 254). In this study of an older adult population who were expected to experience increased frailty and poorer balance, we fastened support rails to the wall in front of the stepper. Although there are some limitations in allowing participants to hold onto rails (discussed later), all participants elected to use the rails for support suggesting they were a valuable addition to the test in the older-adult population. Most of the excluded participants were excluded due to a medical condition. Only 4 participants out of 522 (<1%) declined to exercise because they felt the stepper was not an acceptable activity for them. This compares very favourably with the acceptability of maximal exercise testing using a bicycle ergometer in similar age group, where 20% refused to undertake the exercise.(246) The exclusion of the remaining 113 participants (18%) for health related safety requirements (given in the guidelines(17)) is similar to a previous report in this age group. (246)

30% (n=155) of participants who started the exercise test did not manage to complete 6 minutes of stepping. In 30% (n=47) of these cases, the test was terminated by the technician because of a risk factor such as adverse blood pressure change or instability. This would presumably have been the same outcome regardless of the mode of exercise. Excessive dyspnea (n = 18), muscle fatigue (n =29) and general fatigue or arthritic joint/back pain (n = 61) contributed to 70% of the incomplete tests. This equates to 9% of

the total participants who elected to undertake the test. Therefore, if acceptability of the 6MST is assessed by 'drop-out' rates, then, overall, the 6MST has ~91% acceptability in older adults.

In the German Health Interview and Examination Survey for Adults (DEGS) 57% of participants were eligible to undertake a sub-maximal cycle-ergometer test and 97% of these test-qualified participants agreed to undertake the test.(14) This latter result is similar to the percentage of participants who agreed to undertake the 6MST; however, DEGS was comprised of participants aged 18-64 years old and had a higher exclusion on eligibility criteria.

There was a strong positive relationship between performance (step rate) and measured $\dot{V}O_2$ suggesting the 6MST is sensitive to increases in load (Figure 3.2). The 6MST detected expected gender differences in performance (262) providing some evidence of validity.

3.4.3 Predicting $\dot{V}O_2$max

Submaximal exercise testing with a prediction of $\dot{V}O_2$max is frequently described.(Smith, Evans et al. 2016; Bennett, Parfitt et al. 2016) In this study we used an equation to predict $\dot{V}O_2$max from the sub-maximal heart rate, first described by von Dobeln et al. (263). We selected this equation because the population used to derive this equation included individuals up to 70 years old; unlike some alternative equations.(264) Numerous equations have been reported to predict maximum heart rate with age, we chose two that have been widely used.(231, 232); while all such equations have limitations,(265) in this study they were only used to give an indication of the intensity of the sub-maximal exercise achieved using the 6MST.

3.4.4 Reproducibility of the 6MST and predicted $\dot{V}O_2$max

Steps completed during the second stepper test improved by an average of ~14% with associated improvements in physiological markers of exertion: heart rate & measured $\dot{V}O_2$ (table 3.2). This difference is likely attributable to a 'learning effect'. Other studies report similar findings using other field tests.(266) Despite this, predicted $\dot{V}O_2$max using AVD equations showed very good agreement between the first and the second test (figure 3b), suggesting that the estimate is valid during a primary test. Prediction of $\dot{V}O_2$max using the extrapolation method were higher after from the second test.

3.4.5 Correlation between the 6MST with the 6MWT

The comparison between 6MST and 6MWT was conducted to assess the validity of the 6MST, since the 6MWT is a very widely used test of performance. Results from the 6MWT are usually summarised as distance travelled or walking rate, whereas performance on the 6MST is measured as steps completed or step-rate. Both markers of stepping performance correlated positively with markers of walking performance; meters travelled and walking rate (m/min) (Pearson's r=0.61 and 0.77, respectively). Physiological measurements, $\dot{V}O_2$ and heart rate at the end of exercise, were also positively correlated between the tests although less strongly. Perceived exertion, assessed as a Borg score (0-10), was scored by the participant at the end of each test. Scores after stepping were not correlated with scores after walking. One explanation may be that individuals perceived the severity of the two tasks within a limited range which reduces the correlation.(267) It is also possible that participants experienced these different types of exercise as being more, or less, difficult for reasons unrelated to the intensity of the exercise. Further investigation into the perception of difficulty of an exercise is beyond the scope of this study but further investigation into its contribution to the acceptability of the stepping test would be interesting.

3.4.6 Reproducibility of NIRS for skeletal muscle measurements

NIRS has previously been used for assessment of skeletal muscle blood flow and metabolism in the context of exercise testing and techniques have been developed to assess various features of muscle function.(256) The findings in this study are in line with previous studies that have reported good reproducibility of measurements of muscle$\dot{V}O_2$(235) and good reproducibility of the time constant.(215, 219, 220) One study examined reproducibility of the time constant in a group of older adults approaching the same mean age as the participants enrolled in this study (60±7 years old); they reported an ICC of 0.93.(220)

The measurements of post-exercise muscle$\dot{V}O_2$ were lower on the second test (D2) compared to the first test (D1). This appears out of line with the previously described ~30% improvement in exercise performance (Chapter 2). However, participants in the sub-group who also underwent muscle measurements, only completed an average of 15 more steps in the second stepper test compared to the first; ~6% improvement from the 1st test (D1). Compared with the results presented in Chapter 2 which included 20 participants, this was a smaller improvement in performance from D1 to D2 and therefore less likely to yield a higher muscle$\dot{V}O_2$ response.

ATT-corrected values of resting and post-exercise muscle$\dot{V}O_2$ were higher and had a greater scatter around the mean than the uncorrected values. The median uncorrected values for resting and exercise muscle $\dot{V}O_2$ are ~40% smaller than the corrected values; this is similar to other groups who have reported mean values ~50% lower.(240) In figure 3.9 corrected and uncorrected values are plotted against ATT. One previous study also made this comparison, although they present their values in different units and fewer participants were enrolled, the relationship between corrected and uncorrected tracks the changes in ATT with a similar trend.(242)

3.5 Limitations

3.5.1 The 6MST

The 6MST is a self-paced test with an instruction to maintain stepping pace throughout. While this allows the protocol to be simple and increases acceptability, performance on the test is limited by a combination of physiological and psychological factors. Therefore, the physiological response, measured peak $\dot{V}O_2$, will be determined, to varying degrees, by the participants' self-efficacy in performing exercise, previous experience of exercise and perception of how hard the exercise is. However, self-perception of intensity also depends on physiological efficiency – the ability to deliver and effectively utilize oxygen influences the perception of muscle fatigue and therefore, effort; a typical example is accumulation of lactic acid in muscle.(44)

Although performing an incremental, maximal exertion exercise test would circumvent this limitation, maximal exertion cardio-pulmonary exercise tests (CPETs) are also not exempt from the effects of varying participant effort. When conducted according to current guidelines,(268) the incremental, maximal exertion CPET has been considered to under-represent cardio-respiratory $\dot{V}O_2$max.(66)

A further limitation of a self-paced test is that we cannot be certain that the participant has maintained pace throughout the test and, therefore, kept the work load constant. Participants were asked to try to select a pace from the onset of exercise that they felt could be maintained but the technician did not control the pace at any point during the test.

We fixed support rails to the wall in front of the stepper. This may also compromise the accuracy of calculating workload because we cannot be sure to what extent each participant used the rails to assist themselves during exercise. We found that all participants opted to support themselves by holding on to the rails during the test. Holding onto rails may have altered the measured $\dot{V}O_2$ as the participant exerts force from their

arm muscles to grip. An obvious limitation is that we did not directly validate the predicted $\dot{V}O_2$max by conducting maximal exercise tests in our study sample. However, as discussed above many older people are unwilling to undertake maximal exercise testing(246) and, in those who do, exercise to exhaustion is often not achieved(67); moreover the use of maximal exercise testing has safety implications. Previously, performance on the 6MST has been shown to correlate well with $\dot{V}O_2$max in younger COPD patients (254).

3.5.2 NIRS measured skeletal muscle oxidative capacity

The influence of skin perfusion on the NIRS signals remains unclear and no attempt was made to correct for this. Carrying out NIRS measurements following a period of exercise where skin perfusion is likely to increase to allow body cooling is likely to introduce some error and increased variability to the measurements.(194) Although this has previously been considered to influence the muscle measurements only minimally,(195) future muscle studies would merit including an adjustment or correction for increased skin perfusion during exercise. This could potentially be addressed by including a channel with a short source-detector distance; previous estimates suggest that, for adults 8.4mm is the ideal distance.(269) The shortest source-detector distance available in the device applied here was 30mm.

Absolute values of oxy- and deoxy-Hb cannot be measured using continuous wave NIRS because the Beer-Lambert law assumes that the medium which the photons are passing through is homogenous (non-scattering). As explained in Chapter 1, biological tissue is not homogenous (comprised of layers: epidermis, dermis, fat and muscle) and differences in thickness of adipose tissue above the area of interest alters the light-scattering properties of the tissue.(168) Variance in ATT between participants is therefore a major technical limitation for NIRS. Values of muscle$\dot{V}O_2$ presented here were corrected for the ATT using equations previously described.(240) There are some limitations to using these equations:

First, derivation of the original correction curves were based on simulated data and experimental data obtained from 15 participants,(243) in these studies 12 participants were male and 3 female; the 12 men had ATT ranging from 0.3-0.8cm(239) while 2 of the women represented the upper limit of measured ATT (~1.1cm). This leaves some uncertainty whether the relationship between ATT and muscle muscle$\dot{V}O_2$ described in this study could have been partially attributed to a gender difference.(242) Second, using ATT-corrected values of muscle$\dot{V}O_2$ to examine differences between groups of participants potentially risks attenuating differences between groups that could be the result of generally greater adiposity. For example, if we are interested in the difference in muscle$\dot{V}O_2$ between people with and without diabetes, correcting for ATT is appropriate in technical terms because it corrects for the increase in scattering; however, having greater adiposity in general may influence the 'true' value of oxygen consumption in the muscle, and therefore, correcting the muscle$\dot{V}O_2$ measurement for ATT has the potential to attenuate underlying 'true' differences between people with and without diabetes. Alternatively, if we have some prior knowledge that adiposity influences 'true' skeletal muscle$\dot{V}O_2$, it would be appropriate to conceptually model ATT/adiposity as a confounder, in which case using corrected values is appropriate.

3.6 Conclusion

In conclusion, the 6MST is an appropriate method of assessing fitness that is well accepted by older adults and has some advantages compared with the 6MWT. $\dot{V}O_2$max can be estimated reproducibly using the relationship between workload and submaximal heart rate and a previously described predictive equation. Conducting a study to compare $\dot{V}O_2$max results of an exhaustive CPET with the 6MST may confirm this. The results from the 6MST test correlate with the 6-minute walk test in terms of outcome for exercise

capacity. Skeletal muscle metabolic function can be examined reproducibly using NIRS in this population-based group of older adults undertaking a 6MST.

Chapter 4: Exercise capacity and skeletal muscle metabolic function in the presence of diabetes

4.1 Introduction

4.1.1 Overview

Type 2 diabetes mellitus (T2D) is a debilitating, chronic disease and public health problem.(110) The prevalence of diabetes has increased over the last few decades and is predicted to rise to as much as 642 million by 2040.(111) People with T2D consistently have excess cardiovascular and all-cause mortality despite aggressive cardiovascular risk factor intervention.

T2D is associated with impaired cardiorespiratory fitness compared to age, weight and activity-level matched people without diabetes.(270-272) This impairment in fitness is clinically relevant as it potentially contributes to the increased cardiovascular and all-cause mortality seen in T2D.(273-275) Evidence from epidemiological studies suggests that higher levels of physical activity reduce morbidity and mortality in people with diabetes.(109) Thus, it is important to understand which aspects of the components that make up cardio-respiratory fitness can be improved via increasing physical activity and which activities are most effective. Cardio-respiratory fitness, measured by CPET, provides an overall, total-body measurement of maximal oxygen uptake (as described in Chapter 1). This type of test does not usually incorporate an assessment of oxygen uptake kinetics and oxidative capacity specifically in the muscle.(276)

As described in Chapter 1, evidence suggests that impaired skeletal muscle oxidative capacity exists in people with T2D; whether this is due to reduction in the number of mitochondria or the intrinsic functional capacity of the mitochondria is still open for debate. An association between impaired mitochondrial function and development of insulin resistance and disease progression has also been described(107) although the cause-effect relationship remains unclear.(129)

While muscle metabolic disruptions associated with T2D are not likely to be the primary limiting factor for exercise capacity, evidence suggests that exercise intervention improves metabolic function of skeletal muscle(277) and attenuates lipid-induced insulin resistance.(105) It is therefore valuable to consider, in the same individuals, the relationships between exercise capacity, cardio-respiratory fitness ($\dot{V}O_2max$) and local skeletal muscle oxygen kinetics. Because of the technical limitations of conducting skeletal muscle measurements (described in Chapter 1), previous studies have tended not to include a simultaneous measure of cardio-respiratory oxygen consumption and to employ small sample sizes prohibiting multivariable analyses. Previous studies that include an exhaustive CPET tend to recruit younger participants who are more able to achieve maximal exertion.

Submaximal exercise tests are a valuable way of assessing exercise capacity and predicting cardio respiratory fitness (VO_2max). As well as having improved ease of administration, submaximal exercise tests are more representative of everyday exercise. NIRS is an emerging tool that permits non-invasive skeletal muscle measurements to be carried out in a dynamic environment.

4.1.2 Objectives

(1) To compare sub-maximal exercise capacity, in terms of steps completed and step-rate selected on the self-paced 6MST, and muscle strength, measured by grip-strength, between older adults (>65 years old) with or without T2D.

(2) To describe reasons for drop-out on the 6MST before completion of 6 minutes and compare reasons for drop-out between older adults with, or without, T2D.

(3) Compare perception of exertion (Borg score) during exercise between individuals with, or without, T2D.

(4) Compare measured sub-maximal oxygen uptake (measured peak VO_2) and predicted VO_2max between older adults with or without diabetes. Other markers of cardio-respiratory exertion measured during exercise; heart rate (HR), HR response (HRR), oxygen uptake efficiency slope (OUES) and blood pressure are also compared in the presence and absence of T2D.

(5) Compare locally measured skeletal muscle oxygen consumption (muscle $\dot{V}O_2$) and skeletal muscle oxidative capacity between older adults with and without T2D

(6) Determine the association (or disassociation) between submaximal exercise capacity and skeletal muscle oxidative capacity and if this relationship is different in the presence of T2D.

4.2 Methods

4.2.1 Participants

Participants in this study were recruited from the SABRE study as described in Chapter 2.(223).

All procedures were in accordance with the principles of the Helsinki declaration, all participants gave written informed consent and the study was approved by the National Research Ethics Service (NRES) Committee London – North Fulham.

4.2.2 Anthropometrics and questionnaires

Height was measured barefoot using a standard stadiometer (Seca217, Hamburg, Germany). Weight, body fat mass and body fat percentage were measured using digital bio-impedance scales (BC-418,Tanita, USA).

Diabetes was defined as self-reported physician diagnosis or reported use of anti-diabetic medication, hypertension was defined as self-reported physician diagnosis, or reported use of medication for hypertension. Details of medication, years of education, ethnicity, smoking habit, alcohol intake and physical activity were obtained by questionnaire.

4.2.3 Exercise testing

Participants were invited to undertake the 6MST; this was carried out as described in Chapter 2. Measurements of exercise capacity were recorded as; steps completed, stepping rate and the power achieved on the stepper which is calculated via equation 5 (Chapter 2).

At the end of the exercise test participants were asked to rate how hard they perceived their effort to be on a modified Borg score (0-10).(227) Two scores were recorded; one for their perceived cardio-respiratory effort (how hard they perceived their breathing to be) and one for leg muscle fatigue.

Muscle strength was measured as grip strength, carried out using a hand held pneumatic bulb dynamometer (Baseline, Patterson Medical). Grip strength was measured 3 times at 1 minute intervals and the highest value achieved was accepted as the final maximum grip strength.

4.2.4 Cardio-respiratory fitness

Heart rate and expired gas variables were measured using a heart rate monitor and portable gas analyser (K4b^2, COSMED). Measures of cardio-respiratory fitness were; peak

measured oxygen consumption ($\dot{V}O_2$, ml/min/kg), peak heart rate, oxygen uptake efficiency slope (OUES) and predicted $\dot{V}O_2$max.

Peak measured $\dot{V}O_2$ is calculated as the highest value determined from a rolling 60-second average calculated across the duration of exercise. Peak heart rate (peak HR) was the highest measured heart rate during exercise. Heart rate response (HRR) is the difference between the peak heart rate and resting heart rate. $\dot{V}O_2$max was predicted using the equations presented in Chapter 2 (for men; equation 2.3, for women; equation 2.4). OUES is the slope of the measured $\dot{V}O_2$ (ml/min) regressed against the log of ventilation (ml/min).(278)

Blood pressure (BP) was measured using a motion insensitive monitor (Tango M2, SunTech Medical, NC, USA) at rest and during the 2nd, 4th and 6th minute of exercise. The highest measured BP during exercise was assigned as the maximum exercise BP.

4.2.5 Skeletal muscle measurements

Skeletal muscle measurements were carried out as described in Chapter 3 using NIRS (Portamon, Artinis Medical Systems, Netherlands) to measure changes in oxy-Hb and deoxy-Hb from the lateral gastrocnemius. Position and orientation of the device was standardized between individuals. The device was attached using tape and covered completely using a neoprene sleeve.

During the muscle measurements the participant stood with their weight distributed equally between both feet. Arterial occlusions were performed at rest and immediately following the exercise test as described in Chapter 3, figure 3.1. Participants were excluded from skeletal muscle measurements and arterial occlusions for the following reasons: restrictive clothing which prevented the device or cuff being attached appropriately, intolerance of the arterial occlusion or if no trained staff were available to conduct the measurement.

Parameters marking skeletal muscle metabolic function were calculated as described in Chapter 3, figure 3.3. Resting muscle$\dot{V}O_2$, post-exercise muscle$\dot{V}O_2$ were reported as measures of skeletal muscle oxygen consumption. Exercise-related increases in muscle$\dot{V}O_2$ (Δmus$\dot{V}O_2$) were calculated as the absolute difference between resting muscle$\dot{V}O_2$ and post-exercise muscle$\dot{V}O_2$. The time constant (τ) was calculated as the recovery rate of muscle$\dot{V}O_2$ and represents the oxidative capacity of the skeletal muscle.

4.2.6 Statistical analysis

Categorical data are presented as n (%). Continuous descriptive data were examined for normality and participant characteristics are presented as means ±standard deviation or median (interquartile range) if skewed; results are presented as means (95% confidence interval). Comparison of means was done using an unpaired Student's *t*-test for continuous data and chi squared test for categorical data.

Multivariable linear regression was used to adjust for potential confounding factors. All models included a basic adjustment for age and sex because of the generally accepted influence of these factors on exercise capacity, cardio-respiratory fitness and muscle function. Markers of exercise capacity were further adjusted for ethnicity and BMI based on previous evidence suggesting ethnic-differences in cardiorespiratory fitness and physical activity exist.(158, 161) BMI is adjusted for in these models as an index of adiposity to account for differences that could confound the differences in exercise capacity, cardio-respiratory fitness and muscle function in the presence of T2D. Models of the differences in cardio-respiratory fitness markers in the presence of T2D were adjusted for β-blocker use because of higher β-blocker use in participants with T2D and the known effect of β-blockers on HR and oxygen uptake. Differences in skeletal muscle measurements were also adjusted for age, gender, ethnicity and BMI.

4.3 Results

4.3.1 Participants

Participant characteristics are shown in table 4.1 for 580 participants who undertook an exercise test. Participant characteristics are shown in table 4.2 for 139 participants who underwent all skeletal muscle measurements.

Mean±SD or n(%)	Diabetes absent n=448	Diabetes present n=132	P value
Male n(%)	225(57)	79(60)	0.55
Age (years)	71.3±6.4	71.8±5.8	0.39
Ethnicity (E/A/AC/OTH)	226/143/71/8	37/64/28/3	<0.001
Education (years) (n=335/93)	12.4±3.5	12.3±4.4	0.82
Height (cm)	166.5±8.9	165.6±8.7	0.92
Weight (kg)	74.6±12.9	78.0±13.4	0.01
BMI (Kg/m^2)	27.2±4	28.4±4.3	0.004
Resting heart rate (bpm)	65±11	71±13	<0.001
B-Blocker use n(%)	65(15)	29(22)	0.041
Resting systolic blood pressure (mmHg)	138±16	141±14	0.127
Resting diastolic blood pressure (mmHg)	77±9	76±9	0.167
Smoker (never/ex/current) (n=373/102)	228/132/13	67/34/1	0.355
Alcohol intake (units/day) (n=370/103)	2.0±1.2	1.8±1.1	0.138
Cardiovascular disease diagnosed n(%)	68(15)	32(24)	0.015
Hypertension diagnosed n(%)	234(52)	96(73)	<0.001
Arthritis in knee/hip n(%)	108(24)	48(36)	0.005
Lipid lowering medication n(%)	204(46)	102(77)	<0.001
Diabetes duration (years) (n=66)	-	15.2±8.2	-
Diabetes medication n(%)	-	115(87)	-

Table 4.1 Participant characteristics for all participants who undertook an exercise test. Data are stratified by presence of diabetes. Where information was missing from self-reported sources of smoking, alcohol consumption and diabetes duration; n is given in parenthesis for the number of participants with available data in each group. P-values indicate significance of the difference between participants with and without diabetes. For Ethnicity E; European, AC; Afro-Caribbean, OTH; all other ethnicities (excluding south Asians).

Mean±SD or n(%)	Diabetes absent n=112	Diabetes present n=27	P value
Male n(%)	84(75)	22(82)	0.48
Age (years)	72.1±6.7	73.2±4.9	0.31
Ethnicity (E/A/AC/OTH)	49/48/12/3	6/18/2/1	0.14
Education (years) (n=84/16)	13.1±3.9	13.3±3.8	0.80
Height (cm)	167.4±8.8	167.3±8.6	0.94
Weight (kg)	73.5±12.4	75.5±8.7	0.33
BMI (Kg/m^2)	26.1±3.5	27.0±3.4	0.22
Resting heart rate (bpm)	61±10	69±13	0.007
B-Blocker use n(%)	20(18)	7(26)	0.34
Resting systolic blood pressure (mmHg)	138±16	142±15	0.21
Smoker (never/ex/current) (n=95/17)	60/32/3	13/4/0	0.50
Alcohol intake (units/day) (n=93/18)	2.0±1.2	1.8±1.1	0.45
Cardiovascular disease diagnosed n(%)	25(22)	8(30)	0.42
Hypertension diagnosed n(%)	62(55)	21(78)	0.03
Arthritis in knee/hip n(%)	16(14)	9(33)	0.02
Lipid lowering medication n(%)	57(51)	20(74)	0.03
Diabetes duration (years)	-	15.2±8.1	-
Diabetes medication n(%)	-	23(85)	-

Table 4.2 Participant characteristics for all participants who undertook muscle measurements. Data are stratified by presence of diabetes. Where information was missing from self-reported sources of smoking, alcohol consumption and diabetes duration; n is given in parenthesis for the number of participants with available data in each group. P-values indicate significance of the difference between participants with and without diabetes. For Ethnicity E; European, A; Asian, AC; Afro-Caribbean, OTH; all other ethnicities (excluding south Asians).

4.3.2 Exercise capacity, drop-out & muscle strength

Step-rate was lower in the presence of diabetes (difference = -3.6 steps/minute, CI:-5.3,-1.8, p<0.001; table 4.3) and people with diabetes completed fewer steps during the 6MST (difference = -27steps, CI:-41,-13, p<0.001) (table 4.3). The lower overall step number can be explained by a combination of a lower step rate and a greater proportion of participants with diabetes terminating exercise prior to completion of a full 6 minutes of stepping (27% versus 36%, p=0.063).

Reasons for terminating exercise early included; a systolic blood pressure drop by >20mmHg, a blood pressure increase (diastolic BP>115mmHg or systolic BP>230mmHg), dizziness, excessive heart rate, instability, arthritic or joint pain, excessive dyspnea, muscle fatigue, general fatigue or for an undefined/unspecified reason reported by the participant. Differences in the reasons for stopping exercise early between participants with or without diabetes are shown in figure 4.1. There were no differences between people with or without diabetes in reason for stopping the test early (p=0.82).

Grip strength was lower in people with diabetes (difference = -3.7KPa, CI:-7.0,-0.4, p<0.01) (table 4.3).

	Age and sex adjusted mean (95%CI)		Difference (95%CI)		
Outcome	**Diabetes absent (n=448)**	**Diabetes present (n=132)**	**Model 1 Age, sex**	**Model 2 M1+ethnicity**	**Model 3 M2+BMI**
Steps completed	204.9(198.3,211.5)	177.9(165.8,190.0)	-26.7(-40.5,-12.9)***	-23.8(-37.8,-9.9)**	-22.6(-36.0,-8.0)**
Step rate (steps/min)	38.6(37.7,39.4)	35.0(33.5,36.5)	-3.6(-5.3,-1.8)***	-3.4(-5.2,-1.6)***	-3.4(-5.1,-1.6)***
Grip strength (KPa) (n=577)	70.7(69.1,72.2)	67.4(64.5,70.3)	-3.7(-7.0,-0.4)**	-4.4(-7.7,-1.1)**	-4.8(-8.2,-1.8)**

Table 4.3 Exercise capacity and muscle strength. Data in columns 2 and 3 are age and gender adjusted means (95%CI) for each outcome in column 1 stratified by presence/absence of diabetes. Data in columns 4-6 are the differences between individuals with and without diabetes with adjustment for confounders (see statistical methods section). P-values for the difference between people with and without diabetes are as follows: p<0.05*, p<0.01 or p<0.001***. BMI; body mass index, kPa; kilopascals**

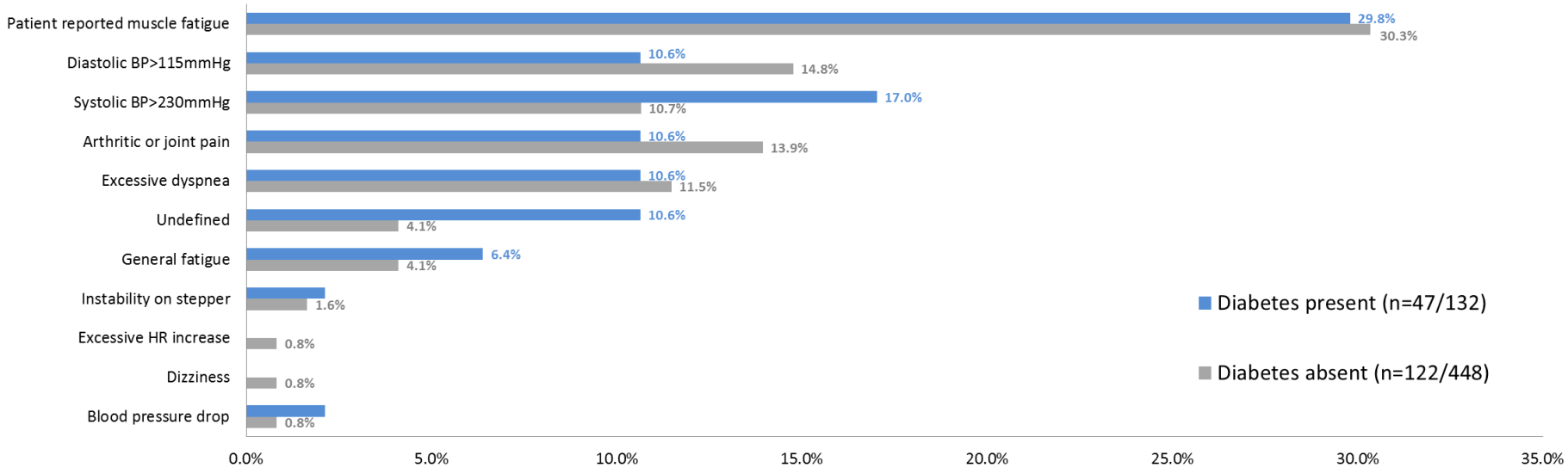


Figure 4.1 Reasons for stopping the exercise test prior to 6-minutes. Data are given as percentages of the total number of participants in each group who stopped exercising before 6 minutes was completed. Results are stratified by absence of diabetes (grey bars) or presence of diabetes (blue bars). The total number of participants who did not complete 6 minutes of stepping and the total for each group is given in parenthesis in the key. Chi^2 tests were used to test for differences in the reasons for stopping before 6 minutes was complete; there were no differences in the reasons for stopping early between people with and without diabetes (p=0.82).

4.3.3 Perceived exertion

Perceived exertion quantified by the modified Borg score (0-10) was similar between people with and without diabetes (mean±SD; 4.9±2.3 & 5.2±2.2, p=0.19, respectively). This was unaffected by adjustment for age and gender (adjusted means(CI); 4.9(5.0,5.4) & 5.2(5.0,5.4), p=0.15). Perceived leg fatigue (0-10) was also similar between people with and without diabetes (mean±SD; 5.0±2.6 & 5.1±2.2, p=0.47, respectively). This was also consistent after adjustment for age and gender (adjusted means(CI); 5.0(4.9,5.3) & 5.0(4.6,5.4), p=0.59).

4.3.4 Cardiorespiratory fitness in the presence of diabetes

530 (91%) participants underwent cardio-respiratory monitoring during the 6MST. People with diabetes achieved lower peak measured $\dot{V}O_2$ than those without (difference = -2.0ml/min/kg, CI:-2.7,-1.2, p<0.001) (table 4.4). Predicted $\dot{V}O_2$max was calculated in 446 participants (84 participants were excluded from this calculation because they were taking a β-blocker). Predicted $\dot{V}O_2$max was lower in the presence of diabetes (difference=-2.5ml/min/kg, CI:-3.6,-1.3, p<0.001) (table 4.4).

Other markers of cardiorespiratory effort were not markedly different between participants with and without diabetes (table 4.4). Peak HR reached during exercise was similar in older adults with T2D compared to those without (difference=1.4, CI:-3.3, 6.1, p=0.57; table 4.4), however, the difference between resting and peak HR (HRR), was lower in older adults with diabetes (difference =-6.6 bpm, CI:-11.3,-1.9, p=0.006; table 4.4) reflecting the higher resting heart rate in people with T2D (Tables 4.1 & 4.2). OUES was not different between individuals with T2D compared to those without (difference=-0.05, CI:-0.11, 0.03, p=0.23; table 4.4).The rise in systolic blood pressure during exercise was slightly lower in participants with diabetes (difference =-1.3, CI:-6.6, 4, p=0.62).

	Age and sex adjusted mean (95%CI)		Difference (95%CI)			
Outcome	**Diabetes absent (n=406)**	**Diabetes present (n=124)**	**Model 1 Age, sex**	**Model 2 M1+ethnicity**	**Model 3 M2+BMI**	**Model 4 M3+β-blocker**
Pk HR (bpm)	123.0(120.8,125.3)	122.3(118.0,126.4)	-0.6(-5.4,4.12)	0.15(-4.7,5.0)	0.62(-4.3,5.5)	1.4(-3.3,6.1)
HRR (bpm)	58.9(56.7,61.2)	52.0(48.0,56.2)	-6.6(-11.3,-1.9)**	-6.8(-11.6,-2.0)**	-5.9(-10.8,-1.1)*	-5.6(-10.5,-0.8)*
Pk measured VO_2 (ml/min/kg)	16.4(16.0,16.8)	14.4(13.7,15.1)	-2.0(-2.7,-1.2)***	-1.8(-2.6,-1.0)***	-1.4(-2.2,-0.7)***	-1.4(-2.1,-0.6)***
Pred VO_2 max (ml/min/kg) (n=349/97)	28.4(27.9,29.0)	25.8(24.8,26.9)	-2.5(-3.6,-1.3)***	-2.7(-3.9,-1.6)***	-2.0(-3.1,-0.9)***	-
OUES (ml/min/(logml/min))	1.6(1.6,1.7)	1.6(1.5,1.6)	-0.06(-0.1,0.01)	-0.02(-0.1,0.1)	-0.05(-0.1,0.02)	-0.05(-0.1,0.03)
$\Delta SBP_{exercise}$ (mmHg) (n=426/125)	49.4(46.9, 51.9)	45.4(41.0, 50.1)	-3.5(-8.8,1.8)	-2.5(-7.8, 2.9)	-2.0(-7.4, 3.4)	-1.3(-6.6,4.0)

Table 4.4 Cardiovascular measurements during exercise. Data in columns 2 and 3 are age and gender adjusted means (95%CI) for each outcome in column 1 stratified by diabetes present or absent. Data in columns 4-6 are the differences (i.e. β-coefficients) for diabetes in models 1-4 respectively. P-values for the difference between people with and without diabetes are as follows: p<0.05*, p<0.01 or p<0.001***. BPM; beats per minute, Pk HR; peak heart rate, Pred $VO_{2\ max}$; predicted VO_{2max}, HRR; heart rate response, OUES; oxygen uptake efficiency slope, SBP; systolic blood pressure.**

4.3.5 Skeletal muscle oxidative capacity

Skeletal muscle function was measured in 139 participants who undertook exercise. In this sub-group of participants the number of steps completed and the cardio-respiratory response to exercise were found to follow similar trends as reported above in tables 4.3 and 4.4.Table 4.5 briefly describes differences in exercise capacity between people with, and without, T2D in this smaller group.

	Age and sex adjusted mean (95%CI)		
Outcome	**Diabetes absent (n=112)**	**Diabetes present (n=27)**	**p-value**
Steps completed	229(218,241)	173(150,196)	<0.001
HRR (bpm)	60(56,64)	48(40,56)	0.024
Pk measured VO_2 (ml/min/kg)	18.2(17.5,18.9)	15.5(14.0,16.9)	0.002

Table 4.5 Exercise capacity and cardio-vascular response to exercise in the sub-group of participants who undertook skeletal muscle measurements. Data are age and gender adjusted means (95%CI) for each outcome in column 1 stratified by presence/absence of diabetes. P-values for the difference between people with and without diabetes are as follows: p<0.05*, p<0.01 or p<0.001***. HRR; heart rate response, Pk; peak.**

Resting and post-exercise muscle oxygen consumption were little different between participants with, versus without, diabetes (difference= 0.05, CI:-0.03,0.13, p=0.22 & -0.15, CI:-0.63,0.32, p=0.53 for resting and post-exercise values, respectively; table 4.6). The time constant was longer in older adults with diabetes (difference =10.88s, CI: 0.21, 21.54, p=0.046).This difference was marginally attenuated by adjustment for ethnicity and BMI such that the p-value for the difference in the presence of T2D did not reach significance;

however, the difference was still a greater than 10 seconds longer time constant in the presence of T2D (difference =10.39 s, CI: -0.44, 21.21, p=0.06; table 4.6).

Outcome	Age and sex adjusted mean (95%CI)		Difference (95%CI)		
	Diabetes absent (n=112)	Diabetes present (n=27)	Model 1 Age, sex	Model 2 M1+ethnicity	Model 3 M2+BMI
Resting mus$\dot{V}O_2$ (ΔHb_{diff}µMol/s)	0.30(0.26,0.33)	0.32(0.26,0.33)	0.02(-0.06,0.10)	0.04(-0.04,0.12)	0.05(-0.03,0.13)
Post-Ex mus$\dot{V}O_2$ (ΔHb_{diff}µMol/s)	1.56(1.35,1.77)	1.38(0.95,1.81)	-0.21(-0.70,0.28)	-0.24(-0.73,0.25)	-0.15(-0.63,0.32)
Time constant (s)	45.6(40.9,50.3)	56.2(46.7,65.7)	10.88(0.21,21.54)*	10.19(-0.54,20.91)	10.39(-0.44,21.21)

Table 4.6 Skeletal muscle measurements. Data in columns 2 and 3 are age and gender adjusted means (95%CI) for each outcome in column 1 stratified by presence/absence of diabetes. Data in columns 4-6 are the β-coefficients for the effect of presence of type-2 diabetes (T2D) on the outcome variable with adjustment for the variables in models 1-3 respectively. P-values for the difference between people with and without diabetes are as follows: p<0.05*, p<0.01 or p<0.001***. BMI; body mass index, Post-Ex; post-exercise.**

4.3.6 Oxidative capacity and exercise capacity

There was no convincing association between the time constant and the number of steps completed during the 6MST (age and gender adjusted β-coefficient=-0.20steps, CI: -0.63, 0.23, p=0.367).There was no association between the time constant and predicted $\dot{V}O_2$max (age and gender adjusted β-coefficient:0.001ml/min/kg, CI: -0.003, 0.005, p=0.60).

There was no detectable difference in the relationship between the time constant and steps completed when analyses were stratified by presence of diabetes (T2D absent: difference=-0.04steps, CI: -0.51, 0.44, p=0.874, T2D present: difference=-0.17steps, CI: -1.15, 0.80, p=0.716).

Further adjustment of the diabetes-associated reduction in number of steps completed (table 4.6; -53steps, CI: -79, -27, p<0.001) for the time constant did not alter the differences (age/gender/time constant-adjusted difference=-52steps, CI: -79, -26, p<0.001).

4.4 Discussion

4.4.1 Key findings

In this study, older adults with T2D had a lower submaximal exercise capacity than similarly aged participants without diabetes despite a similar score on perceived exercise intensity and leg fatigue. T2D in this population-based sample was also associated with markers of poorer cardiovascular response to exercise; including lower cardio-respiratory peak measured $\dot{V}O_2$ and lower predicted $\dot{V}O_2$max. Skeletal muscle oxidative capacity (the time constant) was impaired in T2D but there were no detectable differences in muscle $\dot{V}O_2$ at rest or post-exercise. Skeletal muscle oxidative capacity did not correlate with

exercise capacity in either the presence or absence of T2D or overall in this older adult population.

4.4.2 Exercise capacity and cardiorespiratory fitness

The findings reported here of lower exercise capacity in people with T2D are in line with previously conducted studies which suggest maximal exercise capacity is impaired in the presence of T2D and cardio-respiratory oxygen uptake (peak $\dot{V}O_2$) is lower.(270, 279) Cardio-respiratory peak $\dot{V}O_2$ was previously assessed during submaximal exercise workloads; the authors reported the relationship between $\dot{V}O_2$ and workload was 16% lower in the presence of T2D.(280) Bauer et al,(271) demonstrated poorer skeletal muscle oxygen uptake kinetics in the presence of T2D; they used NIRS measured deoxy-Hb as a measure of O_2 extraction and estimated skeletal muscle blood flow attributed differences in exercise capacity to inferior vascular function in the presence of T2D.

4.4.3 Oxidative capacity

The finding that oxidative capacity is poorer in older adults with T2D supports findings from other studies.(208) These studies measure oxidative capacity from biopsy samples as; activity of rotenone-sensitive NADH:O_2 oxidoreductase and citrate synthase,(126) and activity of succinate dehydrogenase .(104) However when O_2 flux was measured in permeabilised muscle fibre biopsy samples (keeping the cells more intact), correction for mitochondrial DNA content or citrate synthase activity completely attenuated the difference between people with and without T2D; the authors therefore concluded that differences in oxidative phosphorylation between people with T2D can be attributed to lower mitochondrial content.(281) The findings presented here also agree with previous *in vivo* work which demonstrated ~45% longer time constants for PCr recovery post-exercise, measured using ^{31}P-MRS, in the presence of T2D.(131) Although the differences presented here were comparatively modest, at ~20% longer. This concurs also with a study demonstrating good agreement between recovery time constants measured with

NIRS and ^{31}P-MRS.(259) However, no previous studies have assessed oxidative capacity using NIRS with the objective of detecting differences in people with T2D, as has been reported here.

The association between impaired oxidative capacity and T2D was only slightly attenuated by adjustment for cardio-respiratory peak measured $\dot{V}O_2$ (Chapter 4; table 4.7) suggesting that, at sub-maximal exertion, the measurement is uninfluenced by workload of the exercise. This is in line with previous studies that have applied different methods of exercise.(258)

There was no discernible difference in resting muscle$\dot{V}O_2$, post-exercise muscle$\dot{V}O_2$ or Δmus$\dot{V}O_2$ between older adults with and without diabetes. Peak measured $\dot{V}O_2$, assessed by analysis of expired gases, positively correlated with post-exercise muscle $\dot{V}O_2$. Locally measured muscle $\dot{V}O_2$ is not expected to be directly proportional to cardio-respiratory $\dot{V}O_2$, however, it is useful to acknowledge the positive direction of the correlation of these two marker of oxygen consumption immediately following a period of exercise.

4.5 Limitations

In this study submaximal exercise capacity was assessed using a self-selected paced test rather than maximal exercise capacity via an incremental exhaustive protocol. As discussed in Chapter 2, the issue of voluntary effort limits these findings because psychological factors could influence the participants elected level of exertion which would also influence the physiological responses measured. It is possible that people with diabetes participate in less physical activity in general and have less wide range of exercise intensity experience; this may result in lower effort during testing or selection of an inappropriate stepping speed. The latter would explain the higher rate of test termination before 6 minutes in people with diabetes. Previously, older women with T2D

were reported to have higher scores of perceived exertion when undertaking exercise workloads that were predicted to achieve equivalent cardio-respiratory exertion.(282)

Although differences in oxidative capacity were seen in the presence of diabetes, it remains unclear to what extent oxidative capacity contributes to any limitation in exercise capacity in the presence of diabetes.

This study found no relationship between exercise capacity and oxidative capacity (the time constant); differences in this relationship between people with diabetes were also considered. This is not in line with previous studies that suggest that, over a range of values, there should be a positive association between cardio-respiratory fitness and skeletal muscle metabolic function. There are several other potential explanations for this: (I) we measured sub-maximal exercise capacity and the predicted maximal aerobic capacity. This may not accurately reflect the true maximal aerobic capacity (II) there are potentially other factors in this age group that disturb the relationship between metabolic function and CR-fitness measures (III) Our measurement of skeletal muscle metabolic function is not accurately detecting metabolic function in this age group.

This study examined cross-sectional data of older adults with and without diabetes. Therefore it is not possible to ascertain whether reduced oxidative capacity was causally linked to T2D. The study design is observational and, at the time of this analysis, only 27 people with diabetes could be included. This fairly small sample size may have resulted in the study being underpowered for some of the outcomes examined.

4.6 Conclusions

Older adults with diabetes have lower submaximal exercise capacity and lower predicted maximal $\dot{V}O_2$ despite reporting similar levels of cardio-respiratory effort and leg fatigue.

Skeletal muscle oxidative capacity is poorer in the presence of diabetes but, in older adults, it is not associated with submaximal exercise capacity.

Chapter 5: Ethnic differences in exercise capacity, cardiorespiratory fitness and skeletal muscle oxidative capacity in older adults of South Asian, African Caribbean and European origin

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5.1 Introduction

5.1.1 Overview

The prevalence of T2D is higher is people of South Asian origin compared to those of European origin, this is particularly evident after migration out of their home country.(139, 140) T2D is also more prevalent in men and women of African-origin living in the UK and also in North America (as described in Chapter 1).(139, 141) Risk factors for development of T2D are present at a lower BMI in South Asians than in Europeans(139) and South Asians may also be more susceptible to the adverse effects of T2D; South Asian people with diabetes are at a higher risk of developing subsequent cardiovascular disease than Europeans with diabetes.(149) Overall, morbidity and mortality from stroke and coronary heart disease is higher in South Asians than in their European comparators.(146, 148) Individuals of African origin also have increased incidence of stroke compared to Europeans, but, they do not experience the same elevated risk of coronary heart disease that is seen in South Asians.(149, 164)

5.1.3 Cardiorespiratory fitness and grip strength

Cardiorespiratory fitness is poorer in South Asians.(155, 156) Lower cardiorespiratory fitness has previously been shown to account for 61% of the ethnic difference in fasting glycaemia seen in south Asians.(157) In general, physical activity levels are lower among South Asian populations.(158) However, lower physical activity could not account for the differences in cardio-respiratory fitness seen in South Asian men.(157) Lower cardio-respiratory fitness has also been described, independent of physical activity levels, in non-Hispanic Black men and men from other racial/ethnic groups compared to non-Hispanic White and Mexican American men (data from NHANES, described in Chapter 1).(159)

Measuring grip strength is accepted as a simple way of capturing overall limb skeletal muscle strength previously shown to correlate strongly with leg strength.(283) Recently, Ntuk et al compared grip strength by ethnicity in the presence and absence of T2D in >400 000 participants from the UK Biobank study.(284) They showed that in men and women of White European, African and South-Asian descent, living in the UK, there was an independent association between grip-strength and prevalence of T2D. Participants were middle-older aged adults (40-69 years old) and, although the greatest proportion were White (~26:1), the absolute number of African and South Asian participants was high (n= 7266 & 8540, respectively). In South Asians (men and women) and also in African men, lower grip strength predicted a higher prevalence to a greater extent than the relationship in Europeans and African women. The authors suggest that specific resistance exercise intervention should be considered as part of future randomized trials in these groups. Interestingly, in the PURE study, described briefly in chapter 1, although lower grip strength was associated with cardiovascular disease and all-cause mortality, the association between grip strength and T2D did not reach significance.(63) Some small studies, have found positive correlations between grip-strength and cardio-respiratory fitness(285) and between grip-strength and exercise capacity, measured as distance walked during a 6-minute walk test.(285) However, ethnic differences in grip strength have

not previously been compared alongside differences in cardio-respiratory fitness in older adult populations.

5.1.4 Skeletal muscle oxidative capacity

The impaired fat oxidation seen in skeletal muscle of South Asians suggests an underlying link to skeletal muscle function;(161) although longitudinal studies would need to be carried out to discern the direction of this relationship. Observations have been made that the metabolic profile in people with and without T2D differs depending on their ethnicity.(108, 164, 165) Several other studies have observed skeletal muscle metabolic disruptions in South Asians compared to Europeans.(144) Although the evidence regarding overall impairments in skeletal muscle oxidative capacity is inconclusive.

Focus is typically given to the determinants of excess insulin resistance/fasting glycaemia and increase risk of T2D that is seen in South Asians. Poor cardio-respiratory fitness is included in analysis as an explanatory variable (a mediator).(157, 161) The determinants of poor cardio-respiratory fitness have not previously been well investigated.

Limitations of the current evidence are that, so far, ethnic differences in cardiorespiratory fitness have been assessed in young to middle-aged populations that include mostly male participants.(161) Previous studies have enrolled fairly small sample sizes (with the exception of Ghouri et al; described above). Skeletal muscle measurements have previously all been based on analysis of biopsy samples, and an *in vivo* assessment of oxidative capacity by ethnic group has not been described.

5.1.5 Objectives

The objectives of this chapter were:

(1) Compare ethnic differences in sub-maximal exercise capacity (6MST and grip-strength) in a population of older adults (>65 years old) comprised of people

originating from South Asia, the Caribbean and Europe, all resident in West London.

(2) Compare measured oxygen uptake during sub-maximal exercise and predicted $\dot{V}O_2$max between ethnic groups of older adults

(3) Compare skeletal muscle oxygen consumption at rest and recovery rates of muscle oxygen consumption post-exercise (a marker of skeletal muscle oxidative capacity) between ethnic groups.

(4) Determine if differences in oxidative capacity can explain, to some extent, differences in sub-maximal exercise capacity observed in South Asian older adults

We hypothesised that in people from ethnic minority groups, South Asians and African-Caribbean's, sub-maximal exercise capacity and predicted $\dot{V}O_2$max will be lower than in Europeans and poor exercise capacity will be associated with impaired skeletal muscle oxidative capacity.

5.2 Methods

5.2.1 Participants

Participants were older adults recruited from the Southall and Brent Revisited (SABRE) study (as described in Chapter 2). Only participants who reported their ethnicity as European, South Asian or African-Caribbean were included in this analysis.

5.2.2 Anthropometrics and questionnaires

Height was measured barefoot using a standard stadiometer (Seca217, Hamburg, Germany). Weight, body fat mass and body fat percentage were measured using digital bio-impedance scales (BC-418, Tanita, USA).

Diabetes was defined as self-reported physician diagnosis or reported use of anti-diabetic medication, hypertension was defined as self-reported physician diagnosis, or reported use of medication for hypertension. Ethnicity was obtained by questionnaire.

5.2.3 Exercise test and skeletal muscle measurements

A 6MST was carried out to determine sub-maximal exercise capacity and to predict $\dot{V}O_2$max as a measure of cardio-respiratory fitness (as described in Chapter 2). Steps completed and step-rate were both recorded to describe sub-maximal exercise capacity. $\dot{V}O_2$max was predicted according to both the equation and extrapolation methods described in Chapter 2.

Near Infrared Spectroscopy (NIRS) was used to non-invasively assess skeletal muscle oxidative capacity using changes in oxygenated and deoxygenated haemoglobin concentrations during arterial occlusions at rest and post-exercise (as described in Chapter 2).

5.2.4 Statistical analysis

Categorical data are presented as n (%). Continuous descriptive data were examined for normality and participant characteristics are presented as means ±standard deviation or median (interquartile range) if skewed; results are presented as means (95% confidence interval). Comparison of means was done using an unpaired Student's *t*-test for continuous data comparing 2 groups or one-way ANOVA for multiple-group comparisons and chi squared test for categorical data. Multivariable linear regression was used to adjust for potential confounding factors. The effect of ethnicity on exercise capacity and cardio-respiratory fitness included a basic adjustment for age and sex to account for possible sampling bias, and further adjustment for presence of T2D as a potential mediator since this condition is known to be more frequent in South Asians and African-Caribbean's and may affect outcomes. Further adjustment for oxidative capacity (τ) as a potential mediator of associations between ethnicity and exercise capacity was also performed.

5.3 Results

5.3.1 Participants

183 participants (mean age=72±6 years, male=137) undertook the both the exercise test and the skeletal muscle measurements. Participant characteristics, stratified by ethnicity and unadjusted for confounders, are show in table 5.1. There were fewer men, and participants had a higher BMI, in the African-Caribbean group compared to both the European and the South Asian group. Prevalence of T2D was highest in the South Asians and lowest in Europeans. Age, resting heart rate and use of β-blocker medication was similar across the groups (table 5.1).

	Ethnicity			p value (pairwise comparison)			
Mean±SD or n(%)	European n=79	South Asian n=74	African Caribbean n=30	P value	E-SA	E-AC	SA-AC
Male n(%)	59(75)	62(84)	16(53)	0.005	0.17	0.032	0.001
Age (years)	73.5±6.7	71.8±5.1	70.6±7.5	0.07	0.09	0.069	0.410
BMI (Kg/m^2)	26.6±3.4	25.1±3.1	28.2±3.4	<0.001	0.005	0.03	<0.001
Resting HR (bpm)	63.1±9.3	62.23±10.5	63.0±14.7	0.74	0.403	0.851	0.802
β-blocker use n(%)	11(14)	19(26)	4(13)	0.126	0.067	0.169	0.936
Diabetes n(%)	9(11)	19(26)	6(20)	0.074	0.022	0.244	0.539

Table 5.1 Participant characteristics for all participants who undertook an exercise test. Data are stratified by ethnicity. P-values in column 5 indicate differences between the groups by Chi^2 test for categorical data and analysis of variance (ANOVA) for continuous data. Between group pairwise comparisons were made using students t-tests or chi^2 tests; p-values for pairwise comparisons are given in columns 6-8. E; European, SA; South Asian, AC; African-Caribbean, HR; heart rate.

5.3.2 Exercise capacity & cardio-respiratory fitness

Compared to Europeans, after adjustment for age and sex, South Asians achieved fewer steps during the 6MST (difference (95%CI): -39.5 (-61, -18) steps, p<0.001) whereas African-Caribbean's achieved a similar number (difference (95%CI): -10.9 (-39, 17) steps, p=0.5). These differences persisted when T2D was included in the model (South Asians: -32.4 (-53, -12) steps, p=0.002; African-Caribbean: -5.6 (-33, 22) steps, p=0.7). South Asians completed fewer steps than African-Caribbean's although this difference did not reach significance (-27 (-55, 0.9) steps, p=0.06).

Grip strength was lower in South Asians compared to Europeans after adjustment for age, sex and T2D (difference (95%CI): -8.6 (-13.9,-3.4) KPa, p=0.001). Compared to African-Caribbean's grip strength was lower in South Asians (difference (95%CI): -16.2 (-23.3, -9.2) KPa, p<0.001) and lower in Europeans (difference (95%CI): -7.6 (-14.5, -0.67) KPa, p=0.03). Table 5.2 shows marginal means for the 3 ethnic groups for exercise capacity and grip strength.

Outcome	Adjusted mean (95%CI)								
	Unadjusted mean			Model 1 (Age + sex)			Model 2 (M1+T2D)		
	E	SA	AC	E	SA	AC	E	SA	AC
Steps completed	239(223,256)	213(196,230)*	235(209,261)	246(231,260)	206(191,221)***	235(211,259)	242(228,256)	210(195,224)**	236(213,260)
Step rate (steps/min)	43(41,45)	39(37,41)**	45(42,49)	44(42,45)	38(36,40)***	45(42,48)	43(42,45)	39(37,41)***	46(37,48)
Grip strength (KPa)	76(72,81)	72(67,77) †	81(73,88)	78(74,81)	69(65,72)***†††	85(79,91)*	77(74,81)	69(65,72)***†††	85(79,91)*

Table 5.2 Adjusted means comparing ethnic differences in submaximal exercise capacity (steps completed and step rate) and maximal grip strength. Adjustment was made for potential confounders in model 1 (age and sex) and model 2 (age, sex and type 2 diabetes (T2D)). Significance differences between South Asians (SA) and African-Caribbean's (AC) compared to Europeans (E) is denoted by stars: *p≤0.05, **p≤0.01, ***p≤0.001 and differences between South Asians compared to African-Caribbean's is denoted by dagger symbols: †p≤0.05, ††p≤0.01, †††p≤0.001. AC; African-Caribbean, E; European, SA; South Asian, T2D; type-2 diabetes.

Measured peak $\dot{V}O_2$ was lower in the South Asians compared to Europeans after adjustment for age, sex and T2D (difference (95%CI): -1.5(-2.7,-0.3) ml/min/kg, p=0.019) but similar in African-Caribbean's compared to Europeans (difference (95%CI): -0.6(-2.3,1.1), p=0.487). There was no significant difference in measured peak $\dot{V}O_2$ between South Asians compared to African-Caribbean's (difference (95%CI): -0.9(-2.6,0.82), p=0.304).

Compared to Europeans, the predicted $\dot{V}O_2$max (using equations) was not different in South Asians (difference (95%CI): 1.5(-0.2, 3.2), p=0.08) or African-Caribbean's (difference (95%CI): -1.5(-3.8,0.8), p=0.19). Predicted $\dot{V}O_2$max using the extrapolation method was also not different between the ethnic groups (South Asians difference (95%CI): -0.17(-1.1,0.7), p=0.70); African-Caribbean difference (95%CI): -0.51(-1.7,0.66), p=0.39). There were no differences in predicted $\dot{V}O_2$max when South Asians were compared to African-Caribbean's (South Asians difference(95%CI); 0.64(-0.44,1.74), p=0.24).

5.3.3 Skeletal muscle oxidative capacity

Compared to Europeans, after adjustment for age, sex and T2D, resting muscle oxygen consumption was not different in South Asians (difference (95%CI): 0.05(-0.12,0.01) $\Delta Hb_{diff}\mu M/s$, p=0.10) or African-Caribbean's (difference (95%CI): 0.03(-0.05,0.12) $\Delta Hb_{diff}\mu M/s$, p=0.45). Resting muscle oxygen consumption was lower in South Asians compared to African-Caribbean's, after adjustment for the same confounders (-0.09(-0.17,-0.001) $\Delta Hb_{diff}\mu M/s$, p=0.05).

Compared to Europeans, after adjustment for age, sex and T2D, South Asian ethnicity was associated with longer time constants (difference (95%CI): 10.1 (2.3, 17.9) s, p= 0.011). The time constant was similar in African-Caribbean's compared to Europeans (difference (95%CI): 5.8(-4.4, 16.0), p=0.264). There was no difference between South Asians and African-Caribbean's (difference (95%CI): 4.3(-6.1,14.7), p=0.416).Table 5.3

shows marginal means for the 3 ethnic groups for resting muscle oxygen consumption (muscleVO_2) and the time constant (τ).

Outcome	Adjusted mean (95%CI)								
	Unadjusted mean			Model 1 (Age + sex)			Model 2 (M1+T2D)		
	E	SA	AC	E	SA	AC	E	SA	AC
Resting muscleVO_2 ($\Delta Hb_{diff}\mu M/s$)	0.32 (0.28,0.36)	0.27 (0.22,0.31)	0.33 (0.26,0.40)	0.32 (0.27,0.36)	0.26 (0.22,0.31)	0.35 (0.28,0.42)	0.32 (0.27,0.36)	0.26† (0.22,0.31)	0.34 (0.28,0.42)
τ (s)	43(38,48)	53(48,59)**	49(41,58)	43(37,48)	54(48,59)**	49(40,58)	43(38,48)	53(48,59)**	49(40,58)

Table 5.3 Adjusted means comparing ethnic differences in resting muscle oxygen consumption (muscle VO_2) and the time constant (τ). Adjustment was made for potential confounders in model 1 (age and sex) and model 2 (age, sex and type 2 diabetes (T2D)). Significant differences between South Asians (SA) and African-Caribbean's (AC) compared to Europeans (E) is denoted by stars: *p≤0.05, **p≤0.01, ***p≤0.001 and differences between South Asians compared to African-Caribbean's is denoted by dagger symbols: †p≤0.05, ††p≤0.01, †††p≤0.001. AC; African-Caribbean, E; European, SA; South Asian, T2D; type-2 diabetes.

5.3.4 Oxidative capacity and exercise capacity

The reduced exercise capacity observed in South Asian participants was not attenuated when the measure of muscle oxidative capacity (τ) was included in models (Model 3; age, sex, T2D & τ). South Asians still achieved fewer steps compared to Europeans (-36.6 steps, 95%CI=-58, -16, p=0.001), stepped at a slower rate (-5.5 steps/min, 95%CI=-8, -3, p<0.001) and had a lower grip strength (-9.0 KPa, 95%CI=-14, -4, p=0.001).

5.4 Discussion

5.4.1 Key findings

This study demonstrates that older adults of South Asian origin have lower submaximal exercise capacity than Europeans while African Caribbean adults, of a similar age, have a similar capacity for submaximal exercise and stronger grip strength compared to both South Asians and Europeans. South Asians had longer time constants for recovery of muscle $\dot{V}O_2$, suggesting impaired oxidative capacity compared to Europeans; this was independent of presence of T2D. However, when the ethnic differences in exercise capacity were adjusted for the time constant, they could not be explained by the poorer skeletal muscle oxidative capacity. These data indicate that the reduced submaximal exercise capacity and grip strength seen in older adults of South Asian origin is not explained by the higher prevalence of diabetes or impaired muscle oxidative capacity in South Asians. This is consistent with the finding that exercise capacity and skeletal muscle oxidative capacity were similar in African Caribbean's and Europeans.

T2D was more prevalent in South Asians compared to Europeans in this group of older adults (26% versus 11%, p=0.02); this is in line with previous finding from younger participants.(139, 140) Previously, increase prevalence of T2D has been described in African-Caribbean groups compared to Europeans.(141, 284) In this analysis, although

there was a trend towards a higher prevalence of T2D in African-Caribbean's compared to Europeans, this difference did not reach significance (20% versus 11%, p=0.24).

5.4.2 Exercise capacity and cardiorespiratory fitness

The poorer exercise capacity seen in South Asians here is reflected in the fewer steps completed during the 6MST and the lower peak measured oxygen uptake during the submaximal exercise, even after adjustment for age, sex and increased prevalence of T2D. Differences in submaximal exercise capacity are generally in line with findings from other groups who have reported poorer achievement on maximal exertion CPET results and lower physical activity levels in South Asians.(157, 158) Measured oxygen uptake during exercise, compared to Europeans, was lower in South Asians and similar in African Caribbean's. When $\dot{V}O_2$max was predicted using the sub-maximal heart rate and the workload achieved during testing, this was not different by ethnic group which is not in line with previous findings. Thus, we did not find differences in sub-maximal exercise capacity, or predicted cardio-respiratory fitness (predicted $\dot{V}O_2$max) between African Caribbean's and either Europeans or South Asians; this appears contrary to the results of Caeser et al(159) who present lower predicted $\dot{V}O_2$max values in non-Hispanic Black Americans compared to White Americans. These discrepant findings could be explained by the younger age of participants enrolled in the National Health and Nutrition Examination Survey (NHANES) population.(159) It is possible that, in the older adult group, ethnic differences in $\dot{V}O_2$max become more subtle than in younger adults and, therefore, it is not possible to detect differences with a crude predictive measurement. It is also possible that the predictive equations we used here are not appropriate for the South Asian or African-Caribbean groups. These equations were developed using data from European men and women. When the extrapolation method was used to predict $\dot{V}O_2$max, although there was still no significant difference between groups, the values were more in line with previous results.(157) Furthermore, a limitation to our study is that we were only able to recruit half

as many African Caribbean's as Europeans or South Asians giving us less power to detect significant differences.

5.4.3 Skeletal muscle metabolism

Resting skeletal muscle oxygen consumption, measured using NIRS signal changes during a 30 second arterial occlusion, was highest in African Caribbean participants and lowest in South Asians, the difference between these two ethnic groups was significant but only after adjustment for age, sex and T2D. The difference from South Asians to Europeans or Europeans to African Caribbean's did not reach significance (Table 5.3). This suggests that, at rest, metabolic turnover could be higher in African Caribbean's. This could be the result of a difference in the muscle fibre-type, with African Caribbean's having a higher proportion of type 2 fibres, as has previously been described.(286) An alternative explanation is that improved microvascular function in African Caribbean's permits faster extraction of oxygen into the muscle cell, therefore, more rapidly reducing the concentration of oxygenated haemoglobin. This second explanation would be in line with the reduced arterial stiffness, indicating better arterial function, previously described in African Caribbean's compared to Europeans and South Asians.(149)

Skeletal muscle oxidative capacity, measured as the time constant for muscle $\dot{V}O_2$ recovery, was poorer in South Asians compared to Europeans (longer time constants). There were no differences between African Caribbean's and Europeans. This finding appears to directly oppose finding by Nair et al who describe enhanced skeletal muscle mitochondrial function in muscle biopsies from South Asian compared to European-origin participants.(166) They found that mitochondrial DNA copy number, transcription of genes involved in oxidative phosphorylation, citrate synthase activity, and maximal mitochondrial ATP production rate were higher in Indian Asian irrespective of their diabetic status.(166) However, Nair et al examined younger participants (mean age of ~50 years old), they excluded volunteers who had known cardiovascular disease (as well as other co-

morbidities) and, importantly, they did not measure cardio-respiratory fitness or exercise capacity. Therefore, we cannot be certain that there were no differences in fitness or habitual physical activity that may have influenced skeletal muscle measurements or that participant selection introduced bias.

Hall et al, also described poorer oxidative capacity in South Asians compared to Europeans but, like Nair et al, then found this was not due to reduced expression of oxidative and lipid metabolism genes. Hall et al found that expression of carnitine palmitoyl-transferase 1A (CPT1A) and fatty acid synthase (FASN) were nearly 2 times higher in South Asians than Europeans and there was no relationship between whole-body insulin sensitivity and expression of these genes. They also showed that the ratio of mtDNA to nuclear DNA was not different in South Asians. The authors suggest that differences in capacity for lipid oxidation could be a consequence of impaired vascular function. Taken together these findings largely agree with the findings of Nair et al.(205) Evidence from both studies suggests that excess insulin resistance seen in South Asians cannot be explained by reduced expression of genes involved in oxidative phosphorylation. The findings presented here, that non-invasively determined oxidative capacity of skeletal muscle is lower in South Asians independently of T2D, provide additional complementary evidence suggesting that, with age, these differences in skeletal muscle oxidative capacity persist. Oxidative capacity was not different between the Europeans and African-Caribbean's despite a trend towards increased prevalence of T2D in the African-Caribbean group further suggesting a dis-association between oxidative capacity and exercise capacity in the presence of T2D.

5.5 Limitations

In this study we performed non-invasive measurements on skeletal muscle *in vivo;* therefore, conclusions regarding the mechanistic detail underlying impaired oxidative

capacity are not possible. We cannot determine if the impairment in function is due to reduced mitochondria function or reduced mitochondria number.

While our sample size for European and South Asian participants was fairly even (n=79 & 74, respectively), less than half of this number of African-Caribbean's were enrolled (n=30). This limits our power to detect differences between African-Caribbean participants.

We have not included self-reported physical activity as a potential mediator of the effects observed; however, adjusting differences in exercise capacity for habitual physical activity levels would be interesting in future analysis. We do not describe micro-vascular function in this group of older adults.

5.6 Conclusions

Exercise capacity is impaired in older adults of South Asian origin and this effect is independent of the increased prevalence of T2D in this group. Exercise capacity did not differ between Europeans and African Caribbean people despite a trend towards higher prevalence of diabetes in the latter group. Skeletal muscle oxidative capacity is impaired in South Asian people but this does not explain the reduction in exercise capacity.

Chapter 6: The effect of exercise training on skeletal muscle metabolic function

6.1 Introduction

Skeletal muscle metabolic adaptions with endurance exercise training are well recognized.(59, 287) Recent research implicates skeletal muscle metabolic dysregulation in disease and pre-disease states and a potential role for exercise in enhancing mitochondrial biogenesis has attracted recent interest.(104, 288) There is a need to identify sensitive, non-invasive physiological markers of skeletal muscle metabolic function in order to recognize interventions that will deliver positive adaptations.

The effects of exercise training on the cardio-pulmonary system can be quantified using a cardio-pulmonary exercise test (CPET) to assess peak oxygen consumption (peak $\dot{V}O_2$ or $\dot{V}O_2$max) *via* expired gas analysis. Multiple components contribute to peak $\dot{V}O_2$ including: pulmonary diffusion, cardiovascular function (predominantly capacity to increase cardiac output, but also capacity for transport and exchange via the peripheral macro- and micro-circulation), the capacity for oxygen carrying in the blood, and cellular energy metabolism and mitochondrial function, as discussed in detail in Chapter 1.(44) It is generally accepted that in humans, whole body peak $\dot{V}O_2$, as measured by analysis of expired gases, is limited by the rate of oxygen delivery, and not by the rate of uptake/utilization in the muscle.(44)

$\dot{V}O_2$max has an established relationship with exercise capacity, however, within a narrow range of values, performance may differ in terms of exercise capacity.(100) In runners these differences have been described as differences in ‘running economy’ which is quantified as the $\dot{V}O_2$ required for running at a given pace.(100) Therefore, endurance

performance is strongly dependent on good running economy and it is thought that that this could be due to muscle adaptations, specifically increased mitochondrial enzymes, resulting in fewer disturbances to mitochondrial homeostasis.(60) Previously, increased physical activity, but not necessarily heavy exercise training, has been shown to enhance metabolic health independently of cardio-respiratory fitness.(289)

Vigelso et al.(290) have summarized evidence suggesting a direct positive correlation between improvements in cardio-pulmonary peak $\dot{V}O_2$ and muscle mitochondrial enzymatic activity. However, inclusion of a skeletal muscle assessment of oxygen consumption is uncommon within the context of a CPET. This may reflect difficulties, or expense, required to undertake appropriate measurements; the limitations of muscle biopsy analysis and MRS are discussed in Chapter1.

The generalizability of some of the previous work investigating skeletal muscle adaptations to low-functioning participants is debatable. Typically, these kind of performance studies are carried out in young, healthy and usually, relatively fit individuals or athletes who are motivated to undertake maximal exercise tests and rigorous training regimes. The effect of minimal, unsupervised training, in 'real-world' populations, has not previously been examined. Oxidative capacity, measured locally in the muscle, is not typically carried out alongside a CPET and non-invasive measurements using NIRS have never previously been carried out in this kind of setting.

As described in detail in Chapter 1, near infrared spectroscopy (NIRS) is a non-invasive technique that can measure changes in oxy-Hb and deoxy-Hb in skeletal muscle up to a depth of ~1.5cm.(256) Indices previously described in studies using NIRS have been based on response to exercise with, or without, arterial occlusions. Without arterial occlusions it is impossible to differentiate changes in oxygen consumption from changes in blood flow. It is not feasible to perform repeated arterial occlusions to estimate $\dot{V}O_2$ throughout exercise, so a simple alternative is to assume that immediately post-exercise

the $\dot{V}O_2$ determined via arterial occlusion is an acceptable estimate of the $\dot{V}O_2$ during the final stages of the exercising protocol.(215) Short, transient arterial occlusions have also been applied in the post-exercise period to determine the kinetics of muscle$\dot{V}O_2$ recovery(216, 259) these measurements have shown good reproducibility and good agreement(215) with established ^{31}P-MRI measurements of muscle oxidative capacity.(222)

The three objectives of this chapter were:

(1) To compare skeletal muscle oxygen consumption at rest and immediately post-exercise, measured locally using NIRS, with cardio-respiratory fitness, measured using expired gas analysis to determine peak $\dot{V}O_2$ in young, healthy sedentary individuals
(2) To investigate the effect of six months of endurance training in healthy individuals, undertaking preparation for a first marathon, on muscle oxygen consumption (muscle$\dot{V}O_2$) and cardio-pulmonary peak $\dot{V}O_2$ and to explore how these measures relate to first-time marathon running performance.
(3) To investigate the effect of six months of endurance training on oxidative capacity measured as the time constant for recovery of muscle $\dot{V}O_2$ determined using NIRS

6.2 Methods

6.2.1 Participants

Participants were drawn from an observational study investigating the effect of training for a first marathon on cardiac remodeling: The Marathon Study. Inclusion criteria for The Marathon Study were: age 18-35 years old at recruitment, no past significant medical history, no previous marathon-running experience and current participation in running for < 2 hours per week.

Due to availability of equipment it was not possible to study all participants in the Marathon Study, so alternate participants attending clinic for cardiac MRI underwent NIRS. Figure 6.1 shows full details of flow of participants through this study and selection for this analysis. All measurements were repeated after the marathon had been completed; this corresponded to a 6-months period for training (post-training). Figure 6.2 shows the study design, highlighting the timeline of measurements, for all participants included in this analysis.

All procedures were in accordance with the principles of the Helsinki declaration, all participants gave written informed consent and the study was approved by the London – Queen Square National Research Ethics Service (NRES) Committee – 15/LO/0086.

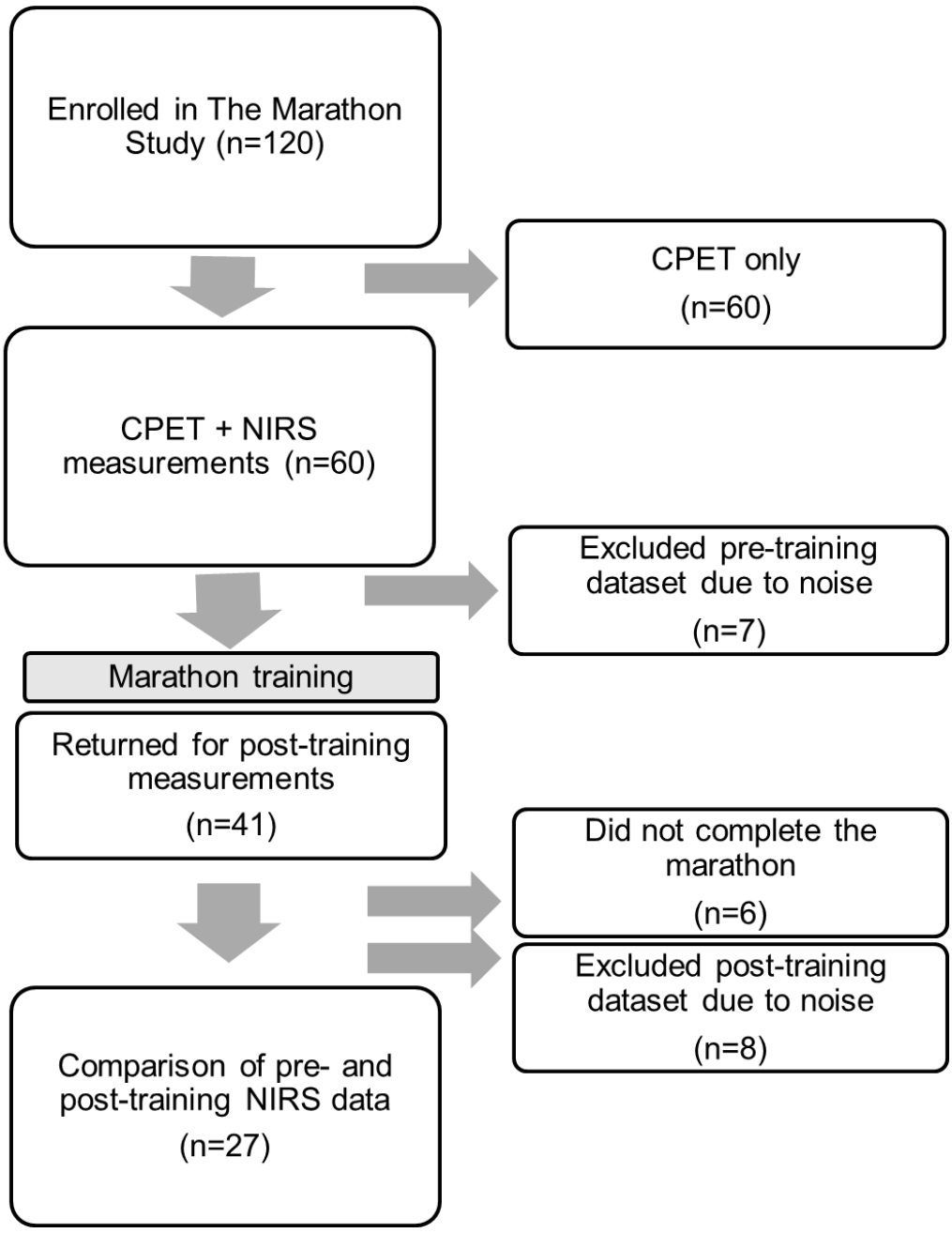

Figure 6.1 Selection process and data flow of NIRS data.

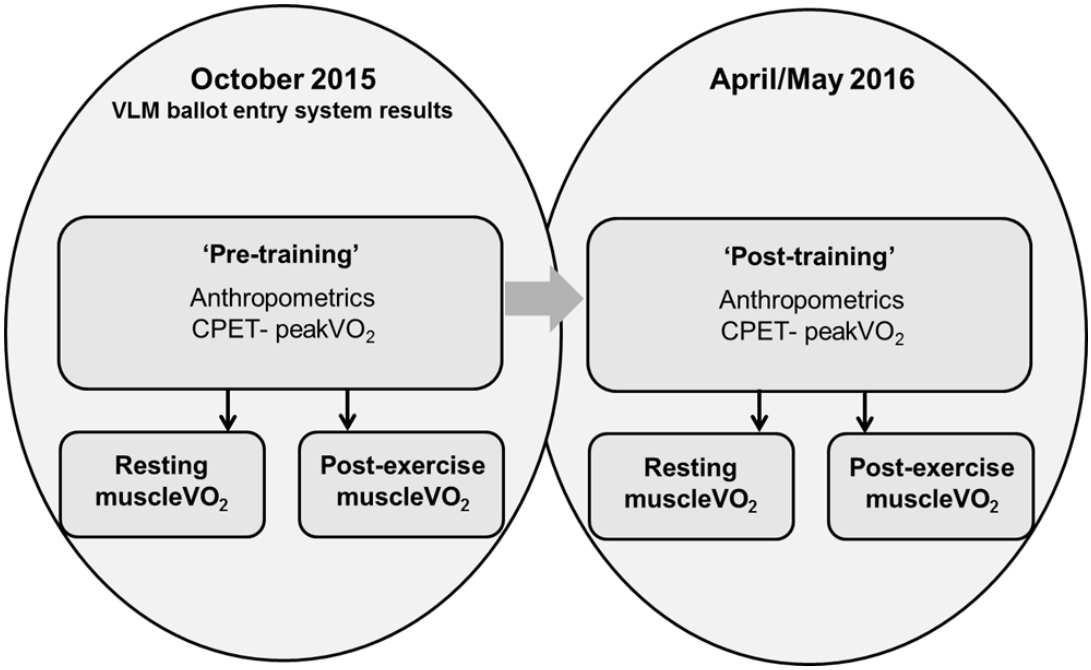

Figure 6.2 Study design highlighting the process of pre-training and post-training measurements. In October 2015 'pre-training' measurements were carried out soon after release of the ballot results for the Virgin London Marathon (VLM) which was due to take place in April 2016. Cardio-pulmonary peakVO_2 was measured pre- and post-training (via cardio-pulmonary exercise testing (CPET)) and muscle measurements were made at rest and immediately post-exercise at pre- and post-training.

6.2.2 Training

Participants were recommended to adhere to the 'First-time finisher' training schedule(291), aiming to run ~3 times per week for the 16-week period prior to the marathon. However, those eager to follow alternative, higher intensity, training plans were not discouraged. Some participants wished to start their training earlier than 16 weeks prior to the marathon and so we elected to conduct pre-training testing immediately following the release of the results from the ballot entry system which was 6 months prior to the marathon. Therefore, the minimum training period for all participants was 16-weeks but some participants may have trained for up to 26-weeks before the repeat assessment.

6.2.3 Anthropometric and blood pressure measurements

Participants were not asked to fast prior to either pre-training or post-training study visit but were asked to abstain from heavy exercise in the 24 hours prior. We did not control for hydration during the study visits, however, participants were offered water throughout their visit but were asked not to have caffeinated drinks.

Height was measured barefoot using a standard stadiometer. Weight, body fat mass and body fat percentage were measured using digital bio-impedance scales (BC-418, Tanita, USA). Body fat was included in this analysis because of the previously described substantial effect of adipose tissue thickness on the NIRS signal.(183)

Brachial blood pressure was measured at rest using an electronic oscillometric monitor (Pulsecor, Cardioscope BP+, Uscom, Sydney). Measurements were made separately from each arm and the mean values are presented.

6.2.4 Cardio-pulmonary exercise test (CPET)

The CPET in the Marathon Study was carried out on a semi-supine ergometer (Ergoselect1200, Ergoline, Germany). Full details of the protocol are described in Chapter 2.

6.2.5 Skeletal muscle measurements

The gastrocnemius was selected for investigation because it is recruited during running and cycling (292), it is therefore likely to undergo adaptations with training. NIRS measurements from this site have been shown to be reproducible(215) and since the calf is covered by less adipose tissue than the thigh or larger locomotive muscles (233) the influence of adipose tissue thickness (ATT) on NIRS measurements is less.

Prior to attaching the NIRS device, ATT was measured at the site of NIRS measurement using B-mode ultrasound (Vivid I, GE healthcare) equipped with a 12L-RS linear array transducer; three measurements were averaged.

As described previously; a continuous wave NIRS device (Portamon, Artinis Medical Systems, Netherlands) was used to measure changes in oxy-Hb and deoxy-Hb from the lateral gastrocnemius. Position and orientation of the device was standardized between individuals. The device was attached using tape and covered completely using a neoprene sleeve. Measurements were acquired at a frequency of 10Hz throughout the protocol.

For NIRS measurements the participant was seated in a semi-upright position with their gastrocnemius relaxed. Arterial occlusions were performed at rest and immediately following the exercise test using a rapid inflation cuff (Hokanson, SC10D/E20, PMS Instruments, UK) placed on the thigh directly above the patellofemoral articulation. The cuff was inflated to a pressure of 250mmHg and inflation or deflation was complete in 0.3 seconds. At rest the cuff was inflated for 30 seconds, post-exercise the occlusion duration was 5-8 seconds.

6.2.6 NIRS post-processing

Analysis of NIRS data was conducted using custom written programs in MATLAB R2014a (MathWorks Inc.). Signals were rejected if visual inspection showed movement artifact or if there was evidence that complete arterial occlusion had not been achieved. Incomplete arterial occlusion was judged on the direction of the oxy-Hb and deoxy-Hb signals; under complete arterial occlusion these are expected to move in opposite directions. Muscle$\dot{V}O_2$ was estimated by fitting the slope of the difference between oxy-Hb and deoxy-Hb signal during each occlusion.(236) More negative values represent higher muscle oxygen consumption; therefore, in order to align our values with the cardio-pulmonary peak $\dot{V}O_2$, we inverted the values to provide a positive index of muscle oxygen consumption. Exercise-related increases in muscle$\dot{V}O_2$ (Δmus$\dot{V}O_2$) were calculated as the absolute difference between resting muscle$\dot{V}O_2$ and muscle$\dot{V}O_2$ measured immediately post-exercise.

Parameters marking skeletal muscle metabolic function were calculated as described in Chapter 2, figure 2.8. Resting muscle$\dot{V}O_2$, and post-exercise muscle$\dot{V}O_2$ were reported as measured of skeletal muscle oxygen consumption. Exercise-related increases in muscle$\dot{V}O_2$ (Δmus$\dot{V}O_2$) were calculated as the absolute difference between resting muscle$\dot{V}O_2$ and post-exercise muscle$\dot{V}O_2$. The time constant (τ) was calculated as the recovery rate of muscle$\dot{V}O_2$ and represents the oxidative capacity of the skeletal muscle. Muscle measurements were corrected for ATT according to equation 3.1 described in Chapter 2.

6.2.7 Statistical analysis

Categorical data are presented as n (%). Continuous data were examined for normality and participant characteristics are presented as mean±standard deviation or median (interquartile range) if skewed. Results are presented as means (95% confidence intervals). Comparison of means was done using a two-tailed, paired Student's *t*-test and comparison of skewed data using a Wilcoxon signed-rank test. Pearson's correlation coefficient (r) was calculated to assess correlations. Partial correlation coefficients ($r_{partial}$) were calculated for relationships between marathon completion time and peak$\dot{V}O_2$ or muscle$\dot{V}O_2$ after adjustment for age and gender. We tested if training for a marathon modified the relationship between variables using an interaction term for statistical significance and also by visual inspection by plotting the data points for each time point with line of best-fit on the same graph. Statistical significance was assigned if $P<0.05$.

6.3 Results

6.3.1 Participants

27 participants provided complete data for analysis from pre- and post-training assessments, the characteristics of these participants are given in table 6.1. The mean duration from completion of the marathon to the post-training visit was 15.8±4.6 days.

Mean±SD or n(%)	Baseline (pre-training) (n=27)
Gender male (%)	17(63)
Age (years)	29.4±3.5
Height (cm)	176.7±10.4
Weight (kg)	72±13
Body fat mass (kg)	15±5.5
$ATT_{gastroc}$ (cm)	0.60±0.22

Table 6.1 Participant characteristics. Data are mean±SD.

6.3.2 Effect of endurance training

The mean marathon completion time was 4.6±0.81 hours. This was longer for women versus men (4.9±1.0 versus 4.3±0.6 hours, p=0.07). Based on weekly mileage data and marathon completion times from 27,000 runners over a 16-week training period,(293) the average times achieved by participants in this study are consistent with a typical training schedule of between 6 – 13 miles/per week.

There was a small reduction in resting heart rate from pre-training to post-training (mean difference=1.4 (95% CI: -4.5, 7.2) bpm, p=0.63; table 5.2) and cardio-pulmonary peak$\dot{V}O_2$ did not differ (mean difference=-0.0 (95% CI: -2.1, 2.0) ml/min/kg, p>0.9; table 6.2). There was a small reduction in systolic blood pressure (mean difference= -2.8 (95%CI: -6.6, 1.1)) and slightly greater reduction in diastolic blood pressure (mean difference= -3.0 (95%CI: -5.6, -0.5)). Post-exercise muscle$\dot{V}O_2$ was greater post-training compared to pre-training

measurements (median value(IQR); 1.04(0.85-1.29) versus 1.48(1.04-2.16) $\Delta Hb_{diff}\mu M/s$, p=0.004; table 6.2 & figure 6.3).

$\Delta mus\dot{V}O_2$ was also greater post-training (mean difference = -0.79 (95% CI:-1.1,-0.46) $\Delta Hb_{diff}\mu M/s$; p< 0.001; table 6.2) and there was little or no reduction in resting muscle $\dot{V}O_2$ (median value(IQR); 0.25(0.19-0.32) versus 0.21(0.16-0.32) $\Delta Hb_{diff}\mu M/s$, p=0.35; table 6.2).

Mean±SD or Median(IQR)	Pre-training	Post-training	P-value
Weight (kg)	72.0±13.0	72.0±12.0	0.98
BMI	22.9±2.9	23.0±2.7	0.84
Body fat mass (kg)	15.0±5.5	14.4±6.2	0.30
Resting HR (bpm)	66±13	64±14	0.63
Peak HR (bpm)	167(161-176)	165(157-171)	0.29
Peak VO_2 (ml/min/kg)	39.2±5.7	39.2±6.7	0.97
Perc. pred. VO_2 (%)	94.1±13.4	94.1±13.5	0.98
Systolic BP (mmHg)	118±9.7	116±10.9	0.16
Diastolic BP (mmHg)	73±5.8	70±5.2	0.02
Resting muscleVO_2 ($\Delta Hb_{diff}\mu M/s$)	0.25(0.19-0.32)	0.21(0.16-0.32)	0.35
ATT-corrected Resting muscleVO_2 ($\Delta Hb_{diff}\mu M/s$)	0.41(0.32-0.53)	0.36(0.31-0.43)	0.25
Post-Ex muscle VO_2 ($\Delta Hb_{diff}\mu M/s$)	1.04(0.85-1.29)	1.48(1.04-2.16)	0.004
ATT-corrected Post-Ex muscle VO_2 ($\Delta Hb_{diff}\mu M/s$)	1.75(1.14-2.49)	2.32(1.89-2.73)	0.004
$\Delta mus\dot{V}O_2$ ($\Delta Hb_{diff}\mu M/s$)	0.88±0.56	1.45±0.94	<0.001
Time Constant (s)	35(22-45)	36(23-60)	0.24

Table 6.2 Physiological measures before and after exercise training in all participants. Data are mean±SD for normally distributed data and median(IQR) for non-normally distributed data. Comparison of means was done using a paired Student's t-test and comparison of skewed data using a Wilcoxon signed-rank test. ATT; adipose tissue thickness, BP; blood pressure, Post-Ex; post-exercise, Hb; haemoglobin.

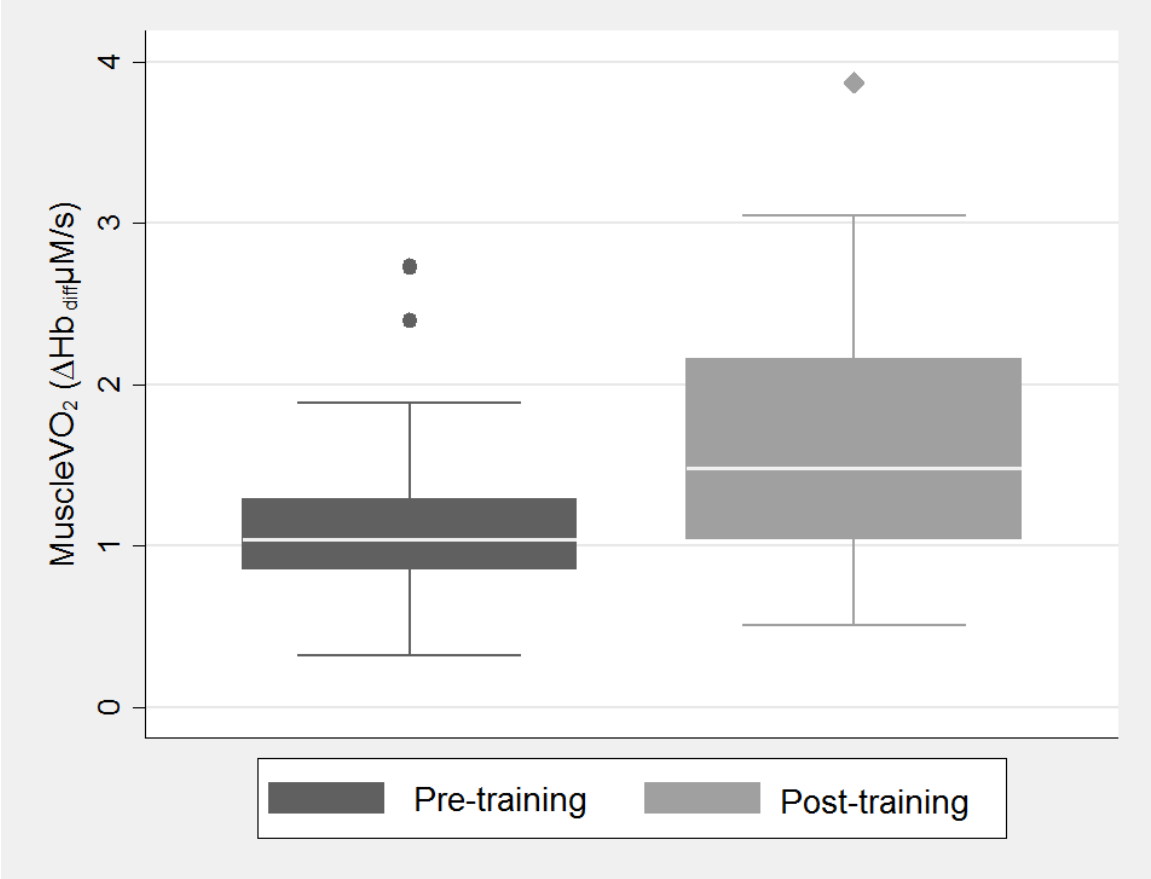

Figure 6.3 Box and whisker plot showing post-exercise muscle$\dot{V}O2$ pre-training (dark grey) and post-training (light grey). **A box and whisker plot is used to describe this data because of its skewed distribution. The bottom and top of the box represent the first and third quartiles, the band inside the box is the median and the whiskers represent the lower and upper adjacent values within 1.5*IQR of the lower and upper quartile, respectively. Medians were compared using a Wilcoxon signed-rank test; post-training values were significantly higher than pre-training. Hb; haemoglobin.**

6.3.3 Muscle$\dot{V}O_2$ and cardio-pulmonary Peak$\dot{V}O_2$

Pre-training higher muscle$\dot{V}O_2$ following the exercise test correlated with higher cardio-pulmonary peak$\dot{V}O_2$ (Pearson's r= 0.64, p<0.001; figure 6.4; red circles). Post-training a positive correlation was also seen but it was less strong (Pearson's r=0.27, p=0.17 (figure 6.4; blue squares)). There was no significant interaction between the visit time (pre- versus

post-training) and the relationship between muscle$\dot{V}O_2$ and cardio-pulmonary peak$\dot{V}O_2$ (p=0.251).

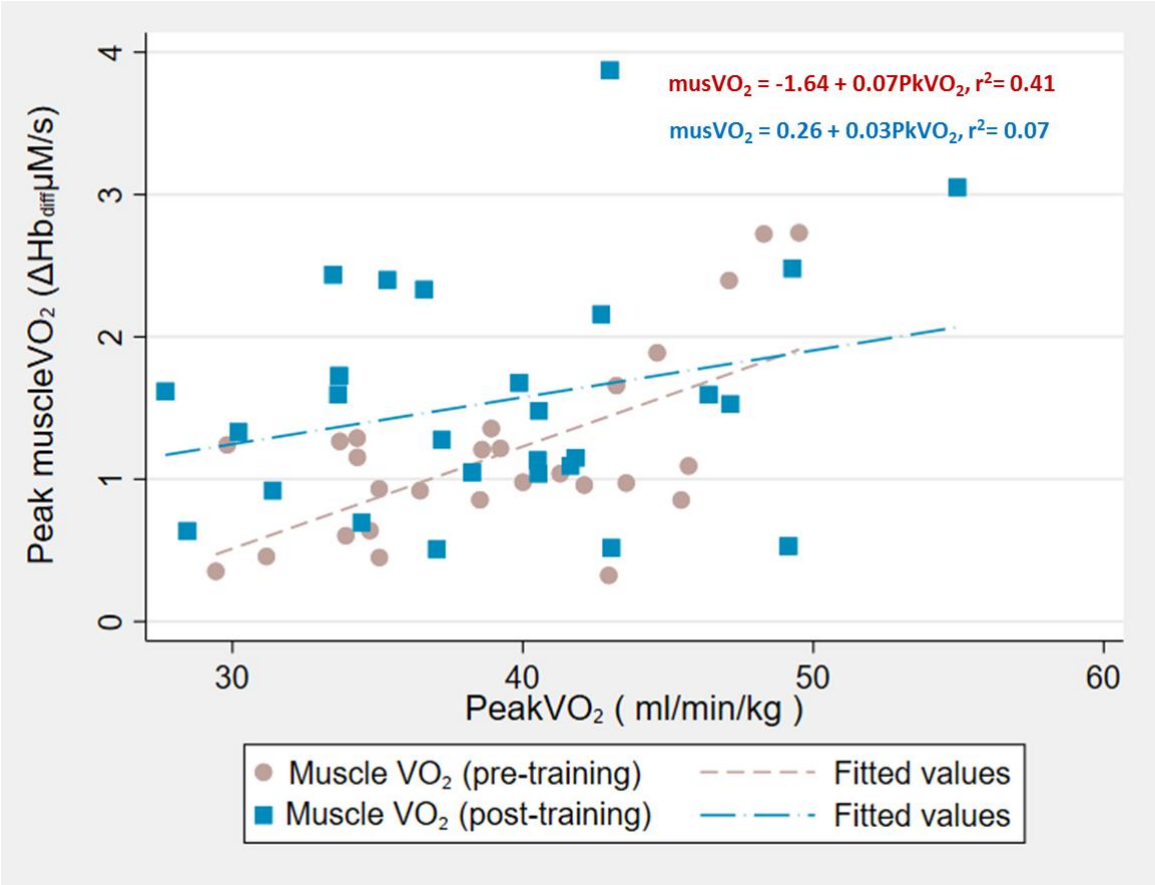

Figure 6.4 Correlation between cardio-pulmonary peak$\dot{V}O2$ and post-exercise muscle$\dot{V}O_2$ for 27 participants assessed pre-training and post-training. Participant data pre-training are represented by rose-coloured circles and a rose line of best fit and post-training by blue squares and a blue line of best fit.

Figure 6.5 shows an example of NIRS signals from a single participant during arterial occlusions performed immediately post-exercise at the (a) pre-training and (b) post-training visits (Figure 6.5 a & b, respectively). The dashed lines indicate the measurement area and green arrow indicates the slope of decline in HbO_2 which represents rate of oxygen consumption in the muscle. The green arrow is shown at both time-points to highlight the change with training.

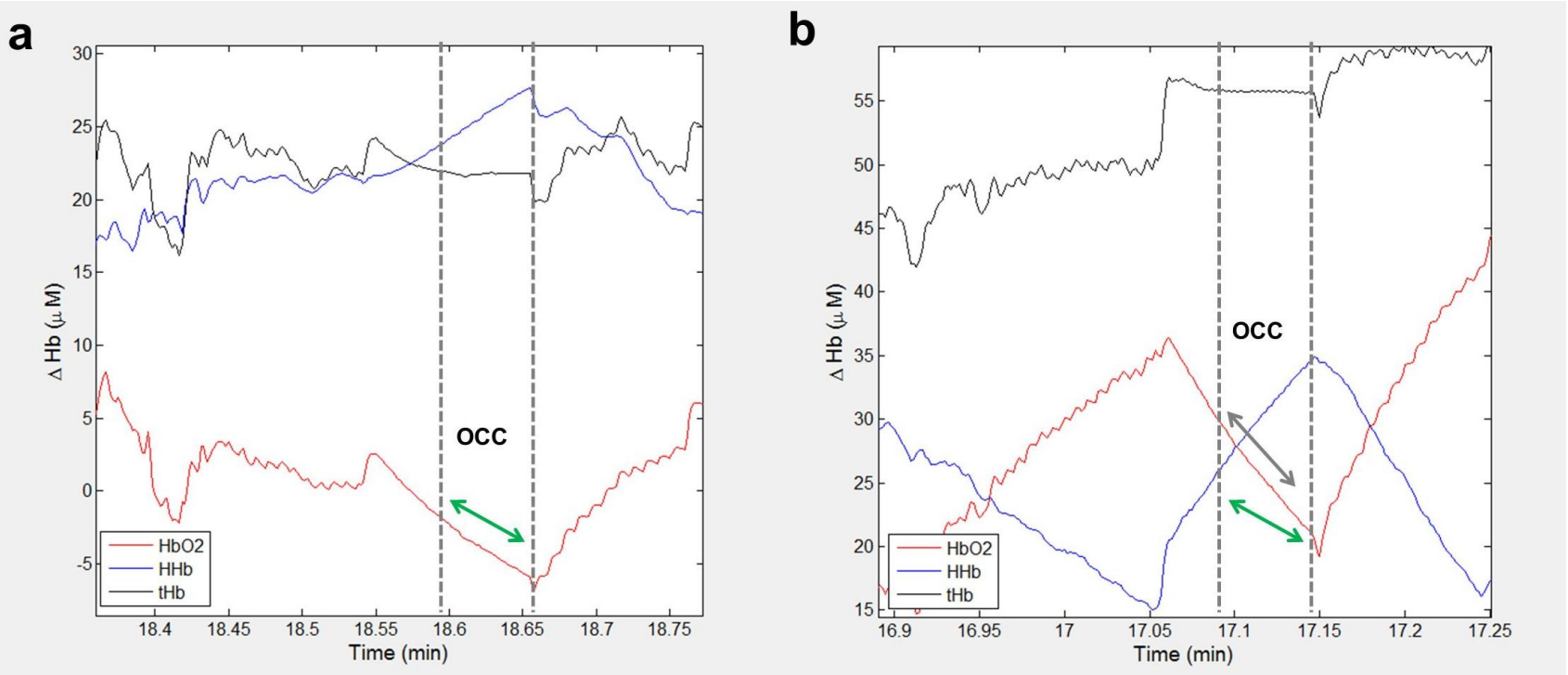

Figure 6.5 Example participant data showing the NIRS signal during arterial occlusions (OCC) performed immediately post-exercise. Changes in oxygenated haemoglobin (HbO2), deoxygenated haemoglobin (HHb) and total haemoglobin (tHb) are shown for one participant at (a) pre-training visit and (b) post-training visit. The dashed lines indicate the measurement area and green arrow indicates the slope of decline in HbO_2 which represents rate of oxygen consumption in the muscle. The green arrow is shown at both time-points to highlight the change with training.

6.3.4 Oxidative capacity with training and performance

There was no difference between the time constant from pre-training to post-training (median value(IQR); 35(22-45) versus 36(23-60) s, p=0.24; table 6.2). Pre- and post-training values were positively correlated (Pearson's r= 0.84, p<0.001; figure 6.6).

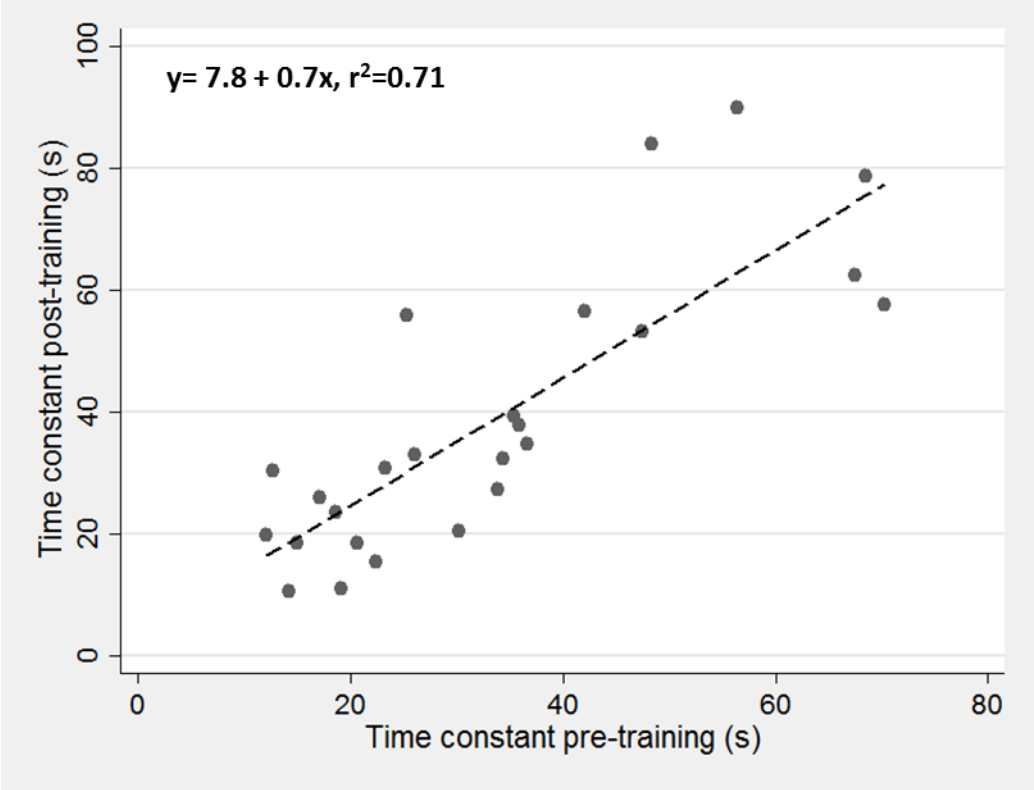

Figure 6.6 Correlation between pre- and post-training measurements of the time constant. **Pearson's correlation coefficient is 0.84, p<0.001.**

There was a positive relationship between the time constant and marathon performance (marathon running time) for both the pre- and the post-training measurements of the time constant, however, this was not statistically significant after adjustment for age and sex ($r_{partial}$=0.37, p=0.09 & $r_{partial}$=0.07, p=0.73, respectively). Unadjusted values for pre- and post-training time constants versus marathon running time are plotted in figure 6.7 (top panel). There was no significant interaction between the visit time (pre- versus post-training) and the relationship between the time constant and marathon running time (p=0.332). The difference in time constant (post- minus pre-training value) showed a

negative trend with marathon time but this also was not statistically significant ($r_{partial}$= -0.24, p=0.32; figure 6.7, bottom panel).

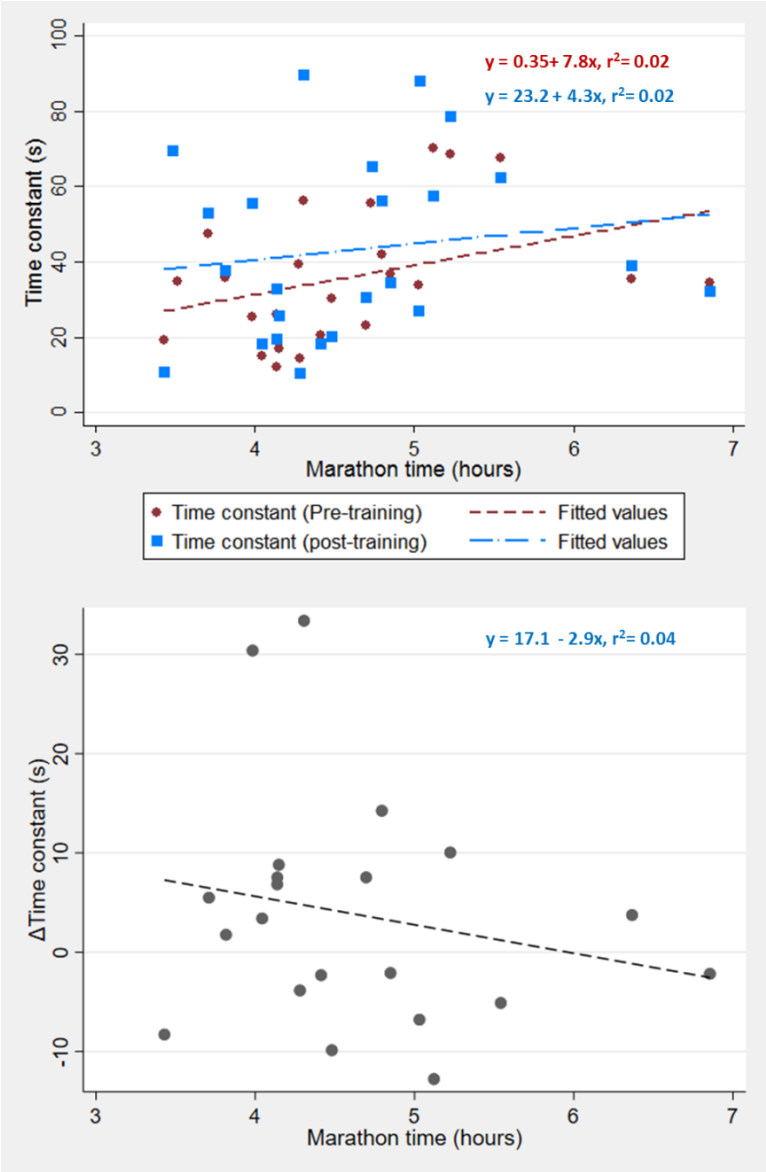

Figure 6.7 Correlation between marathon running time (in hours) and the time constant for muscle$\dot{V}O_2$ recovery. The top panel shows marathon time versus pre-training (red circles and red line of fit) and post-training (blue squares and blue line of fit) time constants. The bottom panel shows marathon time versus the difference between pre- and post-training time constant (the post-training value minus the pre-training value).

6.3.5 Marathon performance and cardiorespiratory fitness

Higher cardio-pulmonary peak$\dot{V}O_2$ values post-training were associated with faster marathon completion times after adjustment for sex and age ($r_{partial}$=-0.58, p= 0.002). Greater increases in peak$\dot{V}O_2$ from the pre-training value also predicted shorter marathon completion times ($r_{partial}$=-0.53, p=0.007). Figure 6.8 shows the unadjusted correlation between marathon running time (in hours) and cardio-pulmonary peak$\dot{V}O_2$ pre- and post-training (top panel) and marathon time versus the difference between pre- and post-training peak$\dot{V}O_2$ (bottom panel). There was no interaction between the visit time (pre- and post-training) and the relationship between cardio-pulmonary peak$\dot{V}O_2$ and marathon time (p=0.744).

There was no convincing relationship between muscle$\dot{V}O_2$ measured post-training and marathon completion time ($r_{partial}$=0.31, p=0.14) or Δmus$\dot{V}O_2$ and marathon completion time ($r_{partial}$=0.31, p=0.14). The post-training recovery time constant was also not associated with marathon completion time ($r_{partial}$=0.18, p=0.41).

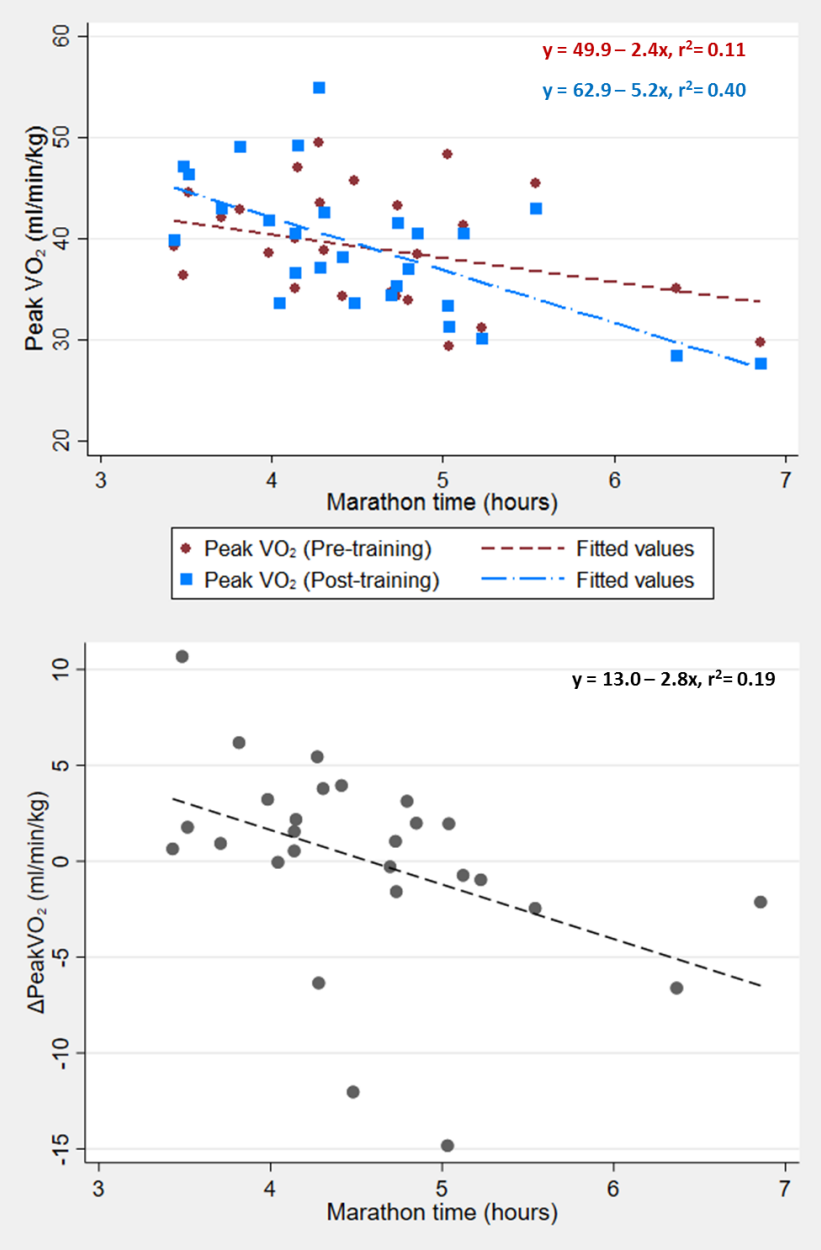

Figure 6.8 Correlation between marathon running time (in hours) and cardio-pulmonary peak$\dot{V}O_2$. The top panel shows marathon time versus pre-training peak$\dot{V}O_2$ (red circles and red line of fit) post-training peak$\dot{V}O_2$ (blue squares and blue line of fit). The bottom panel shows marathon time versus the difference between pre- and post-training peak$\dot{V}O_2$.

6.3.6 Marathon performance and resting muscle oxygen consumption

There were no strong relationships between resting muscle oxygen consumption (resting muscle $\dot{V}O_2$) at either pre- or post-training measurements (figure 6.9, top panel). There was no interaction between the visit time (pre- versus post-training) and the relationship between resting muscle $\dot{V}O_2$ and marathon time (p=0.237).There was no significant change in resting muscle oxygen consumption (Table 6.2, described previously) and there was no relationship between the change in resting muscle $\dot{V}O_2$ and marathon performance ($r_{partial}$=0.26, p=0.22) (figure 6.9, bottom panel).

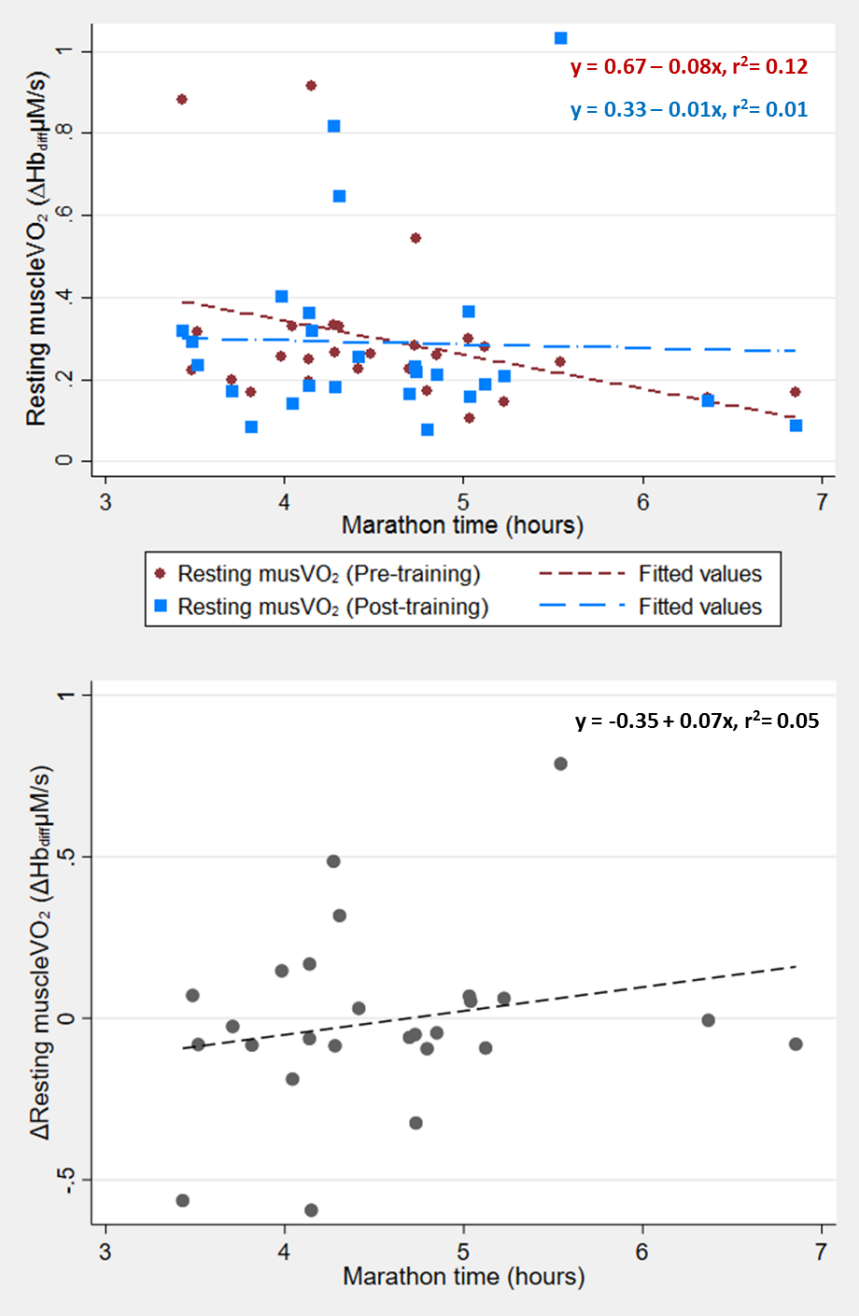

Figure 6.9 Correlation between marathon running time (in hours) and resting musle$\dot{V}O_2$. The top panel shows marathon time versus pre-training resting muscle$\dot{V}O_2$ (red circles and red line of fit) post-training resting muscle$\dot{V}O_2$ (blue squares and blue line of fit). The bottom panel shows marathon time versus the difference between pre- and post-training resting muscle$\dot{V}O_2$.

6.3.7 Cardiorespiratory fitness, resting muscle oxygen consumption and oxidative capacity

Cardiorespiratory fitness (peak $\dot{V}O_2$) was broadly positively correlated with resting skeletal muscle oxygen consumption at the pre- and post-training measurement visits (Pearson's r=0.41, p=0.04 & Pearson's r=0.45, p=0.02, respectively; figure 6.10, top graphs). However, the change in peak $\dot{V}O_2$ and resting muscle$\dot{V}O_2$ were not related.

Cardiorespiratory showed no relationship with the time constant for recovery of muscle $\dot{V}O_2$ post exercise (figure 6.10, bottom graphs). Although, the change in time constant and change in peak $\dot{V}O_2$ with training were positively correlated (Pearson's r=0.49, p=0.02; figure 6.10, bottom right graph).

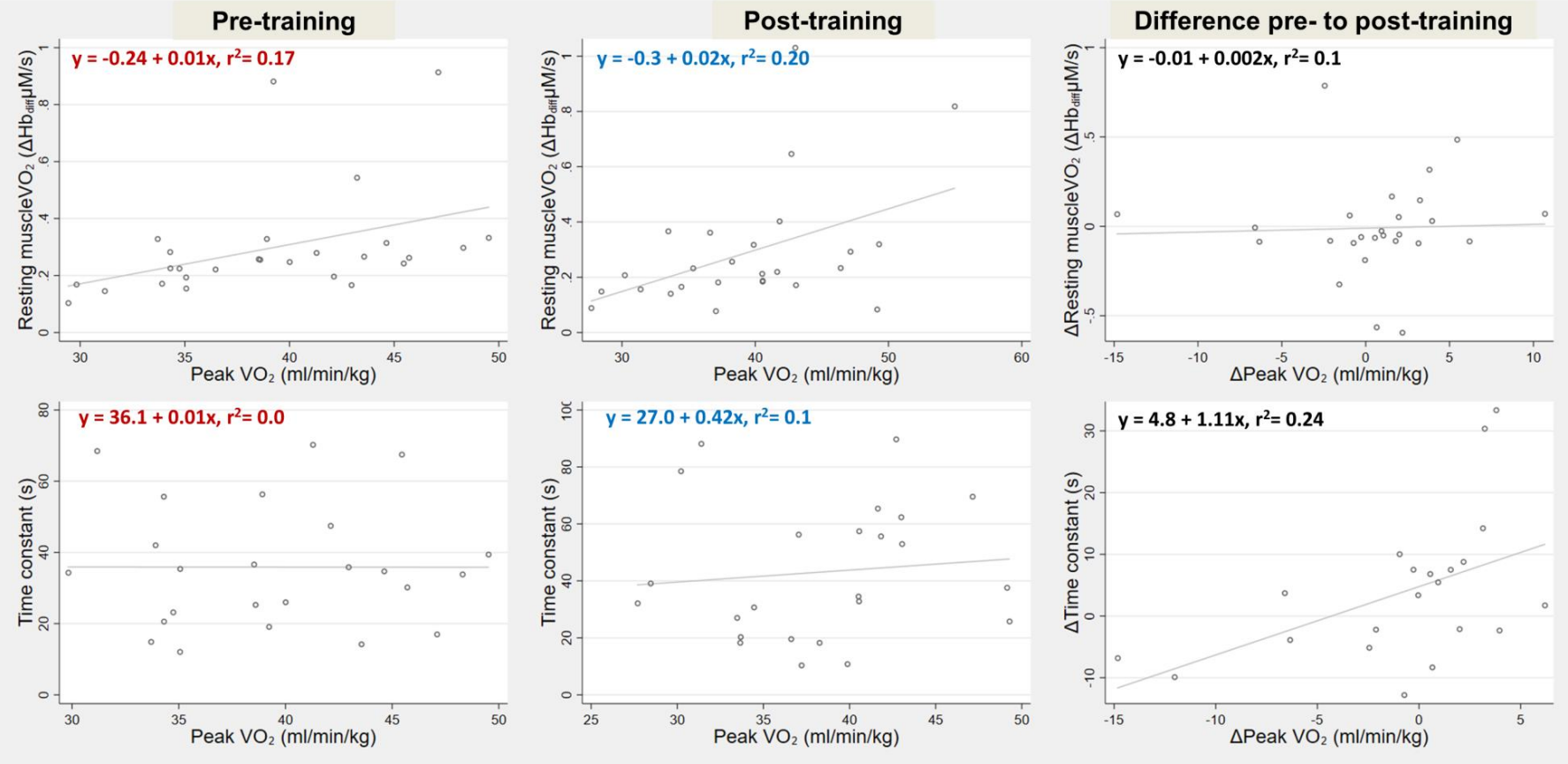

Figure 6.10 six-panel graph plot. The relationship between cardiorespiratory fitness and resting muscle oxygen consumption (muscle $\dot{V}O_2$) is shown in the top 3 panels for; (left to right) pre-training, post-training and the change in resting muscle $\dot{V}O_2$. The relationship between cardiorespiratory fitness and the time constant is shown in the bottom 3 panels for; (left to right) pre-training, post-training and the change in time constant.

6.4 Discussion

6.4.1 Key findings

This study measured resting muscle$\dot{V}O_2$, post-exercise muscle$\dot{V}O_2$ and the recovery time constant of muscle$\dot{V}O_2$ using NIRS. Simultaneous assessment of cardio-pulmonary peak$\dot{V}O_2$ via CPET was carried out in this sample of young, healthy men and women. All measurements were carried out before and after 4 to 6 months of endurance training in preparation for a first-time marathon.

Compared with pre-training, post-training was associated with an increase in post-exercise muscle$\dot{V}O_2$ and Δmus$\dot{V}O_2$, despite no detectable change in cardio-pulmonary peak$\dot{V}O_2$. There was no change in the time constant representing skeletal muscle oxidative capacity. The magnitude of the increase in muscle$\dot{V}O_2$ was similar to that previously reported in healthy volunteers using NIRS following bicycle training (vastus lateralis)(294) or training of arm muscles,(222) or using succinate dehydrogenase(295) or citrate synthase(296) as indicators of muscle oxidative capacity. However, one study failed to observe an increase in maximum muscle oxygen consumption in finger flexor muscles following 6-weeks of training with a dynamic handgrip exercise.(297) Our findings suggest that, in these young, healthy individuals, a comparatively low-level of endurance training, while not sufficient to noticeably improve cardio-pulmonary function, still led to positive skeletal muscle adaptations resulting in improved oxygen extraction following exercise.

6.4.2 Marathon training

It is possible that the detected changes in gastrocnemius muscle$\dot{V}O_2$ reflect changes in muscle group utilization, due to training. Differences in training structure such as inclusion of strength, speed-endurance or interval training in the program are known to differentially affect muscle function.(298-300). For example, it may be the case that participants replaced other activities (gym sessions, cycling etc.) with run-training and therefore

increased conditioning in the gastrocnemius. Post-exercise muscle$\dot{V}O_2$ and the exercise-induced increase in muscle oxygen consumption, $\Delta mus\dot{V}O_2$, were moderately positively correlated with cardio-pulmonary peak$\dot{V}O_2$ pre-training and more weakly with post-training peak$\dot{V}O_2$. Consequently, it seems likely that these correlations relate to inherent participant characteristics (e.g. sex, age) rather than to the 6 months of training. A limitation of this study is that training was not supervised and information about training was not collected (discussed in the limitations section in more detail), therefore, we do not know to what extent participants undertook running, cycling or gym activities in their Marathon preparation.

6.4.3 Marathon performance

Marathon performance was judged as marathon completion time. As expected, faster marathon completion times were associated with higher cardio-pulmonary peak$\dot{V}O_2$,(65) but no relationship was observed between indices of muscle function, post-exercise muscle$\dot{V}O_2$ and $\Delta mus\dot{V}O_2$ in gastrocnemius, and marathon completion time. This is consistent with cardiopulmonary factors rather than mitochondrial oxidative capacity (which typically exceeds maximal O_2 supply in skeletal muscle) being a dominant factor in peak exercise capacity.(44, 301)

6.4.4 Skeletal muscle oxidative capacity

Previously, cross-sectional data have demonstrated improved metabolic function in athletic versus inactive subjects.(60, 99) In the study by Brizendine et al,(99) highly trained endurance athletes, with a mean peak $\dot{V}O_2$= 73.5±9.1 ml/min/kg, were compared to inactive individuals (mean peak $\dot{V}O_2$= 33.7±5.9 ml/min/kg) using the same NIRS methods for deriving a time constant representing skeletal muscle oxidative capacity; they found that in endurance trained individuals mean recovery time constants from the Vastus Laterialis were 18.4±3.2 s versus 32.4±5.2 s in the inactive control group.(99) Other studies have employed NIRS to study skeletal muscle response to run-training, however,

in studies where arterial occlusions are not performed, it is important to note that improvements could be attributed to either, or both, improved O_2 delivery and/or increased mitochondrial capacity.(221)

6.4.5 Cardiorespiratory fitness

Cardiorespiratory fitness (Peak $\dot{V}O_2$) showed a broadly positive correlation with resting skeletal muscle oxygen consumption at both pre-training and post-training measurement times (Pearson's r=0.41, p=0.09 & Pearson's r=0.45, p=0.004, respectively; figure 6.10, top graphs). However, there were no changes detected in resting muscle oxygen consumption with training and there was no relationship between peak$\dot{V}O_2$ and resting skeletal muscle oxygen consumption.

Cardiorespiratory fitness was not correlated with oxidative capacity (the time constant for recovery of muscle $\dot{V}O_2$ post exercise) in the group either before or after training (figure 6.10, bottom left and central graphs). Although, the change in oxidative capacity and change in fitness with training were positively correlated (Pearson's r=0.43, p=0.03) suggesting that improvements in cardio-respiratory fitness, over this wide range in values, was associated with an improvement in skeletal muscle metabolic function, a finding which is in line with previous findings.(100)

6.5 Limitations

We did not observe a significant improvement in peakVO_2 between pre-training and post-training which is contrary to expectation.(65) It is possible that detraining following completion of the marathon may have contributed to this; however, the effect of short detraining periods is minimal(302) and is unlikely to fully explain this finding. It is possible that lack of supervision of a structured training program and/or lack of adherence to training by participants also contributed. Providing structured training programs and support throughout training was beyond the scope of this study. Appropriate methods of

collecting information describing participant adherence to training programs (e.g. wearable activity monitors) would be warranted in future similar studies. Nevertheless, our objective was to observe changes that occur in 'real-world' subjects undertaking their first marathon; therefore, our findings are likely to be representative of young, healthy adults undertaking endurance events. Esfarjani et al, randomized participants to structured high intensity training interventions versus consistent low-intensity running and showed similar, non-significant, increases in peak$\dot{V}O_2$ as we report here in the low-intensity group.(303)

Monitoring training behaviours of the participants in the 6 months prior to the marathon would undoubtedly have provided additional valuable data. However, the objective of this analysis was to investigate if there is an effect of moderate endurance training on skeletal muscle metabolic function in healthy but untrained individuals. The effect of the extent of training was not investigated, although we acknowledge that is likely to have implications for the extent of improvement in skeletal muscle function. All participants included in this analysis completed the marathon and we believe it is fair to assume that this is evidence that, at least minimal, endurance training was undertaken.

Rejection of data due to noise in the NIRS signal was fairly high: ~12% at pre-training and ~19% at the post-training visit. This could be improved in future studies by using maximum cuff inflation pressures to ensure complete arterial occlusions and by preventing movement artifacts through use of a supportive-leg stand to ensure complete muscle relaxation.

6.6 Conclusion

In healthy individuals preparing for their first marathon, participation in a 4 to 6-month training programme is associated with an increase in the ability of gastrocnemius skeletal muscle to utilize oxygen following exercise, implying an improvement in metabolic capacity. Metabolic adaptions can be identified non-invasively using NIRS combined with

arterial occlusions within the setting of a supine CPET, although, caution is warranted regarding movement artifacts and insufficient occlusion pressures when using this technique. Post-exercise muscle$\dot{V}O_2$ was positively correlated with cardio-pulmonary peak$\dot{V}O_2$, particularly pre-training, but, in terms of performance, cardio-pulmonary peak$\dot{V}O_2$ is a better predictor of faster marathon running.

Chapter 7: General discussion and conclusion

7.1 General overview

This thesis investigates skeletal-muscle metabolic function and cardio-respiratory fitness and how they influence exercise capacity. It is useful to understand these relationships in the context of diabetes where skeletal muscle metabolic function has previously been considered important in the physiology of disease development.(107, 277) Understanding the effect of exercise training on metabolic function and the importance of performance to this relationship informs on physiological adaptations of potential preventative/therapeutic targets. Two sample populations were considered; (I) a cohort study comprised of previously well-characterized, older adults known to have at least 23% prevalence of diabetes(223) and (II) a young/healthy adult study designed to investigate the effects of first-time marathon training on the heart.

This thesis also contributes some understanding of NIRS as a muscle measurement tool by applying it to these novel study populations in the context of exercise tests where it has not previously been used.

Eight research questions were initially identified (Chapter 1; section 1.8). These are reproduced in a summary of 5 concise points which will be discussed in the subsequent 5 sections, as follows:

1) *How reproducible are the key methods described in this thesis, namely; submaximal exercise capacity determined via the 6MST and oxidative capacity and oxygen consumption, assessed using NIRS? And how well do the results of the 6MST compare to results of the 6MWT?*
2) *To what extent does the presence of T2D affect submaximal exercise capacity, cardiorespiratory fitness and skeletal muscle oxidative capacity? Is the relationship*

between submaximal exercise capacity and skeletal muscle oxidative capacity different between people with and without T2D?

3) *Are there ethnic differences in sub-maximal exercise capacity, cardiorespiratory fitness and skeletal muscle oxidative capacity? If so, can oxidative capacity explain ethnic differences in exercise capacity/cardiorespiratory fitness?*
4) *Does endurance exercise training in sedentary adults improve skeletal muscle metabolic function?*
5) *Are skeletal muscle metabolic function and cardio-respiratory fitness independently associated with exercise capacity?*

An overview of the design of the studies to address these questions is summarized in figure 7.1. This chapter discusses each question with reference to the studies described in previous chapters and the hypothesis given in Chapter 1, section 1.9.

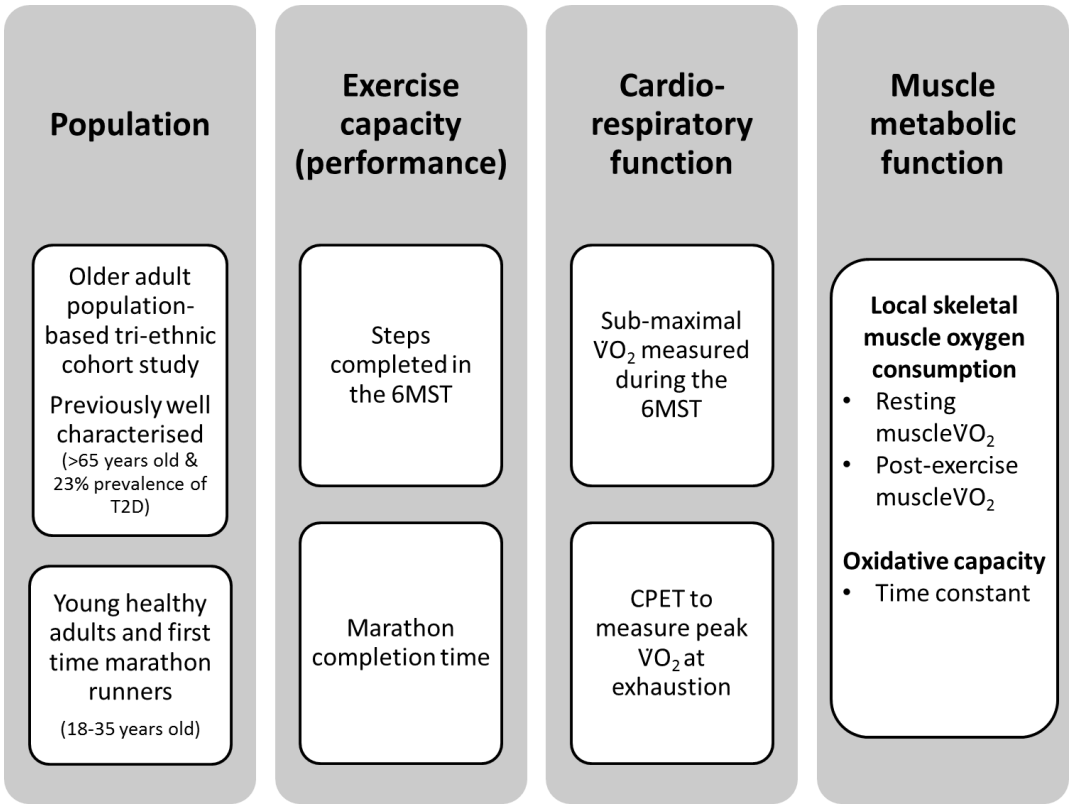

Figure 7.1 Overview of studies designed to answer the research questions.

7.2 Development of methods

How reproducible are the key methods described in this thesis, namely; submaximal exercise capacity determined via the 6MST and oxidative capacity and oxygen consumption, assessed using NIRS? And how well do the results of the 6MST compare to results of the 6MWT?

In the older adult cohort described here, the 6MST was developed to be acceptable for testing submaximal exercise capacity in older adults and was then tested for reproducibility and agreement with the 6MWT. The results presented in Chapter 2 are the first to describe the 6MST for assessment of exercise capacity and prediction of $\dot{V}O_2$max in a large population-based cohort of older adults. Conducting the exercise test in a static environment (without requirement for corridor walking) allowed physiological response to exercise to be accurately monitored, therefore permitting investigations necessary to answer question 2, such as; analysis of expired gasses for determination of peak $\dot{V}O_2$, heart rate measurements throughout to exercise to predict maximum $\dot{V}O_2$ and skeletal muscle measurements immediately post-exercise.

The novel use of the 6MST to permit measurements of skeletal muscle metabolic function is presented in Chapter 3. NIRS was selected as a skeletal muscle measurement tool in these studies because it is non-invasive and relatively inexpensive. The Portamon was selected because, at the time of study design, it was the only commercially available, wireless system providing measurement at a high sampling frequency. The methods for application of NIRS to derive resting muscle$\dot{V}O_2$, post-exercise muscle$\dot{V}O_2$ and for assessment of oxidative capacity are described in Chapter 3. Reproducibility of these muscle measurements was acceptable in older adults.

NIRS methods for measuring skeletal muscle function were also applied in the study designed to examine the training effect on skeletal muscle metabolic function (Chapter 5).

In this study, NIRS measurements were conducted in the setting of a maximal exertion CPET. A direct comparison of resting or post-exercise muscle$\dot{V}O_2$ between the two study populations was not appropriate because of the different designs of the exercise tests; in the older adult population muscle$\dot{V}O_2$ was measured while participants were standing with weight distributed evenly between both legs, while in the young adults, muscle$\dot{V}O_2$ was assessed in the supine position with the gastrocnemius completely relaxed. However, comparison of the time constant between groups revealed longer values in the older adult population (46(41, 50) versus 35(22, 45)seconds), suggesting poorer oxidative capacity in this group; in line with other studies that report age-associated skeletal muscle metabolic aberrations.(304)

NIRS is limited in its ability to provide the detailed physiological assessment of skeletal muscle that can be determined through muscle biopsy samples; such as mitochondrial number or enzymatic activity. However, its ability to provide an assessment of local oxygen consumption, without disruption to the physiological environment, makes it a valuable complementary tool for functional muscle assessments.

7.2 Diabetes

To what extent does the presence of T2D effect submaximal exercise capacity, cardiorespiratory fitness and skeletal muscle oxidative capacity? Is the relationship between submaximal exercise capacity and skeletal muscle oxidative capacity different between people with and without T2D?

Older adults with diabetes had poorer exercise capacity and achieved a lower peak measured $\dot{V}O_2$ during submaximal exercise and lower predicted $\dot{V}O_2$max than adults without diabetes (Chapter 4). This is in line with previous studies that have demonstrated poorer cardio-respiratory fitness, measured as cardio-respiratory $\dot{V}O_2$ at maximal exertion in older adults with T2D.(270, 282) It is also possible that psychological factors influence

the lower self-selected pace achieved by people with diabetes, perhaps reflecting a lower general participation in exercise; less experience of exercise would limit a participants ability for pace control (as discussed in Chapters 2 & 4). The lack of differences in perceived exertion or leg fatigue between people with/without diabetes suggests that older adults with diabetes experience lower workloads as more intense than older adults without diabetes; in line with previous findings.(282)

Muscle oxygen consumption immediately post-exercise (post-exercise muscle $\dot{V}O_2$) was marginally smaller (~15% less) in the presence of diabetes (Chapter 4). While this could represent an impaired ability to effectively increase oxidative phosphorylation in response to sub-maximal exercise, it is also likely that this reflects the lower workload - as discussed above. One further possibility is that the measurement of post-exercise muscle $\dot{V}O_2$ represents slower repayment of the oxygen debt following exercise therefore reflecting poorer micro-vascular function. Bauer et al,(271) demonstrated a slowed response of skeletal muscle blood flow at the onset of exercise in the presence of T2D suggesting microvascular dysfunction in response to exercise may contribute to the overall impairment in $\dot{V}O_2$ kinetics previously reported in the presence of T2D.

In this study we observed poorer oxidative capacity of skeletal muscle in older adults in the presence of T2D. As discussed in Chapter 4, this supports findings from other studies where enzymatic activity(126) or rates of PCr recovery have been assessed.(131) NIRS cannot determine the underlying biochemical mechanisms for this impaired function which could be due to a reduction in mitochondrial number or content or a reduced mitochondrial capacity for fatty acid oxidation (metabolic inflexibility) resulting in lipotoxicity.(161, 305) Nevertheless, NIRS provides a non-invasive, relatively cheap technique to assess skeletal muscle metabolic health. This analysis used cross-sectional data, therefore, conclusions cannot be drawn on the direction of the relationship between skeletal muscle oxidative

capacity and insulin resistance which has previously been subject to heated debate.(129, 306)

7.3 Ethnic differences

Are there ethnic differences in sub-maximal exercise capacity, cardiorespiratory fitness and skeletal muscle oxidative capacity? Can oxidative capacity explain ethnic differences in exercise capacity/cardiorespiratory fitness?

Ethnicity is an important risk for cardiometabolic disease: South Asians and African Caribbean's, the 2 largest migrant ethnic groups in the UK, are at a higher risk for T2D than white Europeans.(139, 142) South Asians are also at a greater risk of developing subsequent cardiovascular disease.(307) Differences in skeletal muscle function have previously been explored in attempts to explain these differences;(161, 308) although results are inconclusive.(309)

In the group of older adults enrolled in this study, we found that oxidative capacity was impaired in South Asians but similar in African Caribbean's compared to Europeans. We found this effect to be independent of the effects of T2D on oxidative capacity, described in Chapter 4 and by others.(161, 166) This suggests an intrinsic impairment in oxidative capacity in South Asians which does not explain differences in prevalence of T2D in this group. This is further supported by the fining in this study that oxidative capacity was unable to attenuate the higher prevalence of T2D seen in African Caribbean's; skeletal muscle oxidative capacity was similar between African Caribbean's and Europeans and resting muscle oxygen consumption was highest in African Caribbean's despite a trend towards higher prevalence of T2D in African Caribbean's. Further work is needed to determine if ethnic differences in oxidative capacity could perhaps explain the ethnic differences in cardiovascular response to T2D; such as poorer cardiovascular outcomes for South Asians.(149)

We found that in our older adult tri-ethnic cohort, exercise capacity was impaired in South Asians compared to Europeans and similar between African Caribbean's and Europeans. This effect was independent of the increased prevalence of T2D in both ethnic groups. Differences in skeletal muscle oxidative capacity did not explain the reduction in exercise capacity.

While NIRS does not provide mechanistic detail at the level of enzymatic activities or mitochondrial number; it is interesting to describe ethnic differences in oxidative capacity captured in vivo in an older adult cohort. The SABRE study cohort provides an ideal opportunity to continue investigation into these differences. Participants have undertaken multiple clinic visits over a period of ~30 years and detailed cardiometabolic phenotyping has been carried out at the most recent 2 visits.

7.4 Exercise training and skeletal muscle metabolic function

Does exercise training improve skeletal muscle metabolic function?

Chapter 6 of this thesis demonstrates the effect of endurance training on skeletal muscle function and cardio-respiratory fitness. In the study sample recruited here, very small gains in peak measured $\dot{V}O_2$ were detected while post-exercise muscle $\dot{V}O_2$ improved and there was no difference in the time constant at the post-training visit.

The lack of overall improvement in cardio-respiratory peak $\dot{V}O_2$ was surprising and contrary to hypothesis 2 that postulated training would impart positive effects on cardio-respiratory function. Completing the marathon in the average finishing time for this study sample suggests an average pace of nearly 11 minutes/mile, at the slowest completion time of 6 hours; pace would be nearly 14 minutes/mile. According to the British Heart Foundation website, suggested speeds for walking are; fast: 12 minutes/mile, moderate: 15 minutes/mile and average: 20 minutes/mile (https://www.bhf.org.uk/get-

involved/events/training-zone/walking-training-zone/walking-faqs). Therefore, in the majority of participants, marathon performance (running time) reflected a running speed not much faster than walking. It is therefore likely that participants enrolled here, predominantly undertook low-intensity training.

It is encouraging that in this study, despite the low-intensity training and lack of improvement in peak$\dot{V}O_2$, skeletal muscle metabolic function improved. Other studies have also demonstrated skeletal muscle metabolic adaptations independent of cardio-respiratory function in response to endurance training.(289, 310) Laye et al,(289) examined metabolic health cross-sectionally in runners and non-runners who were age, gender and BMI matched. They found higher oxidative enzyme content in the runners versus the non-runners; in a sub-group of participants they also matched cardio-respiratory fitness ($\dot{V}O_2$max) and found similar differences in oxidative enzymes between runners and non-runners.(289) Helge et al,(310) observed no difference in $\dot{V}O_2$max following a period of 32-day of low-intensity cross-country skiing but an improvement in blood-lipid profile and a tendency towards improved fat oxidative capacity in the arm muscles suggesting metabolic adaptations occurred without improved cardio-respiratory fitness.

These findings may have important implications for design of exercise therapy for people who are unable, or unwilling, to undertake moderate-high intensity exercise training. It would be interesting to design studies to determine the influence skeletal muscle metabolic (dys)function on subsequent risk of morbidity and mortality, in a similar way to other studies that report the longitudinal implications of cardio-respiratory fitness.(268, 274) It would also be interesting to determine if even lower intensity training has the same skeletal muscle benefits. If mitochondrial dysfunction can be targeted via low-intensity exercise, this could be a valuable finding for older adults who are likely to find low-intensity exercise, such as walking more acceptable.

Understanding the benefits of exercise in older adults is important as it is possible that the effects on skeletal muscle seen here in the young marathon runners may not translate to the older adult group because of differences in plasticity of metabolic function. Still, limited evidence from other studies suggests that the capacity to benefit from exercise may be maintained into older age.(103)

It was not feasible to collect detailed information regarding the type of training program used by the marathon runners recruited here, therefore, the influence of exercise type (e.g. resistance versus endurance exercises) could not be assessed here. The health benefits of low-intensity resistance training, such as yoga, in the presence of T2D have previously been reported.(311) The type and duration of training appears to impact the effectivity of other training programs on muscle metabolic function. For example, a four week endurance training program did not improve oxidative capacity in older adults with heart failure (208), however, an 11 week resistance training program, in a similar group, had beneficial effects on the estimated ATP production rate (312).

7.5 Metabolic function and exercise capacity

Are skeletal muscle metabolic function and cardio-respiratory fitness independently associated with exercise capacity?

One of the initial hypotheses was that impaired skeletal muscle oxidative capacity (a longer time constant) would be associated with poorer exercise capacity at equivalent cardio-respiratory effort. In the older adults shorter time constants were not associated with an increased number of steps completed during the 6MST; this was the case for all participants and when analysis were stratified by presence/absence of diabetes. The difference in the number of steps achieved between people with/without diabetes was also not attenuated by including the time constant in the model. This suggests that while

oxidative capacity differs in people with diabetes, it does not explain their lower capacity to exercise.

In participants undergoing training there was no convincing association between shorter marathon completion times and shorter time constants. Previously a two-fold faster time constant has been reported in endurance-trained athlete compared to non-training matched subjects.(99) This discrepancy is likely due to the inclusion of highly trained individuals (mean peak$\dot{V}O_2$=73.5±9.1) in the study by Brizendine et al and the low-intensity of the training undertaken by the participants enrolled here.

7.6 Future directions

Various diseases, other than diabetes, have been linked to skeletal muscle metabolic dysfunction.(303) Applying NIRS measurements in the older adult population-based cohort study described in Chapter 2 (the SABRE study) provides an opportunity to investigate the relationships between other disease processes or system functionality and skeletal muscle function. For example, participants enrolled in the SABRE study are well characterized in terms of cardiac and large blood vessel function; a further investigation into the relationship between capacity for perfusion of skeletal muscle and the metabolic function of the tissue would be interesting. Appling the methods of Bauer et al. (271) to derive an estimate of skeletal muscle blood flow by applying changes in deoxy-Hb and cardio-pulmonary $\dot{V}O_2$ changes to the Fick equation at onset of exercise would also be of interest in the context of measurements of cardiac function in older adults.

It would be interesting to further investigate the ethnic differences in oxygen consumption and oxidative capacity at future time points to determine changes in oxidative capacity and changes in cardiovascular function with time. To further the comparison between the 3 ethnic groups described here, it may be useful to boost the number of African-Caribbean's included in the study with a push to recruit more participants. An exercise training

intervention carried out in an ethnically diverse cohort could also further understanding into the relationship between exercise capacity and muscle function. If this were carried out in older adult participants, insight into the flexibility of oxidative capacity with age could be gained.

In older adults from an ethnically diverse population sample, with, or without diabetes, NIRS provides an acceptable, non-invasive technique to assess overall skeletal muscle oxidative capacity. Despite having only limited ability to describe detailed physiological mechanisms, such as enzymatic activities or mitochondrial number, NIRS application to skeletal muscle provides a complementary assessment of overall physiological functionality.

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