Eccentric stepping exercise: Acute and chronic physiological responses in young and older adults.

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others

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Abstract

In comparison to concentric exercise, eccentric exercise allows an individual to achieve high mechanical loads for a lower cardiovascular and metabolic requirement. This highlights it as a possible efficacious rehabilitation intervention for exercise intolerant clinical populations, likely stimulating increases in strength and mobility without the development of exercise limiting symptoms that commonly present during traditional exercise. However, the majority of research utilises unnatural forms of eccentric exercise (i.e. reverse cycling), potentially restricting the compliance and translational benefits. Therefore, we have adapted an eccentric stepping ergometer, that may more closely replicate natural activity, and investigated the acute physiological responses and training adaptations within a young and older adult population.

The ergometer adaptations enabled tight control of exercise parameters, and crucially allowed comparison of concentric and eccentric physiological responses on the same device. Consistent with previous literature, we demonstrated a lower eccentric oxygen uptake ($\dot{V}O_2$), heart rate (HR) and blood pressure (BP) at the same power, and a greater eccentric power required to match for concentric metabolic requirement. Interestingly, eccentric $\dot{V}O_2$ and HR progressively increased above a predicted steady state, suggesting a higher exercise intensity at this metabolic rate. Subsequently, we compared concentric and eccentric training within young adults at similar mechanical and metabolic requirements, showing that higher eccentric powers were required to match for concentric $\dot{V}O_2$ and resulted in substantial increases in concentric, eccentric, and isometric strength, not seen with concentric training. Finally, we assessed the feasibility of a short eccentric recumbent stepping programme within an older adult population, showing considerable increases in concentric, eccentric and isometric strength that were maintained at 30-days follow up. Importantly, within both populations, the exercise remained tolerable and resulted in minimal muscle soreness.

These results provide further evidence to support the beneficial neuromuscular adaptions of eccentric exercise, and suggest that eccentric recumbent stepping may provide a safer, more tolerable and effective training modality. Pilot studies with additional measures of physiological function (specifically muscle oxidative capacity and fatigue) suggest that eccentric exercise may promote additional benefits beyond those reported in this thesis. It is hoped that the findings from this thesis will eventually contribute to the implementation of eccentric exercise within exercise intolerant populations that stand to benefit most.

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Abbreviations

1RM – maximum force produced from one repetition

(A-v)O_{2imp} – arteriovenous oxygen difference

ADL – activities of daily living

ADP – adenosine diphosphate

AF – activation fatigue

ATP – adenosine triphosphate

BMD – bone mineral density

BP – blood pressure

Ca^{2+} – calcium ions

CaO_2 – arterial blood O_2 content

CHF – chronic heart failure

CO_2 – carbon dioxide

CON_{MI} – concentric stepping performed at a target $\dot{V}O_2$

COPD – chronic obstructive pulmonary disease

COX – cyclooxygenase

CP – critical power

CPT – constant-power test

CSA – cross-sectional area

CT – computerised tomography

CvO_2 – venous blood O_2 content

DBP – diastolic blood pressure

DEXA – dual-energy X-ray absorptiometry

DOMS – delayed onset muscle soreness

EEIMD – eccentric exercise-induced muscle damage

$ECC_{\dot{V}O2}$ – eccentric stepping performed at a target $\dot{V}O_2$

ECC_{PM} – eccentric stepping performed at a target relative power

ECG – electrocardiogram

EMS – elderly mobility scale

EMG – electromyogram

FDI – first dorsal interosseous

$F_{ET}CO_2$ – end-tidal fractional concentration of CO_2

$F_{ET}O_2$ – end-tidal fractional concentration of O_2

H^+ – hydrogen ions / proton

H_2O – water

HCO_3^- – bicarbonate

HHb – deoxyhaemoglobin

HHb_c – blood volume corrected deoxyhaemoglobin

HR – heart rate

HR_{gain} – rate of HR increase with WR

HR_{peak} – peak heart rate

IGF – insulin-like growth factor

La – lactate concentration

LoT – limit of tolerance

LT – lactate threshold

MAP – mean arterial pressure

MBDS – modified Borg dyspnoea scale

MET – metabolic equivalent of task

MF – muscle fatigue

MISWT – modified incremental shuttle walk test

MRI – magnetic resonance imaging

mTOR – mammalian target of rapamycin

MVC – maximal voluntary contraction

m$\dot{V}O_2$ – muscle oxidative capacity

NGF – nerve growth factor

NIRS – near-infrared spectroscopy

NTS – nucleus tractus solitarii

O_2 – oxygen

O_2Hb – oxyhaemoglobin

PCO_2 – partial pressure of CO_2

PF – performance fatigue

Pi – inorganic phosphate

$\dot{Q}$ – cardiac output

$\dot{Q}_m$ – muscle blood flow

QoL – quality of life

RER – respiratory exchange ratio

RF – rectus femoris

RIT – ramp-incremental test

RMS – root mean squared

RPE – rating of perceived exertion

RPM – revolutions per minute

RPP – rate pressure product

SE – standard error of the mean

SBP – systolic blood pressure

SD – standard deviation

SSC – stretch shortening cycle

tHb – total haemoglobin

TSI – tissue saturation index

VAS – visual analog scale

$\dot{V}CO_2$ – rate of carbon dioxide output

$\dot{V}_E/\dot{V}O_2$ – ventilatory equivalent of oxygen

$\dot{V}_E/\dot{V}CO_2$ – ventilatory equivalent of carbon dioxide

$\dot{V}O_2$ – rate of oxygen uptake

$\dot{V}O_{2gain}$ – rate of $\dot{V}O_2$ increment with WR

$\dot{V}O_{2m}$ – muscle oxygen uptake

$\dot{V}O_{2max}$ – maximal oxygen uptake

$\dot{V}O_{2peak}$ – peak oxygen uptake

$\dot{V}O_{2SC}$ – slow component of oxygen uptake

WR – work rate

Professional activities

Abstracts arising from this thesis.

Renwick NC, Egginton S, Ferguson C. (2016). Reductions in Cardiovascular and Metabolic Demands During Work Rate Matched Eccentric Stepping Exercise. *Medicine and Science in Sports and Exercise*, **48,** 365. American College of Sports Medicine annual meeting, Boston.

Renwick NC, Dale MacLaine T, Ferguson C, Egginton S. (2017). Eccentric exercise in the older adult: Can a short training programme increase strength and mobility, and reduce frailty? *Proceedings of The Physiological Society,* Future physiology conference, Leeds. (*Awaiting publication*).

Manuscripts arising from this thesis.

Renwick NC, Egginton S, Ferguson C. (2018). Reduced cardiovascular and metabolic responses during eccentric stepping exercise. *Medicine and Science in Sports and Exercise.* (*Submitted for peer review*). (*Data arising from chapter 3*).

Chapter 1 Literature review

This thesis aims to validate a novel form of eccentric exercise, and to characterise the physiological response to such exercise within a young and older adult population. Eccentric contractions (muscle lengthening during contraction) are unique in that they allow the generation of large forces at low cardiovascular and metabolic demands. These findings have led to an upsurge in eccentric research, however, the detailed physiological responses of eccentric exercise remain poorly understood in comparison to traditional (concentric) exercise. The majority of eccentric research has been conducted using modalities that likely do not closely represent natural activities or movement, with physiological responses to more ecologically valid forms of exercise having minimal representation within the literature. Despite this, findings suggest that eccentric exercise may provide an effective rehabilitation intervention within exercise-intolerant populations. However, eccentric exercise is not currently routinely utilised within standard health care, highlighting the need for further research utilising natural eccentric exercise modes within healthy and clinical populations, to expand our knowledge and better understand its therapeutic potential.

This literature review will detail the fundamentals of exercise physiology, with a specific focus on comparing the acute and chronic responses of concentric and eccentric exercise. Current eccentric modalities will be critiqued, and the training responses within young and older adults summarised.

1.1 Muscle contraction

A muscle contraction that results in no change in distance between the origin and insertion point of a muscle-tendon unit is termed an isometric contraction. Isometric contractions often occur when a force is applied to a solid, unmovable object and can span from threshold to maximal contractions. Sole isometric

contractions occur infrequently other than under prescribed conditions such as weight training. During these contractions, the muscle force is equal to the opposing force, resulting in no movement and, therefore, no external work. Despite this, there can be significant internal work, with the majority of motor units within the working muscle becoming activated, resulting in a large metabolic (adenosine triphosphate, ATP) cost (Newham et al., 1995; Beltman et al., 2004b). Importantly, although isometric contractions do not shorten the entire muscle-tendon unit, the elastic nature of tendons allow a degree of distension, therefore, allowing limited shortening of the associated muscle (McArdle et al., 2010).

Dynamic muscle contractions (muscle contraction during movement) can be divided into concentric or eccentric muscle contractions. These can be performed throughout the range of movement at the same external load (iso-load contraction) or at the same velocity (isokinetic contraction). Iso-load contractions are generally performed whilst lifting an object, whilst true isokinetic contractions require dynamometers to control the velocity of movement (Hoppeler, 2015). When force generated exceeds the opposing force, the muscle shortens, and a concentric contraction occurs. In contrast, when the opposing force exceeds that generated by the muscle, the muscle lengthens during activation and an eccentric muscle contraction occurs (Abbott and Aubert, 1951; Asmussen, 1953). As concentric muscle contractions accelerate and impart potential energy onto an object they are said to produce positive work, whereas eccentric contractions decelerate or absorb potential energy and therefore produce negative work and do not produce power. To simplify comparisons between concentric and eccentric exercise, recent literature reports a relative positive power for eccentric exercise (LaStayo et al., 2000; Dufour et al., 2004; Elmer et al., 2017), and this method will also be used throughout this thesis. A prime example of concentric and eccentric contractions is when ascending and descending stairs, respectively. During ascent, the quadriceps and gluteal muscles actively shorten to extend the knee and hip, elevating the body to an upper step (Harper et al., 2018), whereas during descent, the muscles are actively lengthening to control the rate of knee and hip flexion, lowering the body to a lower step (Yali et al., 2015).

Concentric and eccentric contractions are rarely isolated during natural locomotion and movement. Instead, they predominantly occur together, with eccentric contractions often enhancing the subsequent concentric power *via* a process known as the stretch-shortening cycle (SSC; Cavagna et al., 1968). For example, during running, eccentric contractions of the gastrocnemius and soleus muscles that occur at initial foot contact are followed by a concentric contraction at take-off, of greater power and lower electrical muscle activity (peak of the rectified and integrated raw electromyogram (EMG) (Komi, 2000) than a pure concentric contraction. The mechanisms responsible for this increased concentric force production remain unclear, but potential mechanisms involve tendon and cross-bridge elongation (Fukutani et al., 2017), the stretch reflex (Komi, 2000), pre-activation (Ettema et al., 1990; Bobbert and Casius, 2005), and residual force enhancement (Fukutani et al., 2017). As the SSC reduces energy demand of subsequent concentric contractions, the muscle efficiency is enhanced, with 50% greater efficiency previously reported compared to concentric contractions alone (Lindstedt et al., 2001). Eccentric contractions are also used to decelerate or brake against an external force, such as landing from a jump (Vogt and Hoppeler, 2014). When the eccentric contraction is not immediately (within ~1s; Wilson and Flanagan, 2008) followed by a concentric contraction, the benefits of the SSC are lost and the stored elastic energy is dissipated as heat (Lindstedt et al., 2001).

1.2 Energy systems and oxygen uptake

The energy required for muscle contractions originates from the hydrolysis of ATP (Szent-Györgyi, 2004). Muscular ATP stores are minimal, only supplying enough energy to provide ~3s of contraction and therefore stores need to be constantly resynthesised by either aerobic (oxidative phosphorylation) or anaerobic (phosphocreatine (PCr) and glycolysis) pathways. The relative contributions of these energy systems depend predominantly on the intensity and duration of the exercise being performed, with continuous low intensity exercise principally relying on oxidative phosphorylation, and short high intensity exercise requiring anaerobic glycolysis and PCr hydrolysis (Figure 1.1; Gastin,

2001). The PCr system can provide energy for ATP resynthesis for ~8-10s of maximal exercise and does so *via* the anaerobic splitting of phosphate in the presence of the catalyst creatine kinase. Oxidative phosphorylation on the other hand is the predominant energy supply during constant load exercise of durations longer than ~60s, providing 36 molecules of ATP from each glucose molecule *via* the transference of electrons from NADH and $FADH_2$ to oxygen (O_2). Anaerobic glycolysis lies between these two systems and in the absence of O_2 can provide energy for ~30s. Glycolysis produces just 2 molecules of ATP by converting glucose into pyruvate, water and NADH and results in accumulation of blood lactate (La), reducing pH and inhibiting further glycolysis.

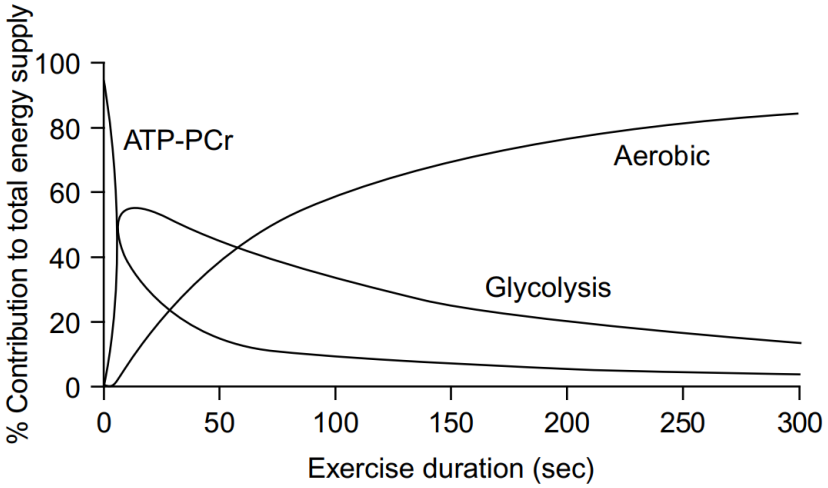

Figure 1.1. Relative energy system contributions to the total energy supply for maximal exercise of set durations (Gastin, 2001).

As muscle oxygen uptake ($\dot{V}O_{2m}$) is a true reflection of the ATP utilisation within the muscle, measuring $\dot{V}O_{2m}$ directly can be used to understand the metabolic requirement of the exercise being performed. Direct measurement of $\dot{V}O_{2m}$, can be determined using the Fick equation; however, this requires invasive techniques to determine the various parameters.

$$\dot{V}O_{2m} = \dot{Q}_m * (C_aO_2 - C_vO_2)$$

Equation 1.

where "$\dot{V}O_{2m}$" is muscle oxygen consumption, "$\dot{Q}_m$" is muscle blood flow, and "C_aO_2" and "C_vO_2" are the muscle arterial and venous blood oxygen content**,** respectively.

As C_aO_2 is generally constant, $\dot{V}O_{2m}$ predominantly represents the ratio of $\dot{Q}_m$ to C_vO_2 (Rossiter, 2011). To obtain $\dot{Q}_m$, Doppler ultrasound and thermodilution methods are predominantly used (Grassi et al., 1996; Hughson, 2009). Direct measurements of C_aO_2 and C_vO_2 are obtained by taking arterial and venous blood samples *via* catheterisation, an invasive and difficult technique. Incorrect catheter insertions can result in only part of the venous blood being sampled, resulting in incomplete mixing and subsequent erroneous values. Such inconsistency and invasiveness of measurements has stimulated non-invasive estimation of $\dot{V}O_{2m}$ *via* breath-by-breath measurement of pulmonary oxygen uptake ($\dot{V}O_2$).

During exercise, utilisation of oxygen within the exercising muscles contributes to the majority of whole body pulmonary $\dot{V}O_2$ (Jones and Poole, 2005), however, contributions from other sources such as the respiratory muscles also exist. Respiratory muscle $\dot{V}O_2$ increases as minute ventilation ($\dot{V}E$) increases, contributing ~ 1.5 to 2 ml of $\dot{V}O_2 \cdot L \cdot min^{-1}$ at moderate intensity work rates (WRs) and increases to ~ 3 ml of $\dot{V}O_2 \cdot L \cdot min^{-1}$ at peak WRs and $\dot{V}Es$ (Scano et al., 2006). Pulmonary $\dot{V}O_2$ increases linearly with increasing WR and plateaus at the limit of tolerance (LoT), whilst respiratory muscle $\dot{V}O_2$ continues to increase as $\dot{V}E$ increases. Therefore, respiratory muscle $\dot{V}O_2$ makes up a greater proportion of total $\dot{V}O_2$ at peak WRs. In healthy humans, at peak $\dot{V}Es$ the respiratory muscle $\dot{V}O_2$ represents ~ 9 to 14% of the total pulmonary $\dot{V}O_2$ (Shephard, 1966; Aaron et al., 1992; Dominelli et al., 2015). Interestingly, respiratory muscle $\dot{V}O_2$ for a given $\dot{V}E$ is greater in women than men, demonstrated by Dominelli et al. (2015) who showed a greater absolute respiratory muscle $\dot{V}O_2$ at a $\dot{V}E$ of 55 $L \cdot min^{-1}$ within women, and a greater relative respiratory muscle $\dot{V}O_2$ as a percentage of total $\dot{V}O_2$ at peak $\dot{V}Es$ (13.8 *vs.* 9.4%). Respiratory muscle $\dot{V}O_2$ further increases with age, likely due to a natural reduction in elastic recoil of the lungs and increased stiffness of the chest wall (Miller, 2010), resulting in an ~ 15%

contribution of respiratory muscle $\dot{V}O_2$ to total $\dot{V}O_2$ at peak $\dot{V}Es$ (Johnson et al., 1991). In patients with chronic obstructive pulmonary disease (COPD) the cost of breathing is further increased. For example, at the same respiratory muscle power output that achieves ~ 120 to 160 $L \cdot min^{-1}$ $\dot{V}E$ in healthy individuals, those with severe COPD only achieve ~ 20-30 $L \cdot min^{-1}$ $\dot{V}E$ (Levison and Cherniack, 1968).

Although respiratory muscle $\dot{V}O_2$ within exercise is likely to alter the $\dot{V}O_2$ measured at the mouth, the contribution is small enough not to alter the $\dot{V}O_2$ gain and delta efficiency of pulmonary and leg muscle $\dot{V}O_2$ during cycle ergometry at WRs up to 90% of maximum (Poole et al., 1992). Therefore, although caution should be taken when inferring muscle $\dot{V}O_2$ from absolute pulmonary $\dot{V}O_2$, pulmonary $\dot{V}O_2$ remains an appropriate method of assessing muscle $\dot{V}O_2$ during exercise. Importantly, at exercise onset, and during progressive exercise such as ramp-incremental tests (RITs), a delay in the $\dot{V}O_2$ response presents due to the transit time of blood from exercising muscles to the lungs (Figure 1.2). This transit time is ~ 20s at low workloads, and reduces at higher workloads to ~10s, predominantly due to the increased cardiac output ($\dot{Q}$) (Rossiter, 2011). This delay has been termed the phase I or cardiodynamic phase (Weissman et al., 1982), and becomes important when setting WRs to achieve a desired $\dot{V}O_2$, based on ramp-incremental exercise. As can be seen in Figure 1.2, the transit delay presents as a delayed pulmonary $\dot{V}O_2$ response, which has been termed tau (τ'). Simply recording the immediate pulmonary $\dot{V}O_2$ at a set WR would result in an elevated pulmonary $\dot{V}O_2$ estimate, as blood from the exercising muscles would have not yet reached the pulmonary capillaries and altered the $\dot{V}O_2$. Following phase I, any changes in pulmonary $\dot{V}O_2$ accurately reflect the changes in $\dot{V}O_{2m}$ (Rossiter et al., 1999; Jones and Poole, 2005).

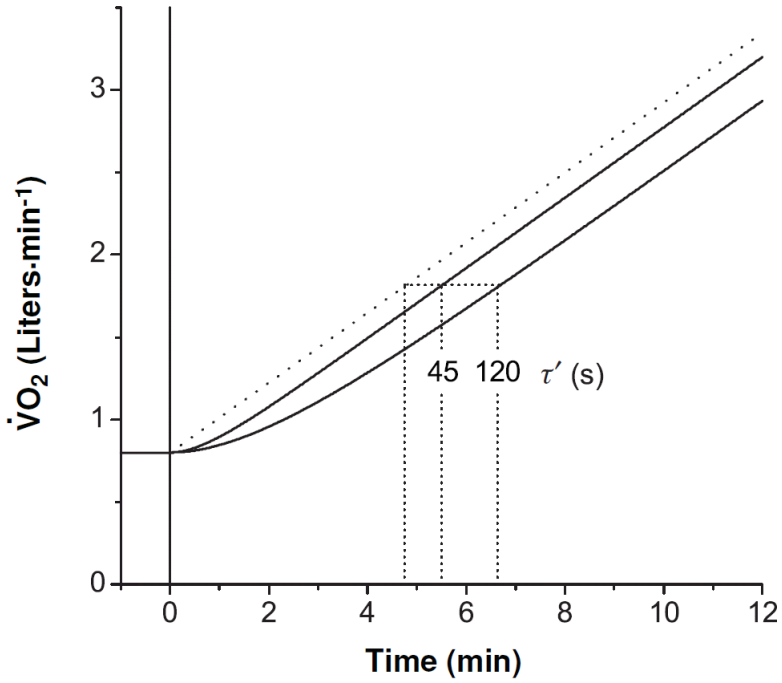

Figure 1.2. A schematic representation of the $\dot{V}O_2$ kinetic response to ramp-incremental exercise. Dotted line represents the expected muscle $\dot{V}O_2$ response (gain of 10 ml·min^{-1}·W^{-1}), and the solid lines represent two theoretical pulmonary $\dot{V}O_2$ responses with time constants (τ') of 45 and 120s. Image adapted from Rossiter (2011).

1.3 Exercise intensity

A variety of methods are used to prescribe the intensity of exercise during constant load exercise, with different intensities being related to different physiological adaptations (Pescatello, 2013) and acute responses (Whipp, 1996). A huge variation exists in the time taken, the subjective nature of the methods used, and range of outcomes measured; For example, intensity can be set *via* a subjective rating, an absolute intensity, a relative intensity or *via* metabolic thresholds. Subjective measures such as the Borg rating of perceived exertion (RPE) (Borg and Noble, 1974; Borg et al., 1987) or OMNI scales (Robertson et al., 2003; Mays et al., 2010) are used to set the intensity of exercise based on the participants feeling of difficulty. This subjectivity results in a wide range of exercise intensities when compared to relative $\dot{V}O_2$ and heart rate (HR) measurements. However, this method is less time-consuming, requires less equipment, and is more applicable for use within a clinical setting.

Setting exercise intensity based on physiological responses such as calorie expenditure, $\dot{V}O_2$, HR and the Metabolic Equivalent of Task (MET) may not take into account the individuals attributes such as age, sex, weight, height or fitness levels and, therefore, often results in misclassification of relative exercise intensity (Byrne et al., 2005; Garber et al., 2011). In order to prescribe a more personally relevant exercise intensity it is important to use an intensity relative to the individuals own maximal exercise capacity (Nelson et al., 2007). The most commonly utilised methods of setting relative intensities are $\dot{V}O_2$ reserve, HR reserve, % of peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$), % of peak HR (HR_{peak}), and % of peak MET (Metabolic Equivalent of Task; MET_{peak}) (Garber et al., 2011). Despite displaying greater variability than reserve values (Swain and Leutholtz, 1997; Lounana et al., 2007), % of $\dot{V}O_{2peak}$ remains the most commonly utilised method (Rossiter, 2011) likely due to the comparative ease of measurement. However, it can result in poor matching of intensities between individuals, for example, Rossiter (2011) demonstrated the differences in $\dot{V}O_2$ and HR responses between two individuals exercising at the same absolute power and relative $\dot{V}O_2$ (85% $\dot{V}O_{2peak}$; Figure 1.3). Despite matching relative work intensities, one of the subjects was working at a higher exercise intensity, culminating in them reaching their maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$), and therefore LoT, far earlier. This mismatching of intensities can be rectified by setting exercise intensity based on metabolic thresholds rather than relative intensities.

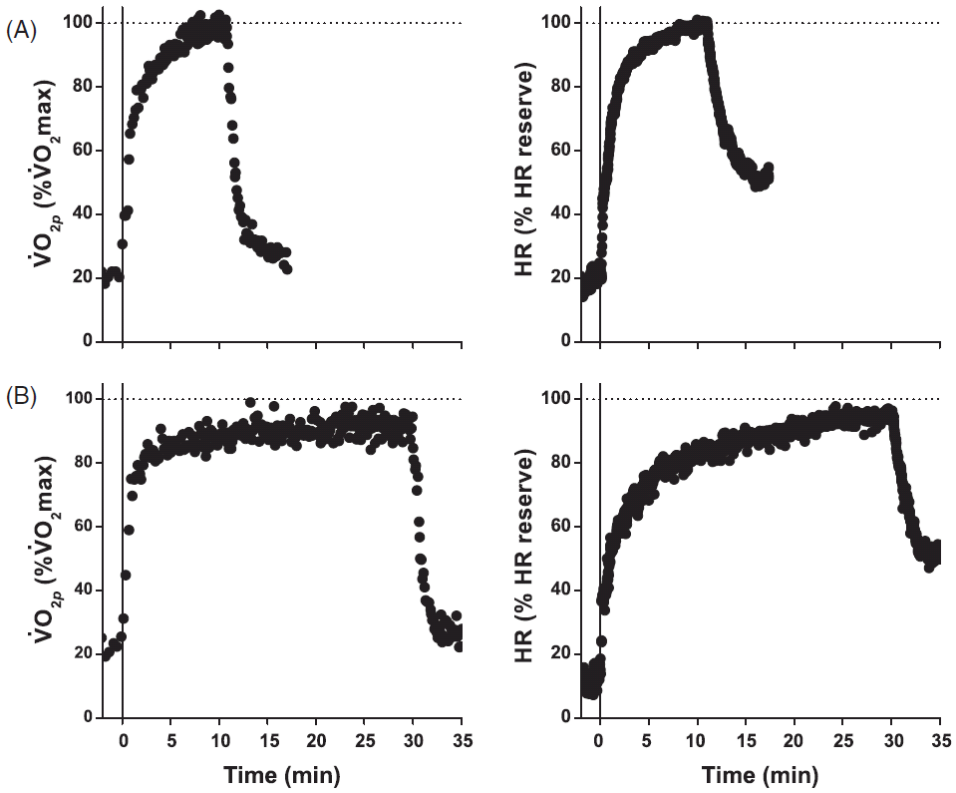

Figure 1.3. Variability of pulmonary oxygen uptake ($\dot{V}O_2$; % of max) and heart rate (HR; % of reserve) during constant-power exercise matched for absolute metabolic demand (85% of $\dot{V}O_{2max}$). Responses of two participants (A and B). Image adapted from (Rossiter, 2011).

Whipp (1996) demonstrated the existence of four exercise intensity domains which display differing pulmonary $\dot{V}O_2$, blood La and proton (H^+) production characteristics (Figure 1.4). Exercise can accurately be placed based on the identification of individual metabolic thresholds (lactate threshold (LT), critical power (CP) and $\dot{V}O_{2max}$. Moderate intensity exercise is characterised by no metabolic acidosis (below LT), i.e. no elevation in La or H^+ above resting values. The $\dot{V}O_2$ response consists of a rapid mono-exponential rise in $\dot{V}O_2$ (phase I and II pulmonary kinetics) (Barstow et al., 1990), that achieves a steady state within 2-3 minutes (phase III). Such activity is fuelled by oxidative phosphorylation (Section 1.2). Phase I (cardiodynamic phase) is largely due to the rapid increase in pulmonary blood flow, and its duration represents the transit delay from working muscle to lungs (Whipp and Casaburi, 1982). Phase II and III are direct reflections of oxygen utilisation at the muscle (Krustrup et al., 2009). The steady-state $\dot{V}O_2$ achieved in phase III increases linearly with increasing power (gain of ~10 $mL \cdot min^{-1} \cdot W^{-1}$ during cycle ergometry; Wasserman and Whipp, 1975;

Rossiter, 2011). Heavy intensity exercise is characterised by WRs between the LT and CP (asymptote of the power-duration curve; Moritani et al., 1981). Within this domain, La and H^+ levels are elevated, but stabilise along with $\dot{V}O_2$ within ~10-15 minutes (Rossiter, 2011), suggesting a balance of aerobic and anaerobic fuel production that eventually limits duration. The upper limit of this intensity domain is the highest WR which results in a steady state $\dot{V}O_2$ and La response (Jones and Poole, 2005). Above the LT, an increase in $\dot{V}O_2$ above the predicted mono-exponential response occurs, termed the $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$) (Poole et al., 1988). This increase in $\dot{V}O_2$ occurs after ~2 minutes into constant-power exercise and is thought to be due to a progressive inefficiency of the exercising muscle (Jones et al., 2011).

Very heavy intensity exercise is characterised by WRs above the CP that result in a progressively rising $\dot{V}O_2$, La, and H^+ that, unlike heavy intensity WRs, do not eventually stabilise resulting in attainment of $\dot{V}O_{2max}$ and cessation of exercise LoT (Poole et al., 1988; Jones and Poole, 2005). The contribution of the $\dot{V}O_{2sc}$ to the total $\dot{V}O_2$ response can be significant, exceeding ~25% in some cases (Jones et al., 2011). Finally, severe intensity exercise is characterised by WRs that result in the attainment of $\dot{V}O_{2max}$ (and cessation of exercise) during the mono-exponential phase II response (Whipp, 1996). Therefore, within this domain, there is not enough time for $\dot{V}O_{2sc}$ to become established.

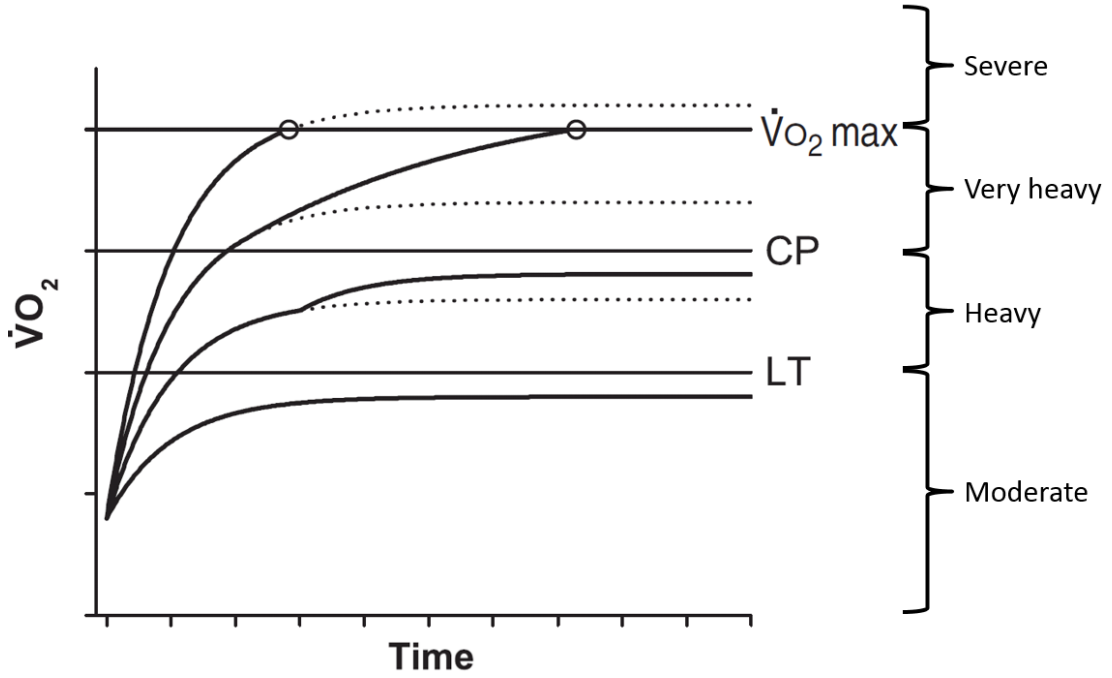

Figure 1.4. Schematic representation of the pulmonary oxygen uptake ($\dot{V}O_2$) response to four exercise intensity domains (moderate, heavy, very heavy and severe). Dotted line represents the predicted mono-exponential $\dot{V}O_2$ response and the solid line represent the actual $\dot{V}O_2$ response. Moderate intensity: exercise below the lactate threshold (LT) results in the attainment of steady state with no $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$). Heavy intensity: exercise between the LT and critical power (CP) present with a $\dot{V}O_{2sc}$ and achieves a delayed steady state. Very heavy: exercise performed above the CP presents with a $\dot{V}O_{2sc}$ and does not reach a steady state, therefore, attaining $\dot{V}O_{2max}$. Severe: Exercise performed above CP that results in the attainment of $\dot{V}O_{2max}$ before a $\dot{V}O_{2sc}$ can present. Image adapted from (Rossiter, 2011).

The presence of a slow component in WRs above moderate intensity exercise suggests care needs to be taken when setting exercise intensity, especially for clinical populations that may be at risk of medical events at higher WRs. WRs may initially match the desired $\dot{V}O_2$ or HR response, but elevate to higher than desired intensities due to physiological inequalities, and potentially result in early cessation of exercise. In order to avoid $\dot{V}O_{2sc}$, the target WR would need to reduce at the same rate as the $\dot{V}O_{2sc}$ increases, i.e. the target WR would be a 'mirror image' of the $\dot{V}O_{2sc}$ (Figure 1.5).

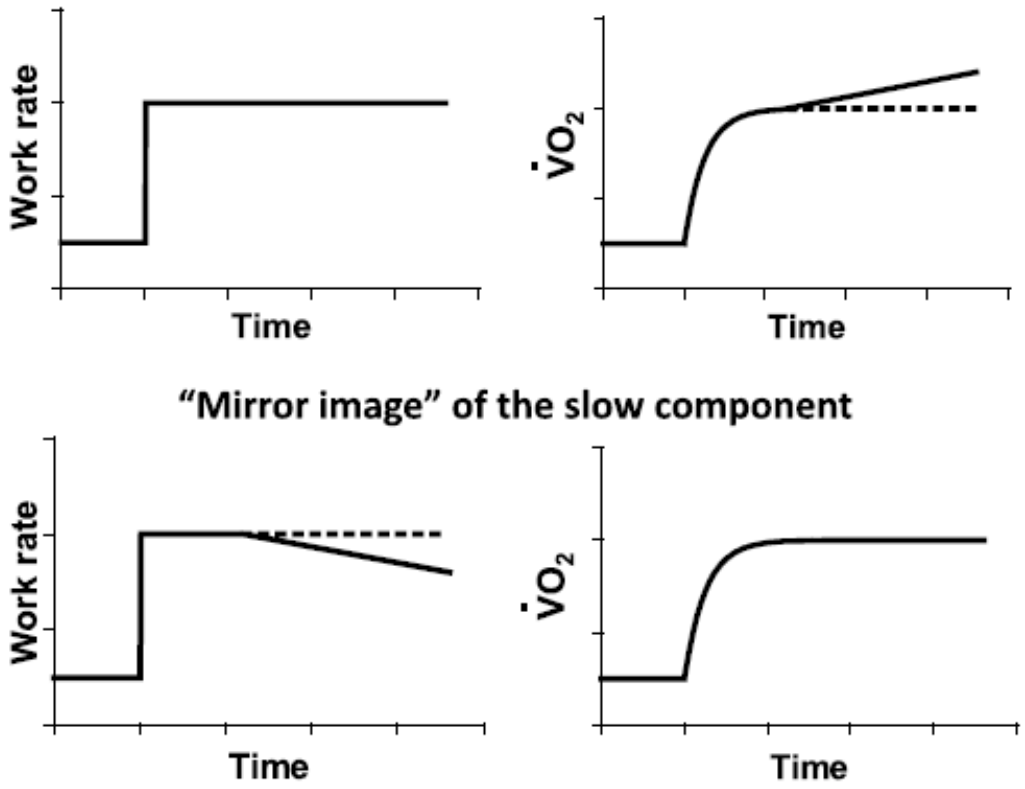

Figure 1.5. Schematic representation of the pulmonary oxygen uptake ($\dot{V}O_2$) response to constant, and declining WRs. In order to eliminate the $\dot{V}O_2$ slow component ($\dot{V}O_{2sc}$), and achieve a steady state response, the WR needs to reduce at an identical ('mirror image') rate to the $\dot{V}O_{2sc}$ (Jones et al., 2011).

1.4 Cardiovascular and respiratory control mechanisms

Before discussing the differences in cardiovascular and metabolic responses to concentric and eccentric exercise, we first need to understand the mechanisms by which these responses are initiated and altered during traditional exercise. The ventilation of alveoli and perfusion of working muscles is constantly adjusted to meet the metabolic requirements of the exercise. This control is orchestrated by the autonomic nervous system which increases sympathetic and reduces parasympathetic outflow in response to increased metabolic and mechanical requirements (Fisher, 2014). The sympathetic and parasympathetic outflow is altered by the integration of various peripheral and central feedback and feedforward mechanisms, namely central command, arterial baroreflexes, chemoreceptors and muscle afferent feedback (Pal et al., 2016).

Central command was first theorised in 1893 (Johansson, 1893) and refers to the coupling of descending input to skeletal muscles with a feedforward alteration in cardiovascular and ventilatory responses at the level of the brainstem. This neurogenic theory helps explain the rapid increase in $\dot{V}E$ and HR that occurs at exercise onset, not possible with slower humoral feedback mechanisms. Early work supporting this hypothesis showed a delayed increase in HR following electrical stimulation of peripheral muscles, proposed to be the result of an absence of central command (Krogh and Lindhard, 1917). Goodwin et al. (1972) provided further evidence *via* his experiments involving tendon vibration of agonist and antagonist muscles. They reduced the amount of central command by vibrating the exercising muscles tendon (less active force required to meet target) and increased the amount of central command by vibrating the tendon of the antagonist (more active force required to meet target). This resulted in an elevated $\dot{V}E$ and HR response during antagonist vibration. Animal models provided even more robust evidence by utilising electrical and pharmacological stimulation of brain regions responsible for descending central command. They stimulated associated regions in the thalamus, hypothalamus and basal ganglia which resulted in increases in $\dot{V}E$ and blood pressure (BP) that mirrored those of voluntary exercise (Orville et al., 1960; Eldridge et al., 1981; DiMarco et al., 1983). This has since been replicated in humans by the implantation of electrodes into the same areas, with stimulation similarly resulting in increases in BP and HR (Thornton et al., 2002). The presence of cardiovascular and ventilatory changes that mirror natural exercise within animals initially suggested that muscle afferent feedback and baroreceptor feedback may be of less importance than central command, however, lesions of the hypothalamus in cats, leading to disruption of descending central command also results in normal cardiovascular and ventilatory responses to exercise (Waldrop et al., 1986). This suggests it is more likely an interplay between these systems that occurs at the level of the brainstem to modulate autonomic nervous system output.

Muscle afferent feedback is also thought to play an important role in the control of cardiovascular and respiratory responses to exercise. Early work demonstrated the influence of muscle afferents on cardiovascular responses by occluding blood flow to the exercising muscle using a supra-systolic cuff (Alam

and Smirk, 1937). This resulted in an elevated BP response post exercise suggesting that the accumulation of exercise metabolites must be triggering feedback to the central cardiovascular control centres. This reflex response has been termed the “exercise pressor reflex” and has been replicated on many occasions (Freund et al., 1979; Fernandes et al., 1990; Iellamo et al., 1999). Further support was presented by Lind et al. (1968) who demonstrated in a patient with unilateral syringomyelia (a fluid-filled cyst or syrinx that compresses and damages one side of the spinal cord), that only contraction of muscles on the unaffected side triggered an exercise pressor reflex, and by Winchester et al. (2000) who showed that electrical stimulation of muscle within Brown-Séquard syndrome patients exhibiting inhibition of sensory afferents resulted in no exercise pressor response.

Type III (thin myelinated) and IV (unmyelinated) muscle afferents are responsible for this feedback response to mechanical and metabolic stimuli, altering the brain stems cardiovascular centre autonomic outflow (Amann et al., 2015). These afferent nerve endings mostly reside within the connective tissue and vascular networks of skeletal muscle respectively (Hayward et al., 1991), with type IIIs firing rate increasing predominantly in response to mechanical stretch (mechanoreflex), and type IVs firing rate increasing in response to a build-up of exercise metabolites (metaboreflex). Increased firing of type III afferents occurs during the application of external force, constriction of the muscle, and stretch of tendons (Kaufman et al., 1983; Mense and Stahnke, 1983), whilst when local blood flow is disrupted by cuff occlusion, exercise metabolites accumulate and the firing rate of type IV afferents increases (Kaufman et al., 1984). It has since been shown that it is likely the accumulation of lactate, ATP, bradykinin and prostaglandins that results in this increased type IV firing rate (Kaufman et al., 1983; Mense and Meyer, 1988; Rotto and Kaufman, 1988). Although type III muscle afferents respond primarily to mechanical stimuli, some cross over exists evidenced by type III afferents displaying a secondary increase in firing rate suggestive of a response to metabolic stimuli, and an increase in firing rate following injection of ATP, lactic acid and bradykinin (Kaufman et al., 1983; Mense and Meyer, 1988). As well as responding to chemical and mechanical stimuli, Hertel et al. (1976) has also demonstrated the increased firing rate of

type III and IV muscle afferents in response to increases in temperature (27-43°C) within the medial gastrocnemius of the cat.

Type III and IV muscle afferents feedback to the central nervous system through afferent fibres synapsing predominantly on lamina I neurones within the spinal cord (Li and Baccei, 2017) and ascending within either the dorsolateral funiculi or dorsolateral sulcus respectively (Chung et al., 1979; Kozelka et al., 1981). Afferent fibres then terminate within the nucleus tractus solitarii (NTS) of the medulla oblongata (Kalia et al., 1981; Iwamoto and Kaufman, 1987; Paton, 1999; Degtyarenko and Kaufman, 2006). Here, mechanoreflex activation of predominantly type III afferents is thought to inhibit vagal neurons located in the dorsal vagal nucleus and nucleus ambiguous, therefore reducing cardiac parasympathetic activity, with a reflex increase in HR. (McMahon and McWilliam, 1992; Al-Ani et al., 1997; Gladwell et al., 2005). Research investigating the muscle metaboreflex activation has demonstrated, with the use of a parasympathetic blockade, that the increase in HR and myocardial contractility with increased metabolic stimuli are likely the result of an increase in sympathetic activity *via* activation of neurones located in the rostral ventrolateral medulla (Fisher et al., 2013), however, a reduction in parasympathetic activity may also contribute (Fisher, 2014).

Recently, it has been highlighted that muscle afferent feedback may play an important role in the cardiovascular dysfunction and dyspnoea that characterise chronic heart failure (CHF) and COPD respectively. Extensive research details an imbalance within the parasympathetic and sympathetic systems within CHF patients, with a reduction in vagal tone and an increase in sympathetic activity (Li et al., 2004). Within CHF patients it has been demonstrated that there may be an exaggerated muscle afferent feedback response that results in an increased sympathetic activation, and reduction in muscle blood flow (Notarius et al., 2001; Piepoli and Coats, 2007). This would act to reduce the O_2 delivery to working muscle, shifting metabolism away from oxidative pathways and contributing to exercise intolerance through an increased reliance on anaerobic energy provision. The significance of this exaggerated afferent response was

recently demonstrated in nine NYHA class-II CHF patients using lumbar intrathecal fentanyl injection, a selective μ-opioid receptor agonist, to inhibit feedback from type III and IV muscle afferents during exercise (Amann et al., 2014). As within healthy subjects' (Amann et al., 2010), inhibition of afferent feedback resulted in a reduced HR, stroke volume (SV), $\dot{Q}$, and mean arterial pressure (MAP) compared to control conditions, however, despite a 4% reduction in MAP, there was an ~12% increase in leg blood flow , contrasting no change in blood flow evident within healthy controls. This suggests that muscle afferent feedback within CHF patients may be exaggerated and be stimulating an increase in sympathetic excitation. This exaggerated response may lie within the processing of afferent feedback within the NTS of the medulla, with nitric oxide shown to be an important regulator of autonomic outflow from the NTS within animal models (Li and Potts, 2001). Blocking nitric oxide within normotensive Wistar–Kyoto rats resulted in significant hypertensive responses matching those of spontaneously hypertensive rats without nitric oxide blockade (Leal et al., 2012), and blocking nitric oxide within these hypertensive rats reduced these hypertensive responses (Leal et al., 2013).

COPD patients similarly display skeletal muscle dysfunction as well as respiratory impairments, together culminating in dyspnoea and a reduced exercise tolerance (Puente-Maestu et al., 2013). It is again hypothesised that COPD patients may exhibit exaggerated muscle afferent feedback that increases $\dot{V}E$ (Puente-Maestu et al., 2013), and intrathecal administration of fentanyl has been shown to effectively reduce afferent feedback, increases in $\dot{V}E$, and subsequent exercise dyspnoea within this patient group (Gagnon et al., 2012). Recent research has focused on the potential role of the metaboreflex in influencing respiratory drive in COPD patients during exercise. The use of blood flow occlusion post-exercise can be used to trap exercise metabolites produced within the working muscles allowing the assessment of the metaboreflex without influence of central drive or mechanoreflex activation (Kaufman et al., 1984). Post exercise cuff occlusion within healthy humans does not prevent the return of $\dot{V}E$ to baseline levels (Rowell et al., 1976; Innes et al., 1989), suggesting that the metaboreflex does not influence respiratory drive during exercise. However, Bruce et al. (2016) demonstrated a metaboreflex mediated increase in $\dot{V}E$ within

COPD patients. They assessed the ventilatory responses of 18 COPD patients and nine age and gender-matched controls during cuff occlusion post exercise. As expected, $\dot{V}E$ returned to baseline levels within the healthy controls, but remained elevated within the COPD patients. Therefore, it is likely that the elevated $\dot{V}E$ seen within COPD patients may be the result of a metaboreflex mediated increase in sympathetic drive.

Finally arterial baroreceptors as well as central and peripheral chemoreceptors play an important role in the maintenance of BP, partial pressure of oxygen (PaO_2), carbon dioxide ($PaCO_2$) and pH (Paton, 1999; McArdle et al., 2010). Arterial baroreceptors are mechanically sensitive unencapsulated free nerve endings situated in the medial-adventitial border of the aortic arch and carotid sinuses (Sheehan et al., 1941). Firing rate increases in response to an increased arterial pressure, and signals are passed to the NTS of the dorsal medulla *via* the glossopharyngeal or vagus nerves (Aicher and Randich, 1990; Kougias et al., 2010). When BP and subsequent stretch of the arterial walls is increased, firing frequency of the baroreceptors increases and results in an elevated parasympathetic outflow and a reduced sympathetic outflow from the NTS. Conversely, a reduction in BP results in a reduced firing rate, a reduced parasympathetic outflow and increased sympathetic outflow (Fadel and Raven, 2012). The balance between parasympathetic and sympathetic activity controls HR, myocardial contractility and blood distribution, with an increased BP seen with exercise resulting in increases in HR and myocardial contractility as well as a redistribution of blood flow to active skeletal muscle *via* vasoconstriction of renal and splanchnic vessels (Mitchell, 1990). Peripheral chemoreceptors reside within the aortic arch (aortic body) and carotid artery (carotid body) and respond primarily to hypoxaemia, hypercapnia and pH (Guo et al., 2002; Jänig and Häbler, 2003; Guyenet, 2006). Peripherally, the carotid body chemoreceptors are thought to provide the majority of feedback, with the glomus cells depolarising in response to hypoxaemia, stimulating an increased discharge of the carotid sinus nerve travelling to the caudal aspect of the NTS (Prabhakar, 1994; Guyenet, 2000). This results in an increased sympathetic outflow, causing vasoconstriction of muscle, splanchnic and renal vascular beds, and an increase in HR and SV, all culminating in an increased $\dot{Q}$, systemic vascular resistance

and BP (Marshall, 1994). Central chemoreceptors are located within the central nervous system at multiple sites in contact with extracellular fluid (Nattie and Li, 2002; Feldman et al., 2003). They are considered distinctly separate to peripheral chemoreceptors as H^+ cannot directly diffuse across the blood-brain barrier, instead increasing in concentration indirectly *via* carbon dioxide (CO_2) diffusing across the blood-brain barrier and producing H^+ *via* reaction with water (H_2O) (Nattie and Li, 2012). As H^+ concentration within the extracellular fluid increases, the firing rate of the central chemoreceptors increases, resulting in a subsequent increase in $\dot{V}E$ which helps to maintain a normal $PaCO_2$ (Nattie and Li, 2012).

The mechanisms controlling $\dot{V}E$ during exercise remain largely unexplained, with a combination of central command, muscle afferent feedback and chemoreception likely at play with a potential for redundancy within these systems (Dempsey, 2006). The initial rapid increase in $\dot{V}E$ at the onset of constant WR exercise is likely neural in origin, as humoral changes are too slow to influence $\dot{V}E$ during this phase (Ward, 2007). Following this initial increase, in the absence of an acidosis, alveolar ventilation closely matches $\dot{V}CO_2$ to maintain a constant $PaCO_2$, and maintain acid-base balance. It was previously hypothesised that peripheral chemoreceptors likely play a role in altering $\dot{V}E$ in response to changes in $PaCO_2$ (Ward, 2007). However, in the absence of any significant change in the arterial blood gases, the mechanism for how humoral mechanisms modulate this response is unclear (Wasserman et al., 1974; Ward, 2007). It is likely that there is an interplay of various mechanisms at play and further research is required to elucidate the exact mechanisms and their relative contributions. Despite this, the majority of evidence points towards changes in $\dot{V}CO_2$ mediating $\dot{V}E$. For example, small transient changes in $PaCO_2$ are consistently demonstrated during step increments in WR and are followed by a proportional increase in $\dot{V}E$ (Whipp et al., 1978), and voluntary hyperventilation, which reduces $PaCO_2$ and $\dot{V}CO_2$, results in a reduced ventilatory response (Ward, 2007).

During exercise in which there is a sustained metabolic acidosis, $\dot{V}E$ increases disproportionately to $\dot{V}CO_2$ and results in a reduction in $PaCO_2$ (Beaver et al., 1986). It is thought that this increase in $\dot{V}E$ occurs *via* stimulation of peripheral carotid body chemoreceptors in response to this metabolic acidosis (Dempsey, 2006). However, it is noteworthy that there is some evidence of hyperventilation in McArdle's disease patient in whom there is no metabolic acidosis, and in whom pH can actually decrease. McArdle's is characterised by a myophosphorylase deficiency which results in the inability to break down glycogen, reducing the accumulation of lactate from glycogenolysis and reducing the resulting acidosis (Ørngreen et al., 2015). Hagberg et al. (1990) assessed the ventilatory responses to incremental cycle exercise within five Mcardle's patients and six participants matched for peak $\dot{V}O_2$ responses. They demonstrated that respiratory compensation occurred at a similar % of $\dot{V}0_2$ max (71 ±7% and 70 ±10% respectively) despite a 20% reduction in blood lactate and H+ levels in the McArdle's patients relative to baseline. This suggests that ventilatory drive in McArdle's patients, and potentially healthy humans, may not be driven by a metabolic acidosis. Similarly, Péronnet et al. (2007) demonstrated the presence of an elevated $\dot{V}E$ with respect to $\dot{V}CO_2$ during high-intensity exercise in healthy humans despite maintaining arterial pH at resting levels *via* bicarbonate infusion. They did, however, show a reduced absolute $\dot{V}E$ (15-30% reduction) suggesting that metabolic acidosis may still partly influence ventilatory changes. These results suggest that there are likely a variety of mechanisms at play, with potential redundancy mechanisms able to compensate and still influence $\dot{V}E$ during exercise.

1.5 Eccentric modalities

Early eccentric exercise research utilised activities such as stair descent and decline treadmill walking to apply eccentric contractions (Chauveau, 1896). These provided initial insight into the physiological characteristics of eccentric contractions but had several limitations, inherently lacking the ability to finely control exercise parameters such as WR, stride length, joint angles, and cadence. Considerable development occurred in the 1950's and led to the first

eccentric cycle ergometer being utilised by Abbott et al. (1952) (Figure 1.6), consisting of two fixed gear bikes placed back to back, connected by a single chain. One rider pedalled forwards (concentric), whilst the other rider resisted the reverse movement of the other bikes pedals (eccentric). Cadence was fixed *via* the use of a metronome, and a strain gauge was used to match the force produced. This provided a valid method of assessing concentric and eccentric exercise with the ability to control WR, cadence, and joint positions; however, it required two riders and tight control of cadence and strain throughout. Asmussen (1953) attempted to eliminate the need for a second rider by placing a fixed gear bike on a decline treadmill, with an extra cog reversing the pedal movements. However, this modality required significant familiarisation and skill in order to use and close supervision throughout. Shortly after this, the first motorised eccentric cycle ergometer was produced by Abbott and Bigland (1953), consisting of a 2.5hp motor that drove bike cranks in reverse, eliminating the need for a second rider. Since then there has been further progression of designs, allowing tighter control of WRs and cadence *via* software control of motor parameters and utilisation of magnetically braked ergometers (Petersen, 1969; Bigland-Ritchie et al., 1973). Currently, all reverse cycle ergometers utilise a similar design, allowing tight control of WR, cadence and joint angles.

Many of the ergometers possess the capacity to switch the direction in which the pedals are driven, requiring the user to resist the forward pedal movement. This eccentric exercise preferentially targets the hamstrings and hip flexors over the quadriceps and gluteal muscles. Eccentric reverse cycle ergometry, however, is the most commonly used modality, likely because it targets anti-gravity locomotor muscle groups, more beneficial to rehabilitation, but also due to the fact that studies report more discomfort during forwards eccentric cycle ergometry (Hoppeler, 2015).

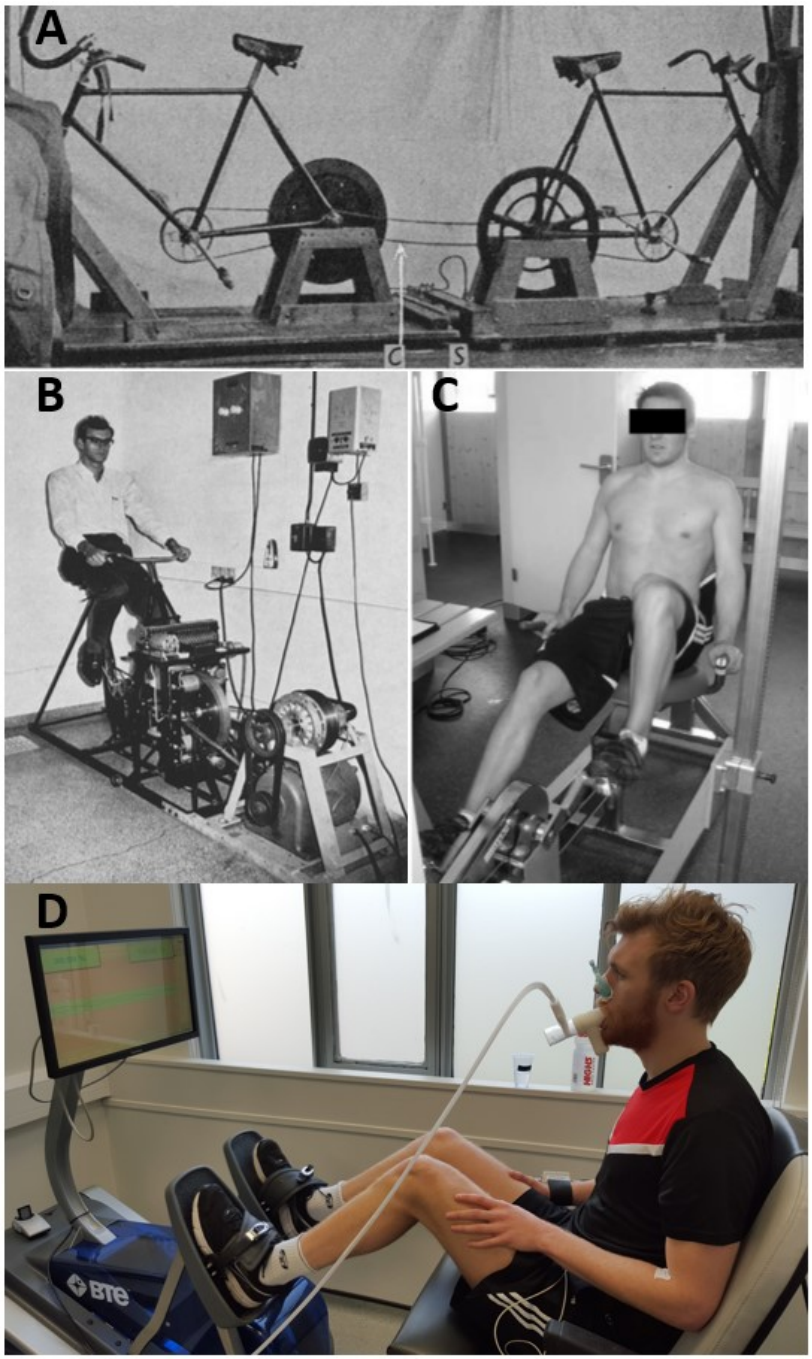

Figure 1.6. Progression of eccentric modalities within research. A) The first reverse cycle ergometer involved two bikes placed back to back connected by a chain so that one participant would resist the forwards pedalling of the other participant (Abbott et al., 1952). B and C) reclined seated ergometers, with a motor driven bike crank (Petersen, 1969; Hoppeler, 2016). D) Commercially available seated eccentric stepper (Eccentron; BTE, Hanover, MD, USA).

The ability to tightly control exercise parameters made eccentric cycling a robust research tool to investigate the physiological responses to eccentric exercise. However, this modality does have its limitations. Firstly, and potentially most importantly, it can be suggested that it does not closely represent natural activity

and movement, with the reverse cyclic motion an unnatural movement not performed in everyday tasks. Training matched to the needs of the activity to be performed consistently demonstrates greater improvements in performance than non-specific training (Millet et al., 2002; Misic et al., 2009; Garber et al., 2011), and training matched to similar joint angles and cadences as an athletic task provides greater increases in voluntary strength and performance outcomes (Morrissey et al., 1995; Stone et al., 2000). For example, isolated running, swimming and cycling training have been shown to only improve performance outcomes of the same specific exercise (Millet et al., 2002; Gamble, 2006). Therefore, training adaptations that occur with reverse cycle ergometry may not transfer as effectively to everyday mobility than a modality that more closely mirrors natural activities of daily living. Secondly, many studies utilising reverse cycle ergometry compare concentric and eccentric exercise performed on different cycle ergometers, with and without a motor driven crank that likely increases variability of results reported due to potential differences in joint angles, cadences, and calibrations. Thirdly, there are inherent safety issues present when performing reverse cycle ergometry (Hoppeler, 2015). The most important being the risk of applying high loads to a hyperextended knee, posing the risk of severe damage to the bone, meniscus, and ligaments associated. Due to this, it is essential that a well-trained operator is present to supervise users. In some instances, safety features have been implemented to reduce the risk of such events occurring, including an emergency stop button, an upper load limit and a dead man's button that needs to be pressed during the session for the motor to work. These, however, are not always sufficient meaning it is unlikely that these ergometers will be used unsupervised in a gym or group rehabilitation setting. Another safety concern is the risk of damage to the bike and injury to the user when excessive forces are applied. The mechanical nature of these cycle ergometer systems means they usually only handle loads of up to 500W (Hoppeler, 2015), restricting their use to exercise intolerant populations that will exercise safely below this upper limit.

Based on the current limitations with reverse cycle ergometry, an eccentric exercise modality that allows for accurate comparisons of concentric and

eccentric exercise, maintains fine control of exercise parameters, is safer to perform, and more closely mirrors natural activities was needed.

An eccentric recumbent stepping ergometer has been developed that may meet these demands (Figure 1.6D). It was first described by Marcus et al. (2008) and is currently manufactured by BTE (Eccentron; BTE, Hanover, MD, USA). This modality attempts to mirror walking downhill or downstairs in a seated position, targeting the major anti-gravity locomotor muscles including the quadriceps (vastus lateralis, intermedius, medialis and rectus femoris) and gluteal muscles (gluteus maximus, medius, and minimus). A motor drives two foot plates backwards and forwards whilst the user resists the foot plate coming towards them. This ergometer is safer than previous eccentric cycle ergometers, with greater preventative measures in place to prevent knee hyperextension (see section 2.3.1), opening the possibility of application to a wider clinical patient population. Despite a lack of kinematic analysis comparing this device to natural activities like walking downhill or downstairs, It is likely that this modality more closely matches activity of everyday life (i.e. is more ecologically valid) than previous eccentric modalities. For example, the manufacturer suggested range of movement (90° to 30° of knee flexion) is similar to that of stair ascent and descent (~100° to 20°; McFadyen and Winter (1988)), and personal communication with a number of individuals that have experienced both reverse cycle ergometry and eccentric recumbent stepping highlights the more natural feel and ease of use that eccentric stepping provides. It is important to highlight that this ergometer, although similar to stair descent, is not identical. Most importantly, the individual is positioned in a recumbent instead of standing position resulting in greater hip flexion and likely different activation patterns of muscles crossing the hip joint (namely rectus femoris and hamstrings). Secondly, like other eccentric modalities (Hoppeler, 2015), you cannot escape the fact that conscious effort is required to achieve a desired force or negative work, therefore, reducing the natural feel of the activity. Regardless, this modality likely provides an activity that more closely matches natural everyday movement than other available eccentric ergometers. Future work comparing kinematics and EMG muscle activity and timing analysis of this modality to natural forms of

activity and other eccentric modalities may provide greater insight (Ivanenko et al., 2004; Macleod et al., 2014).

As supplied, the ergometer cadence, stride length, seat position and target force can all be set prior to each session; however, stride length and target force cannot be altered during testing. Consequently, different protocols such as ramp-incremental testing or interval exercise cannot be set. Also, only force applied as the pedals move towards the user is recorded (eccentric phase), meaning concentric exercise cannot be performed. For this reason, research using this ergometer has only performed eccentric recumbent stepping on this device, lacking comparisons to concentric recumbent stepping (Gerber et al., 2007a; Marcus et al., 2008; LaStayo et al., 2009; Elmer et al., 2017). Instead, these studies either use eccentric recumbent stepping as the sole intervention, or make comparisons to concentric resistance training on separate machines, lacking WR control and comparable relative loads and repetitions (Marcus et al., 2008; Hansen et al., 2009; Marcus et al., 2009; Lastayo et al., 2010; LaStayo et al., 2011; Marcus et al., 2011). At present, only the ergometer software is limiting the capability to record concentric contractions, hence, with modifications it has the potential to become a viable ergometer to compare physiological responses to concentric and eccentric recumbent stepping exercise on the same device. By obtaining the raw signal from the load cells, we reasoned it would be possible to calculate the force applied to the pedals as they move away from the user (concentric contractions) and set different protocols to further compare the physiological responses. With these modifications, this ergometer would possess the ability to maintain tight control of exercise parameters (joint angles, cadence, stride length, force, and relative power), as well as providing a modality that is safe and may more closely replicate natural activities.

1.6 Acute responses to eccentric *vs.* traditional exercise

1.6.1 Increased force

An optimal sarcomere length (~2.2 μm) exists in which maximal cross-bridge formation can occur, with force development reducing at shorter and longer lengths (Figure 1.7a). At short sarcomere lengths, an overlap exists between actin and myosin filaments, resulting in fewer available myosin heads and actin binding sites. At longer sarcomere lengths, actin filaments are pulled away from myosin filaments, again resulting in fewer available cross-bridges. As the sarcomere continues to lengthen, the contribution to force from passive cytoskeletal structures increases. When considering the force produced by a whole muscle-tendon unit, the shape of the length-tension curve changes significantly (Figure 1.7b), and results in a wider optimum length than for single fibres. The curve is smoother due to the contribution of elastic non-contractile tissues, sarcomeres with varying length-tensions, and individual muscle characteristics and architectures (Brughelli and Cronin, 2007). At short muscle lengths, all force produced is the result of cross-bridge formation, whereas at long lengths the majority of force production is the result of passive structure contribution. Muscles have been shown to adapt the number of sarcomeres in series when subjected to prolonged stretch or shortening (Goldspink et al., 2002), potentially to optimise the individual sarcomere architecture to operate at optimal lengths.

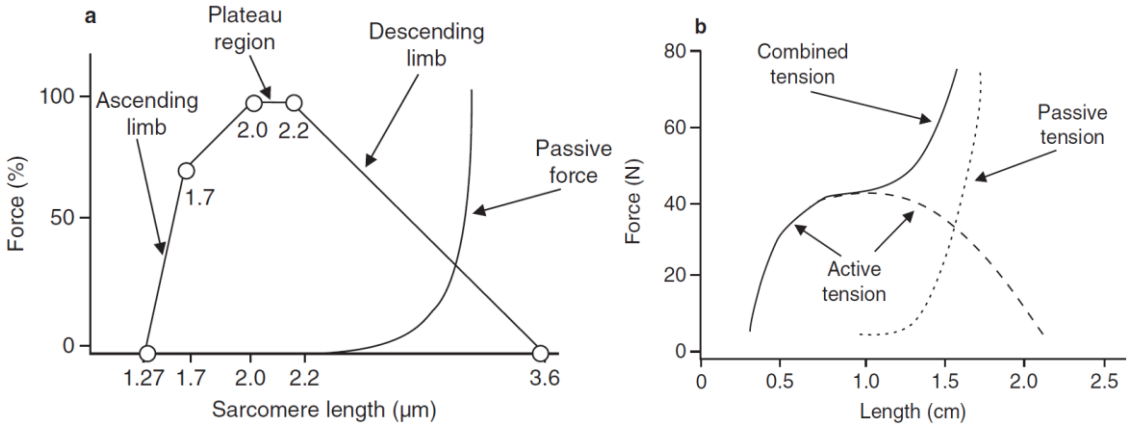

Figure 1.7.Schematic Length-tension curves for an individual sarcomere (a) and whole muscle (b). Image adapted from Brughelli and Cronin (2007).

The generation of force at increasing angular velocities differs greatly between concentric, eccentric and isometric contractions. Hill (1938) showed, using single skeletal muscle fibres from frogs, that as the velocity of a shortening contraction increases the ability to generate force decreases, and this has since been confirmed within single human muscle fibres (Brooks and Faulkner, 1994b), and replicated at the whole joint level (Figure 1.8) (Westing et al., 1990). Huxley (1957) provided a contraction model that, still to this date, provides a simple and effective explanation for these findings (Williams, 2011). His model details how the actin-myosin cross-bridges can either generate a positive or negative force depending on their distortion relative to equilibrium (perpendicular attachment where force = 0). He proposed that, as velocity increases, a greater proportion of myosin heads slide past actin binding sites without formation of a cross-bridge, and that more cross-bridges are distorted further away from equilibrium producing greater negative forces. Therefore, force output reduces with increased velocity and eventually results in positive and negative force contributions being equal and net force output being 0 at maximum velocity.

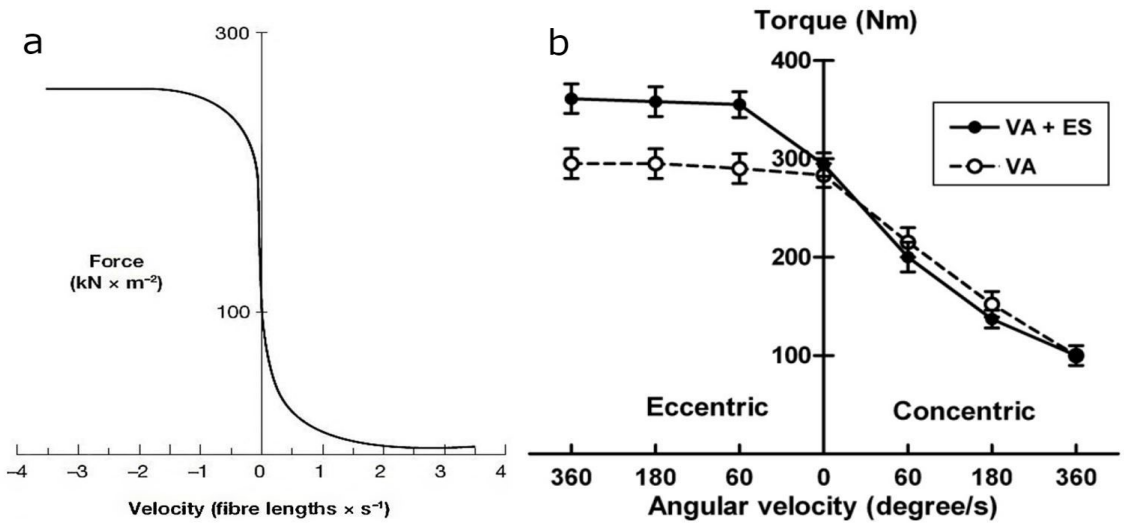

Figure 1.8. Concentric and eccentric force-velocity and torque-velocity relationships. a) Force-velocity relationship during positive (concentric) and negative (eccentric) velocity during stimulated contractions of single muscle fibres (Brooks and Faulkner, 1994a; Hoppeler, 2015), and b); the torque-angular velocity relationship during voluntary concentric and eccentric contractions of the knee extensors, with (VA + ES) and without (VA) additional electrical stimulation (Westing et al., 1990; Hoppeler, 2015). Note that the lack of a sharp rise in torque seen here during voluntary eccentric contractions is reported to be a characteristic of the knee extensor muscles investigated, with other muscle groups exhibiting roughly a 20-30% rise in eccentric torque before levelling off (Hoppeler, 2015).

Eccentric contractions do not elicit decreasing force at increasing contraction velocities (Duchateau and Baudry, 2014; Herzog et al., 2016) (Figure 1.8). In fact, with increasing velocity there is an initial sharp rise in the force generated, with a plateau present at greater velocities (Abbott and Aubert, 1952), suggesting the factors limiting force production within concentric contractions may not fully apply. At the level of the individual sarcomere, Joyce et al. (1969) demonstrated, at an identical velocity (40 $mm \cdot sec^{-1}$) of contraction, that eccentric contraction force was 6.7 times greater than concentric force. It is important to highlight that the pattern and magnitude of force changes when measured *in situ* vary considerably from those measured on isolated skeletal muscle (Figure 1.8). Firstly, there is a similar pattern of decline in torque with increased shortening velocity, however, torque remains elevated at high velocities *in situ* due to the inertial resistance produced by the attached distal limb (Hoppeler, 2015). Secondly, although there is a sharp rise in torque production and a maintenance of this torque at increasing negative velocities, the magnitude of torque discrepancy is less than that observed in single muscle fibres. For example, during concentric and eccentric voluntary contractions of the hamstrings and quadriceps at varying velocities, eccentric torque was ~32% higher at $60°.s^{-1}$ and ~153% higher at $360°.s^{-1}$ (Westing and Seger, 1989). Why the torque discrepancy seen *in situ* is less than that seen at the fibre level remains largely unclear but is likely the result of protective supraspinal and spinal neural mechanisms restricting maximal recruitment of motor units and limiting subsequent damage (Beltman et al., 2004a). This submaximal recruitment and activation of muscle fibres can be evidenced by the application of electrical stimulation on top of a voluntary muscle contraction, which increases torque generation only during eccentric contractions (Figure 1.8b) (Westing et al., 1990; Seger and Thorstensson, 2000). Figure 1.8).

The increased eccentric force at increasing velocities cannot solely be explained by the cross-bridge theory alone and has therefore led to extensive research to elucidate the underlying mechanisms. Five main theories currently exist: changes in neural activation patterns, passive tissue contribution, mechanical breaking of cross-bridges, inclusion of the partner myosin head, and (more recently) addition of the protein titin to passive force contribution. During

concentric contractions, activation of golgi tendon organs results in co-activation of an antagonist muscle, reducing maximal force output of the prime mover. It is thought that during eccentric contractions, this neural inhibitory mechanism may not be as active and may, therefore, allow greater force to be produced by the prime mover (Quiterio et al., 2011). Secondly, it has been suggested that the increased eccentric force may partly be a consequence of increased tension development within passive structures such as the sarcolemma and tendons during lengthening (Ishikawa et al., 2005). However, as increases in eccentric force are present at the sarcomeric level (Leonard et al., 2010), changes in neural activation strategies, or the addition of tension *via* associated passive structures, cannot solely be responsible. Hence, the mechanical breaking of cross-bridges, utilisation of the partner myosin head, and active tension produced *via* titin provide more plausible explanations.

Whilst operating on the descending limb of the length-tension curve, it is hypothesised that the myosin head may be forcibly pulled apart from the actin filament, rather than relying on the binding of ATP for dissociation. (Huxley, 1957; Stauber, 1988). Additional tension may be developed during the breaking of these cross-bridges, increasing the sarcomeric force generated. The myosin head may also be left in its active state, allowing faster re-attachment to actin and further force production. Evidence supporting this theory is lacking, with more research needed to fully elucidate the true tension contribution. Another possible sarcomeric source of additional tension is *via* utilisation of the partner myosin head during eccentric contractions. Every myosin binding site has two motor domains, with only one being attached to actin during concentric and isometric contractions. Using X-ray interferometry, Brunello et al. (2007) and Fusi et al. (2010) have shown that a stretch of just 5nm within a single sarcomere can double the number of attached myosin motors. They suggest that active stretch of the sarcomere may result in distortion of the myosin molecule, allowing the second myosin motor to attach to actin in the presence of calcium (Ca^{2+}). With force being directly proportional to the number of attached cross-bridges, this theory would help to explain the increased eccentric force observed, although it does not explain a reduced ATP requirement.

The final theory for increase force production during eccentric contractions comes from the activation of the protein titin in the presence of Ca^{2+} (Hessel et al., 2017). Titin was not discovered until 1976 (Maruyama et al. (1977) so was previously absent from the sliding filament hypothesis of Hanson and Huxley (1953). Evidence using single frog muscle fibres has shown that only ~34% of the total additional tension (excluding active myofibril contribution) developed during eccentric contractions can be accounted for by passive elastic components within the sarcomere, suggesting a further active contribution that does not directly involve actin and myosin. Leonard et al. (2010) demonstrated that myofibrils stretched past the point of actin and myosin overlap showed greater increases in force during active compared to passive lengthening, and Hessel and Nishikawa (2017) showed that both the rate of force development and total active force was significantly lower in titin deficient mice in comparison to the wild-type controls. A variety of theories have attempted to explain the mechanisms by which titin produces this additional tension (Hessel et al., 2017), with the most plausible being the winding filament hypothesis (Nishikawa et al., 2012). Testing the winding filament hypothesis is extremely challenging, however, research utilising electron tomography, holography, and transgenic mice is currently underway (Nishikawa, 2016). The above observations suggest there should be a difference in efficiency of force production between concentric and eccentric modalities that ought to be reflected in metabolic demand.

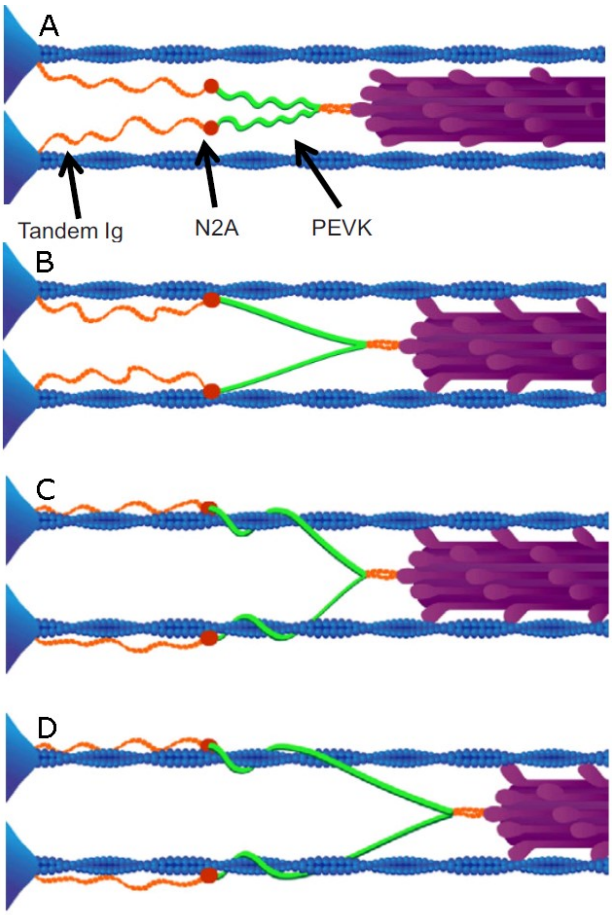

Figure 1.9. Schematic diagram of the winding filament hypothesis. A) Passive sarcomere, with titin folded and bound to the adjacent z discs and associated myosin filament. B) Upon activation, Ca^{2+} influx results in the N2A region of titin binding to the actin filament (blue). C) Cross-bridge (purple) cycling winds the actin filament and PEVK segment of titin. D) Active elongation results in extension of solely the PEVK segment of titin, generating additional tension. Image adapted from (Nishikawa, 2016).

1.6.2 Reduced metabolic demand

In comparison to concentric exercise performed at the same relative power, eccentric exercise exhibits a reduction in the metabolic ($\dot{V}O_2$) demand, and requires a significantly greater relative power to achieve the same concentric $\dot{V}O_2$ (Chauveau, 1896; Abbott et al., 1952; Asmussen, 1953; Knuttgen and Klausen, 1971; Hesser et al., 1977; LaStayo et al., 1999; Perrey et al., 2001; Dufour et al., 2004). It has long been observed that activities that involve eccentric contractions, such as stair descent, are inherently easier to perform than concentric activities like stair ascent (Chauveau, 1896; Hoppeler, 2015). Three pioneering studies provided initial insight that may explain this phenomenon (Abbott et al., 1952; Abbott and Bigland, 1953; Asmussen, 1953). By utilising the first reverse cycle ergometers, Abbott et al. (1952) and Asmussen (1953) showed that, compared to eccentric cycling, concentric cycling required up to 5.9 times more $\dot{V}O_2$ at the same relative power. They also showed that as the velocity of contractions increased, the $\dot{V}O_2$ disparity was accentuated (Figure

1.10). However, these studies although insightful, had poor control of exercise parameters and insufficient power to draw robust conclusions, requiring studies with greater participant numbers to validate these claims. A later study by Bigland-Ritchie and Woods (1976) confirmed previous findings, showing a $\dot{V}O_2$ ratio of 5:1 (concentric:eccentric) at 30 revolutions per minute (rpm), which increased to 10:1 at 100 rpm.

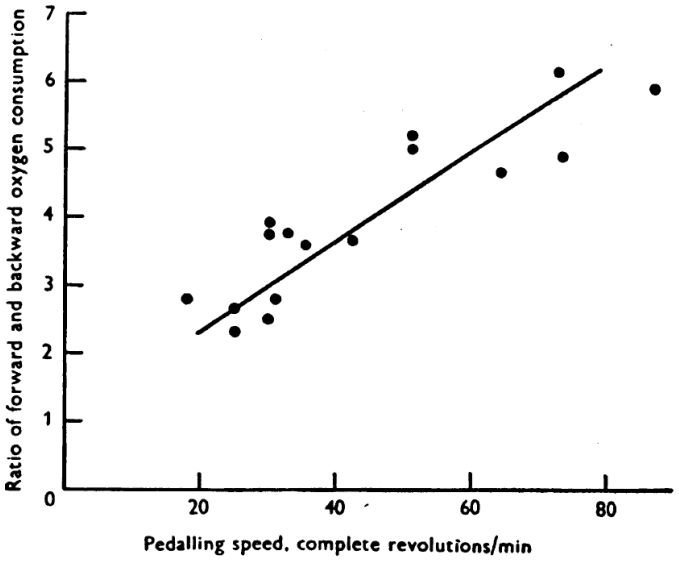

Figure 1.10. The effect of pedalling speed on the ratio of forward (concentric) to backward (eccentric) oxygen uptake ($\dot{V}O_2$) (Abbott et al., 1952).

Perrey et al. (2001) improved on previous studies by more accurately matching metabolic demand to exercise intensity domains. They non-invasively determined LT from a concentric RIT and used that to define concentric WRs that corresponded to a low, moderate and high intensity. They found that ~5 times greater eccentric WRs were required to match for the low-intensity concentric response (Figure 1.11).

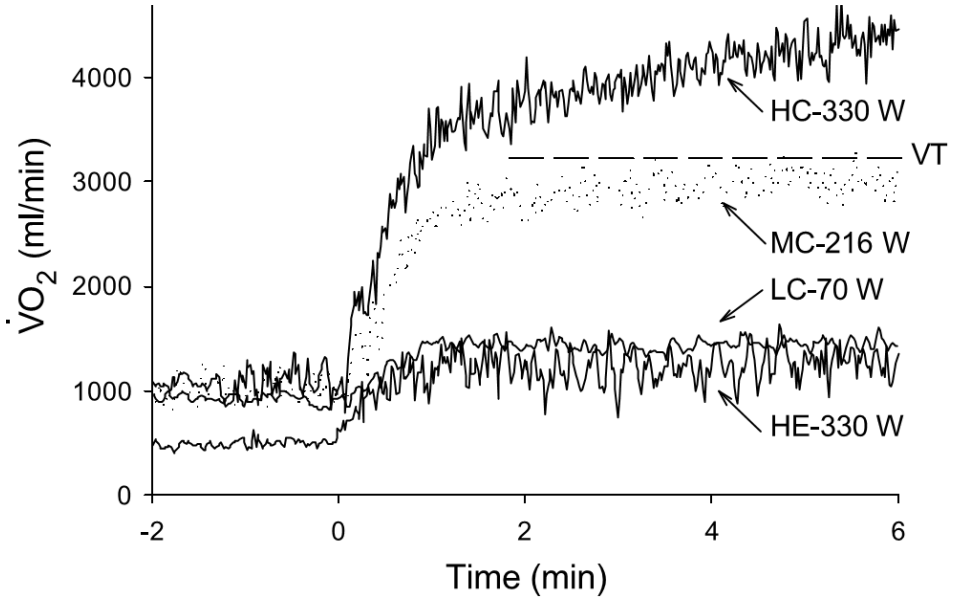

Figure 1.11. Representative subjects concentric and eccentric oxygen uptake ($\dot{V}O_2$) responses to different relative power outputs. Low-intensity concentric (LC, 60W), moderate-intensity concentric (MC, 216W), heavy-intensity concentric (HC, 330W), and heavy-intensity eccentric (HE, 330W). Data shows two minutes of baseline (15W) data and six minutes at the target load. (Perrey et al., 2001).

Dufour et al. (2004) utilised 3-minute step increments of 50W during concentric and eccentric cycle ergometry and compared metabolic responses at the same relative power or metabolic requirement (Figure 1.12). To achieve the same metabolic requirement (~1L·min^{-1} $\dot{V}O_2$), eccentric relative power was ~400% greater than concentric. At the highest possible matched relative power (287 W), eccentric $\dot{V}O_2$, arteriovenous oxygen difference, and blood La were just 37%, 59% and 13% of the concentric values, respectively. It is Important to note, however, that their methodology did not allow for comparison of metabolic responses at higher eccentric WRs, as the upper boundary for comparison was set by the peak concentric WR tolerable for three minutes. Utilisation of a ramp-incremental test protocol may have allowed for comparisons at greater WRs. Based on there being a smaller difference between concentric and eccentric metabolic responses at lower WRs (data extrapolated from Figure 1.12), it would be expected that WRs greater than 287 W would result in a greater disparity.

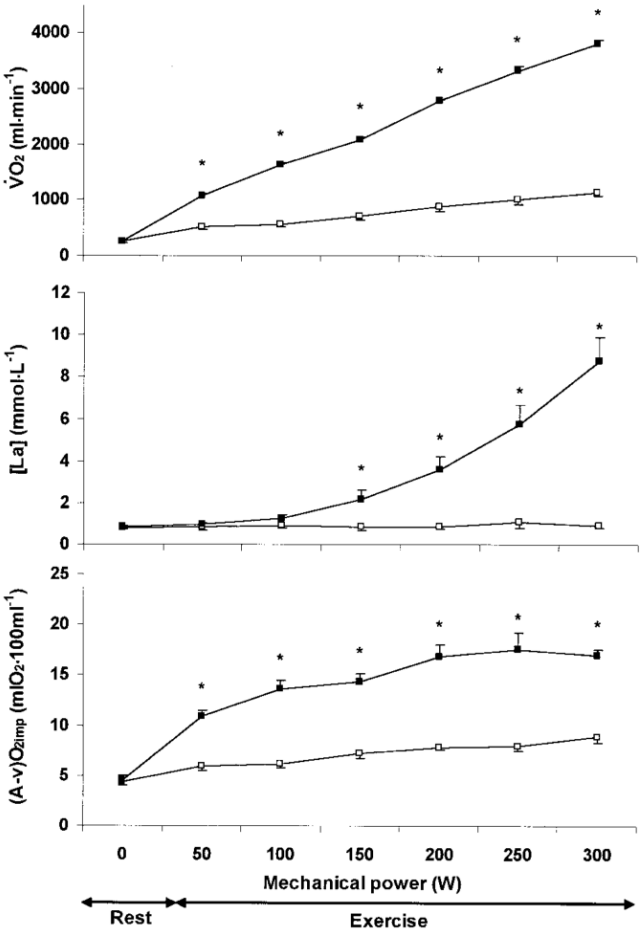

Figure 1.12. Metabolic responses to concentric (CON; ■) and eccentric (ECC; ☐) constant-load exercise matched for relative power (W). Oxygen uptake (top ($\dot{V}O_2$)), blood lactate concentration (middle (La)), and arteriovenous oxygen difference (bottom ($A\text{-}vO_2imp$)) displayed. Values are means ± SE for the last 30 s of each workload. Significant difference between ECC and CON, * P < 0.05. (Dufour et al., 2004).

The majority of eccentric research has utilised reverse cycle ergometry with the metabolic responses to other modalities remaining unknown. It is likely that as with different concentric modalities (e.g. treadmill *vs*. cycle ergometer (Hill et al., 2003)), the efficiency of the exercise (functional gain), as well as the peak metabolic responses will vary. There is a need for further research investigating these responses to expand our understanding and allow better informed exercise prescription.

Following the findings of a reduced metabolic requirement for eccentric *vs.* concentric exercise, researchers aimed to elucidate the energetic cost of force generation at the level of the muscle. Fenn and Wyman's early work on isolated frog muscle demonstrated a lower efficiency (conversion of energy input to mechanical work) during shortening (concentric) than lengthening (eccentric) contractions (Fenn, 1923; Fenn, 1924; Wyman, 1926), with Fenn concluding that this reduction in efficiency is likely a result of additional heat production during

shortening. Research then aimed to estimate the energetic cost of muscle contraction by measuring the heat liberated during conversion of chemical energy stored in high energy phosphates (Hill, 1925; Hill, 1937). Given the technical difficulties and limitations, more recent research has focussed on estimating the cost of eccentric contractions mostly using magnetic resonance spectroscopy to measure the flux of high-energy phosphagens. Results from *in vivo* animal (Beltman et al., 2004b) and human (Ryschon et al., 1997) studies, as well as isolated single rabbit muscle fibres (Homsher et al., 1997), have, in general, confirmed the earlier conclusions and reported a reduction in energy requirements during eccentric contractions compared to concentric. Although insightful, some of these studies failed to accurately control for the force, duration and velocity of contraction and consequently present non-significant results. However, a more recent study by Ortega et al. (2015) developed an *in vivo* force clamp technique that was able to accurately control for these variables whilst measuring the flux of high energy phosphates from the first dorsal interosseous (FDI) *via* magnetic resonance spectroscopy. Their results agreed with previous studies, showing roughly a 65% reduction in ATP cost for eccentric *vs.* concentric contractions at the level of an individual muscle. Although providing an accurate estimation of energetic cost, further research is required to assess the cost of eccentric force production at the level of the muscle throughout a variety of forces and velocities, and within muscle groups that may more closely represent those predominantly active during exercise. Only then will we have access to values that directly map onto experimental protocols pertinent to the current thesis.

1.6.3 Reduction in cardiovascular demand

Coupled with the reduction in metabolic demand is a reduction in the cardiovascular demand of eccentric *vs.* concentric exercise at the same WR (Overend et al., 2000; Perrey et al., 2001; Dufour et al., 2004; Isner-Horobeti et al., 2013). Initial studies using decline treadmill walking and reverse cycling provided strong evidence for reductions in HR, MAP, rate pressure product (RPP) and $\dot{Q}$ during eccentric exercise (Chauveau, 1896; Knuttgen and Klausen, 1971; Pivarnik and Sherman, 1990; Wanta et al., 1993). However, these studies lacked accurate matching between concentric and eccentric WRs making it

difficult to accurately assess the reduction in the magnitude of these variables. Overend et al. (2000) better controlled for relative power using an isokinetic dynamometer, finding that after two-minute bouts of knee extension exercise at 50% of peak concentric force the HR, MAP and RPP were all significantly lower in the eccentric group. Similarly, Dufour et al. (2004) showed a reduction in HR and $\dot{Q}$ at the same relative power, but also showed that at the same $\dot{V}O_2$ there was a significantly higher eccentric HR and $\dot{Q}$ (Figure 1.13). This increase in cardiovascular demand at the same metabolic demand was mirrored by Hesser et al. (1977) in an earlier study, which suggested that there was a lower oxygen pulse ($\dot{V}O_2$/HR) eccentrically. These results suggest that care needs to be taken when prescribing eccentric exercise based on metabolic responses, as the cardiovascular demand may increase above its predicted and safe limits.

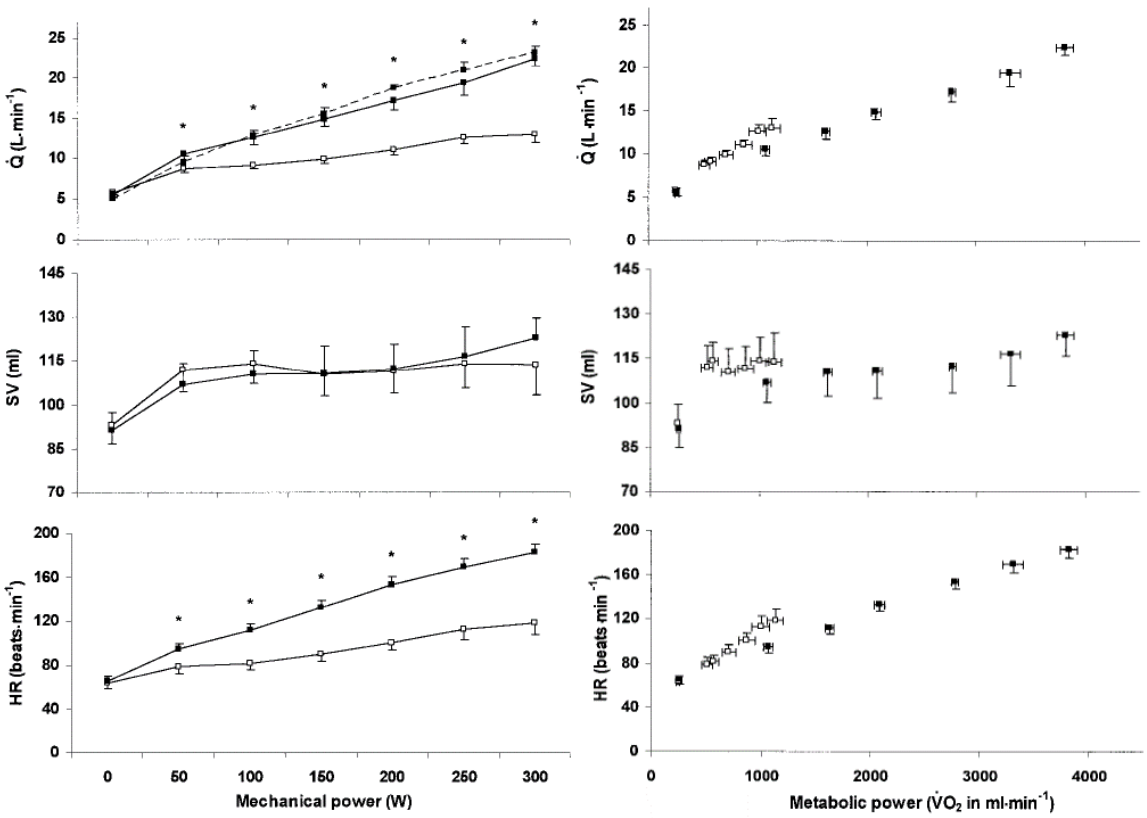

Figure 1.13. Cardiovascular responses to concentric (■) and eccentric (□) constant-load exercise matched for relative mechanical (left; (W)) and Metabolic (right) ($\dot{V}O_2$) power. Cardiac output (top ($\dot{Q}$)), stroke volume (middle (SV)), and heart rate (bottom (HR)) displayed. Values expressed as means ± SE of the last 30 s of each workload. Significant difference between eccentric and concentric, * P < 0.05. Adapted from Dufour et al. (2004).

Although the exact mechanisms leading to a higher HR at the same $\dot{V}O_2$ remain unclear, they are likely due to the higher forces required to achieve the same metabolic demand during eccentric exercise (Thomson, 1971). The resulting increased muscle tension may stimulate an increased rate of firing in type III (responsive to mechanical stimuli) and IV (metabolic stimuli) muscle afferents, producing an increased HR response *via* the suppression of parasympathetic autonomic outflow from the brainstem (Kaufman et al., 1983; Mense and Stahnke, 1983; Hayward et al., 1991). Eccentric contractions are also thought to stimulate greater intramuscular heat production (Sargeant and Dolan (1987), again thought to increase type III and IV muscle afferent firing (Hertel et al., 1976). It may also be postulated that the increased muscle tension would increase intramuscular pressure and occlude blood flow within the working muscle, thereby reducing venous return and $\dot{Q}$ which would result in a reflex increase in HR *via* a reduction in parasympathetic and increase in sympathetic outflow (Fadel and Raven, 2012). However, Meyer et al. (2003) matched for metabolic demand between concentric and eccentric cycling and demonstrated the same $\dot{Q}$ despite a 4 x greater eccentric WR. It has been demonstrated within both human quadriceps (Sjøgaard et al., 1988) and calf muscles (Barcroft and Dornhorst, 1949) that isometric contractions as low as 15-20% of a maximal voluntary contraction (MVC) can reduce intramuscular blood flow, meaning it is likely that both contraction modes restricted blood flow and caused similar reductions in venous return and $\dot{Q}$. Therefore, it is unlikely in this instance that the elevations in eccentric HR are a result of increased eccentric intramuscular pressure and reduced venous return. HR response may also be affected by cadence and duration of contraction influencing the muscle pump effect and venous return. For example, Gotshall et al. (1996) showed that as cycling cadence increased from 70 to 100 rpm, $\dot{Q}$ increased to a greater degree than $\dot{V}O_2$. However, as cadence further increases, the short relaxation time between contractions may result in the next contraction occurring prior to the peak hyperemic response (Ohmori et al., 2007). Concentric cycling cadence was higher than eccentric within Meyer's study (80 *vs.* 50 rpm) but again didn't increase $\dot{Q}$, suggesting that the hyperemic response during relaxation and subsequent emptying of venous circuit is similar between contraction modes in this cadence range. These results suggest that muscle afferent firing in response

to pressure and temperature are the most plausible explanations for the increase eccentric HR response observed.

1.6.4 Neuronal control

Compared with concentric muscle contractions performed at the same relative power, eccentric muscle contractions demonstrate up to 50% lower EMG activity (most commonly assessed *via* the average or peak of an amplified, rectified and integrated raw EMG signal; (Bigland-Ritchie and Woods, 1976; Fang et al., 2001; Grabiner and Owings, 2002; Fang et al., 2004)). Conflicting results have been reported, and are likely due to investigation of different muscle groups and utilisation of different joint angles, but research supporting a reduced EMG activity is far more plentiful (Duchateau and Baudry, 2014). This reduction is consistent with the findings of greater eccentric forces at all velocities, as it would be expected that fewer muscle fibres are required to be active eccentrically to produce the same force (Hoppeler, 2015). It has also been shown that it is harder to achieve full activation of a muscle during an eccentric contraction than during a concentric or isometric contraction. For example, Beltman et al. (2004a) using superimposed electrical stimulation showed that there was a 21% *vs*. 8% voluntary activation deficit with eccentric *vs.* concentric contractions. This may be related to functional capacity, with Amiridis et al. (1996) showing no deficit in eccentric voluntary contraction in highly trained athletes.

Studies comparing the firing frequency of motor units between concentric and eccentric contractions have utilised mean frequency analysis of surface EMG and single motor unit intramuscular EMG recordings. Results suggest that at low WR intensities, the firing frequency of motor units during eccentric contractions are higher than that during concentric contractions (McHugh et al., 2002), and only slightly increase at higher intensities (Pasquet et al., 2006). This results in lower eccentric firing frequencies at high WR intensities, present across varied loading conditions (Duchateau and Baudry, 2014), and remains present even when the integrated EMG amplitude is matched (Del Valle and Thomas, 2005).

It was previously hypothesised that eccentric contractions may not follow the Henneman size principle (Henneman, 1957) which states that there is orderly recruitment of motor units with increased force, in the order of smallest to largest. Recordings of soleus and gastrocnemius single motor units showed that only 15-50% of high threshold motor units were recruited during eccentric contractions, and that low threshold motor units active during concentric contractions were de-recruited during eccentric contractions (Nardone et al., 1989). This suggests that high threshold motor units were preferentially recruited during eccentric exercise. Alternative methods such as EMG power spectrum analysis and mechanomyography have not supported this theory; an in-depth review by Chalmers (2008) concluded that the majority of evidence is in support of the Henneman size principle. However, it remains possible that an altered recruitment order may exist with eccentric exercise, and further fine wire EMG studies are required to investigate a variety of muscle groups, velocities and loading conditions to fully test this theory. An altered recruitment order may rely on inhibitory Renshaw cells, preferentially activated by high threshold motor units, de-recruiting low threshold motor units (Wakeling et al., 2006).

1.6.5 Eccentric exercise induced muscle damage

It is well documented that eccentric exercise results in significantly more exercise-induced muscle damage than traditional (concentric) exercise (Kanda et al., 2013). The initiating event of eccentric exercise induced muscle damage (EEIMD) is thought to be the overstretching of sarcomeres termed the ‘popping sarcomere hypothesis’ (Morgan and Proske, 2004). As sarcomeres in series within one muscle fibre do not present with identical lengths, some act on the descending limb of the length-tension relationship making them more susceptible to stretching and subsequent damage (Lieber et al., 1991). Injury to type II muscle fibres is more commonly reported, likely due to their narrower and weaker Z disks as well as the potential preferential activation during eccentric contractions (Macaluso et al., 2012). Within animal models, sarcomeres have been shown to stretch past the point of myofilament overlap, resulting in loss of active force and reliance on all further tension being generated by passive sarcomeric structures. The repetition of this applied force at long sarcomere

lengths is thought to lead to damage of the sarcomeres and failure to re-align, commonly presenting as z-line streaming and disruption to the A band region, t-tubules, and sarcoplasmic reticulum (Friden and Lieber, 1998; Takekura et al., 2001), (Figure 1.14). Increased permeability results in an uncontrolled influx of Ca^{2+} into the sarcoplasm, resulting in a local contracture and activation of proteolytic degradative pathways which break down damaged myofibrillar proteins and sarcolemma, as well as the later phagocytic inflammatory phase involving removal of damaged tissue and repair of the damaged regions (Kuipers, 1994; Peake et al., 2005).

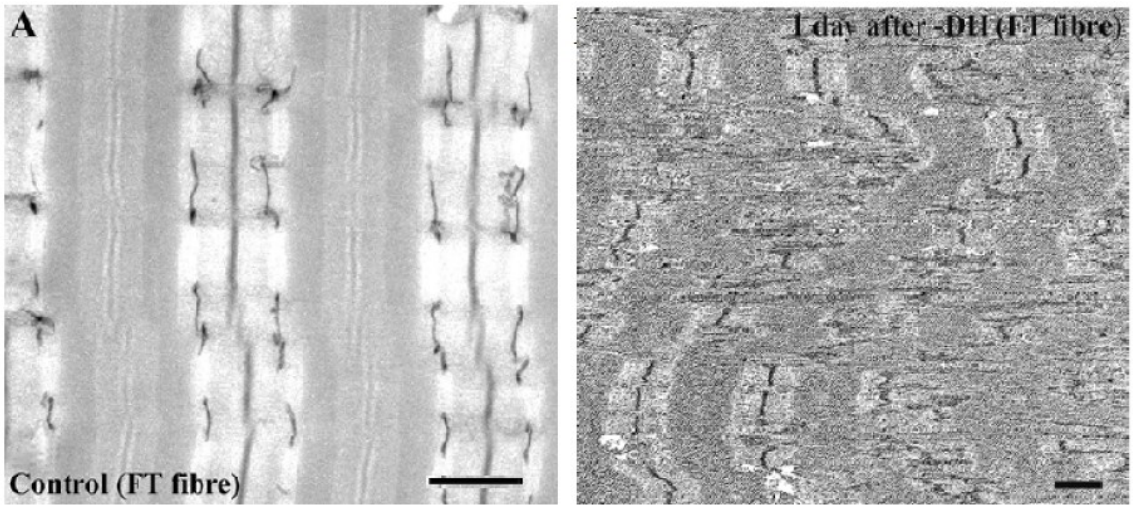

Figure 1.14. Sarcomere damage of a rat triceps brachii muscle 1-day post downhill running (DH). Scale bars 1μm. Note the Z band streaming and A band disruption. Image adapted from Takekura et al. (2001).

The muscle damage, inflammation, and repair occurring with EEIMD can acutely change subsequent physiological function, with reports of altered length-tension relationships, reduced voluntary muscle strength (Byrne et al., 2004), reduced range of motion, and increased HR and blood La responses (Byrne et al., 2004; Brughelli and Cronin, 2007; Tee et al., 2007). Voluntary strength reductions can be as high as 50 to 65% (Chapman et al., 2011), with the magnitude of decline similar across contraction types (concentric, eccentric and isometric, Byrne & Eston, 2002). Voluntary strength declines are greatest at higher velocities, consistent with type II fibres being preferentially damaged (Chapman et al., 2011), and loss of voluntary strength following eccentric exercise is most

pronounced immediately following the exercise bout, with a linear recovery occurring after this stage (Byrne et al., 2001).

EEIMD not only affects physiological function but may also contribute to the occurrence of delayed onset muscle soreness (DOMS), characterised by the delayed feeling of stiffness and soreness follow an eccentric exercise bout (Hough, 1900), that peaks at ~48 hrs (Byrnes and Clarkson, 1986; Tee et al., 2007; Isner-Horobeti et al., 2013), and disappears after some five to seven days (Cheung et al., 2003). Symptom intensity is variable, from the feeling of slight stiffness, to excruciating pain which prevents excessive movement or force (Cheung et al., 2003). Pain is usually worse in regions close to the distal myotendinous junction, potentially due to the higher concentration of muscle pain receptors (nociceptors) within this region (Newham et al., 1983), and the greater oblique insertion point of muscle fibres (Tidball, 1991; Noonan and Garrett, 1992; Malm et al., 2000; Nosaka, Kazunori et al., 2002). There are a variety of proposed mechanisms that attempt to explain the development of DOMS, including microdamage to muscle fibres and associated connective tissue, as well as inflammation and the subsequent oxidative stress that occurs (Armstrong, 1984; Cheung et al., 2003; Kanda et al., 2013). Interestingly, the magnitude of muscle soreness has been shown to not correlate with the magnitude of muscle damage (Nosaka, K. et al., 2002; Fujii et al., 2008), stimulating research into other pathways that may be involved such as the bradykinin like substance to nerve growth factor (NGF) (Murase et al., 2010), and cyclooxygenase (COX) 2 to glial cell line-derived neurotrophic factor (GDNF) (Murase et al., 2013) pathways. Murase et al. (2010) administered a β2 bradykinin antagonist within male Sprague Dawley rats finding that muscle soreness was completely eradicated when administered before an eccentric exercise bout, but not when administered 30 minutes following. This shows that the bradykinin like substance (Arg-BK in rats), a substance upregulated following eccentric exercise (Blais et al., 1999), likely plays a key role in the stimulation of muscle soreness following eccentric exercise but is not responsible for the maintenance of soreness. They also demonstrated that NGF mRNA and protein was upregulated 12 hours to two days following eccentric exercise, a similar time course of soreness presenting with DOMS, and by injecting an antibody to NGF

two days following the exercise bout, all muscle soreness was reversed. Following this, Murase and colleagues investigated the prostaglandins COX-1 and 2 pathways (Murase et al., 2013), secondary to evidence of nonsteroidal anti-inflammatory drugs, which act *via* inhibition of COX (Gan, 2010), effectively reducing the severity of DOMS (Tokmakidis et al., 2003; Rahnama et al., 2005). They demonstrated that inhibitors of COX-2, but not COX-1 administered before eccentric exercise suppressed the magnitude of muscle soreness but failed to reduce the severity once the muscle soreness was established. Therefore, as with bradykinin, it is likely that COX-2 is involved in the initial pathways that generate muscle pain, rather than in subsequent pathways that maintain soreness. COX-2 was shown to upregulate the neurotrophic factor GDNF, and by blocking GDNF's actions *via* an antibody, they were able to reduce the magnitude of muscle soreness. Recently the same research group have determined the receptor subtype that is responsible for this upregulation of GDNF as being the EP2 receptor by utilising an EP2 knockout mice model (Ota et al., 2018). The process by which upregulation of NGF and GDNF *via* Arg-BK and COX-2 respectively results in a pain response is likely *via* sensitisation of thin-fibre muscle nociceptors afferents (Lewin et al., 1993; Svensson et al., 2003; Reinold et al., 2005; Murase et al., 2014).

The pain experienced from DOMS, as well as reductions in force previously reported (Chapman et al., 2011), could severely impair function within exercise intolerant populations which may deter healthcare professionals and ethical review boards from advising this exercise modality for rehabilitation (Hoppeler, 2015). It has been shown, however, that you can eradicate or minimise development of DOMS by gradually increasing the eccentric load resisted (LaStayo et al., 1999; Lastayo et al., 2010). It is well known that the pain experienced in a second bout of eccentric exercise is less than that during the first bout, termed 'the repeated bout effect'. Producing just two maximal eccentric contractions reduces the severity of DOMS after subsequent exercise (Nosaka et al., 2001), and the protective effect increases with increased force and repetitions (Chen et al., 2007; Chen et al., 2010). However, lower intensity eccentric contractions have been shown to still result in a protective effect; just 10% of the participants' maximal isometric force resulted in no increase in

measured markers of muscle damage or any muscle soreness, and yet still attenuated DOMS with subsequent higher intensity eccentric exercise (Lavender and Nosaka, 2008; Chen et al., 2013). This low-intensity pre-conditioning exercise not only reduced the sensation of pain, but also reduced the levels of creatine kinase (a marker of muscle damage), and reductions in force and range of movement. This suggests that with correct implementation, eccentric exercise could be used clinically to reduce soreness and potentially increase adherence levels. The mechanisms responsible for the repeated bout effect remain largely unknown, with neural, cellular and mechanical adaptations potentially being at play (Hyldahl et al., 2017). Again, it is likely a combination of these mechanisms that conditions against subsequent damage and more research is needed to fully understand the mechanisms.

1.7 Training responses to eccentric *vs.* traditional exercise

1.7.1 Resistance training

Traditional resistance training involves applying high external mechanical loads (60-100% of one repetition maximum (1RM) concentric contraction) to target muscle groups, with the aim of increasing muscle strength and mass (Pescatello, 2013). Exercises are typically performed at relatively low angular velocities and involve both a concentric (generally lifting of an object) and eccentric (generally lowering of an object) phase of movement. The external mechanical load is identical during each phase, meaning that, due to the fact higher forces can be produced during eccentric contractions at all velocities (Duchateau and Baudry, 2014; Herzog et al., 2016), the relative load (as a % of 1RM) applied during the eccentric phase is significantly smaller. Therefore, it could be hypothesised that by increasing the external mechanical load during the eccentric phase, greater adaptations in muscle strength and mass could be stimulated.

A simple method of increasing load during the eccentric phase is *via* the use of a spotter who reduces external load during the concentric phase, allowing a greater eccentric load to be resisted (for example, a spotter lifting dumbbells

during bicep curls). The need for two people and the potential benefits of increasing eccentric load have led to the development of purpose-built training machines that increase load during the eccentric phase of movement, or ergometers that completely isolate the eccentric phase of movement. Roig et al. (2009) conducted a systematic review with meta-analysis, investigating the effects of eccentric resistance training on outcomes of voluntary strength and muscle mass in young healthy participants (18-65 yr). They solely included randomised controlled trials and clinical controlled trials that compared between concentric and eccentric exercise with a minimum training duration of four weeks. Their inclusion and exclusion criteria ensured the elimination of studies with poor methodology, e.g. those utilising unilateral training (resulting in bilateral transference, Carroll *et al*., 2006), or those using eccentric resistance training as solely an additive intervention. They concluded that the ability to resist higher loads eccentrically translated into superior increases in muscle mass and voluntary strength (average concentric, eccentric and isometric), showing that when concentric and eccentric intensity were similar, differential voluntary strength gains disappeared. Interestingly, when velocity of contractions were controlled, the relative voluntary strength increases with eccentric training were more pronounced due to the greater force disparity between concentric and eccentric force at faster contraction velocities (see section 1.6.1).

When assessing changes in concentric, eccentric and isometric voluntary strength that occurred with different contraction modality training, Roig et al. (2009) found that isometric strength increased similarly with concentric or eccentric training, regardless of differences in intensities. Concentric strength increased more when participants completed concentric training, and eccentric strength preferentially increased with eccentric over concentric resistance training. These contraction mode specific increases in strength suggest a neural mechanism for increases in strength, due to the fact that if solely muscle hypertrophy were at play, you would expect identical voluntary strength increases with all modes of contraction (see below for potential neural mechanisms). Similarly to increases in voluntary strength, muscle mass, measured *via* leg girth, magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DEXA), ultrasound or computerised tomography (CT) increased

preferentially with eccentric over concentric training (Roig et al. (2009), independent of intensity or velocity of training. However, caution needs to be taken with interpreting these results due to the large variability in methods used; when assessing results only from the gold standard MRI and CT studies, 3 out of the 4 studies reported significant increases in muscle mass with eccentric exercise.

The mechanisms by which traditional resistance training (concentric, isometric, or mixed modes) results in muscle strength and mass gains are well described, yet mechanisms for differential increases in voluntary strength and mass with eccentric training remain unclear. The factors resulting in increased strength following traditional resistance training are considered to be a result of muscle hypertrophy, neural adaptations and muscle architectural changes (Kawakami et al., 1993). There is minimal research investigating the architectural changes that occur with eccentric *vs.* concentric training with just two studies to date comparing changes in fascicle length and pennation angle utilising ultrasound imaging (Timmins et al., 2016). Increases in fascicle length greatly influence shortening velocity of muscle (Bodine et al., 1982; Reeves et al., 2009), whilst increased pennation angle allows for an increase in the physiological cross-sectional area (CSA) of muscle, i.e. the number of cross-bridges that can be activated in parallel, increasing the force-generating capacity (Aagaard et al., 2001). The studies by Reeves and Timmins demonstrated that both concentric and eccentric training increased fascicle length, and that only concentric training increased pennation angle significantly. The greater increases in fascicle length suggest that more sarcomeres were added in series, a common observation following prolonged periods of stretch (Holly et al., 1980; Williams et al., 1988). They propose that the higher forces resisted during eccentric training resulted in a greater stretch stimulus to induce sarcomere addition. The observation that pennation angle only increased within the concentric group suggests that the stimulus for adding sarcomeres in parallel may be different to adding in-series. Reeves postulates that fascicle length may increase in response to mechanical stimulus whilst pennation angle may increase in response to a metabolic stress. To further investigate these hypotheses, studies with greater control of both mechanical force and metabolic stress are required.

Traditional resistance training results in complex signalling events that result in structural and functional adaptations allowing the muscle cells to better tolerate future stressors (Hoppeler et al., 2011). Potential triggers for adaptations involve hormonal disturbances, neuronal changes, metabolic stress, and mechanical load, with the latter being most applicable to resistance type exercise (Hoppeler, 2015). High loads are sensed by the proprioceptors (muscle spindles) in skeletal muscles and tendons (Golgi tendon organ) resulting in increased transcription and translation of myofibrillar proteins *via* altered signalling cascades (Figure 1.15). This results in a greater density of myofibrils, allowing a subsequently greater force to be produced (Griffin and Cafarelli, 2005; Gabriel et al., 2006).

The key modulator controlling muscle protein synthesis with resistance training is the enzyme mammalian target of rapamycin (mTOR) (Drummond et al., 2009). Its activation is strongly correlated with the magnitude of load applied (Bodine et al., 2001), with increased translation of myofibrillar protein mRNA and increased cytoskeletal organisation occurring (Rivas et al., 2009). mTOR is increased in the presence of plasma insulin-like growth factor (IGF) (Hoppeler et al., 2011), which itself is regulated predominantly by the levels of growth hormone and leucine (amino acid) content, activated by high mechanical load and a high protein diet, respectively (Fujita et al., 2007b). On the other hand, myostatin is the predominant down regulator of protein synthesis, causing inhibition of mTOR activity *via* myostatin receptor binding; myostatin knock out mice had muscles two to three times larger than wild type controls (Hoppeler et al., 2011). Other negative regulators of skeletal muscle hypertrophy include the pro-inflammatory peptides TNF alpha and interleukins, which again downregulate mTOR activity (Sacheck et al., 2004).

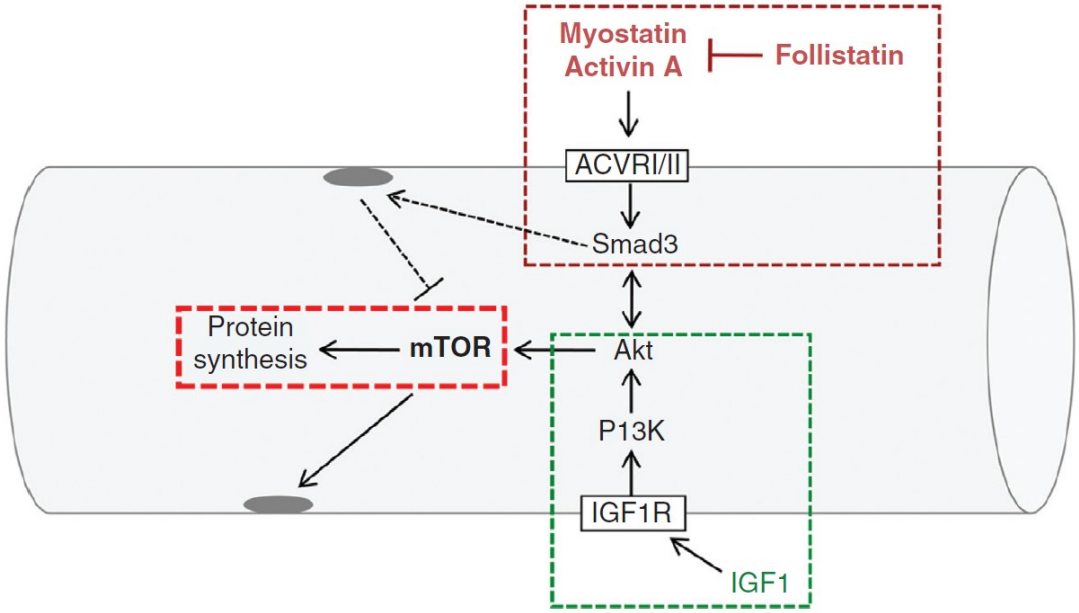

Figure 1.15. Major pathways involved in regulation of muscle protein synthesis. Insulin-like growth factor (IGF) and myostatin pathways up or down regulate mTOR activity respectively, which itself increases muscle protein synthesis (Blaauw et al., 2013).

Very few studies have investigated differences in molecular signalling between concentric and eccentric exercise. Initial animal studies in rodents have shown conflicting results; Garma et al. (2007) showed no differences in the expression of myostatin and IGF, compared to Heinemeier et al. (2007) who demonstrated greater downregulation of myostatin and increased upregulation of IGF with eccentric *vs.* concentric or isometric exercise. Garma et al. (2007) controlled for the force being exerted under each contraction load, whereas Heinemeier et al. (2007) did not. It may, therefore, be the increase force occurring with eccentric contractions that resulted in these expression changes, rather than the contraction type *per se*. Human studies have shown that eccentric stepping, performed at the same load as concentric stepping, results in superior expression of genes related to protein synthesis (Kostek et al., 2007), suggesting that different molecular signalling pathways may be activated. mTOR can be activated *via* integrin connections to the cytoskeleton during high mechanical loads (Lueders et al., 2011). This further supports the theory that increased mechanical tension provides an additional hypertrophic stimulus. The results of these studies are by no means conclusive, and further research is required to ascertain the primary mechanisms responsible for increased voluntary strength with eccentric *vs.* concentric exercise.

Early evidence suggested that increases in muscle hypertrophy were correlated with the magnitude of the mechanical stress applied, with the American College of Sports Medicine recommending the application of a load equating to 60-80% of 1RM for roughly 8-12 repetitions to maximise muscle hypertrophy (Pescatello, 2013). However, it has now been demonstrated that similar increases in muscle mass can be achieved by increasing the magnitude of local metabolic stress. Key evidence for this arises from low-load resistance (Mitchell et al., 2012; Ogasawara et al., 2013) and blood flow restriction exercise studies (Takarada et al., 2000b; Abe et al., 2005; Abe et al., 2010; Ozaki et al., 2010). For example, Mitchell et al. (2012) investigated voluntary strength and MRI measured muscle mass changes within 18 young men that completed 10 weeks of knee extension resistance exercise three times a week at varying mechanical loads to failure. They found that those performing three repetitions of 30% 1RM to failure achieved the same increases in muscle mass as those performing one repetition of 80% 1RM to failure. The magnitude of metabolic fatigue would have been greater within those exercising at lower mechanical loads (Ozaki et al., 2015) potentially providing an additional compensatory stimulus for muscle growth. Blood flow restriction during exercise has also shown promising results for at stimulating muscle hypertrophy at low intensities (as low as 20% 1RM) within both young and older adults (Abe et al., 2005; Madarame et al., 2008). Ozaki et al. (2010) demonstrated this within 18 older adults performing 20 minutes of treadmill walking, four times a week, for 10 weeks, at an intensity equating to 45% of HR reserve. A pressure belt applying pressures of between 160 to 200 mmHg was applied to both legs of half of the participants to reduce venous return and increase localised metabolic stress (Loenneke et al., 2010). They showed that muscle CSA, volume and isokinetic strength increased by 3.1%, 3.7% and 22% respectively only within those that received blood flow restriction, providing evidence for an increased metabolic stress providing the stimulus for increases muscle hypertrophy. The mechanisms by which metabolic stress triggers a hypertrophic response remain largely unknown, however, mechanisms that may be involved include; upregulation of the mTOR pathway (Fujita et al., 2007a; Drummond et al., 2008), an increased growth hormone response (Takarada et al., 2000a; Reeves et al., 2006), an increase in heat shock proteins (Kawada and Ishii, 2005), and nitric oxide synthase (Kawada and Ishii, 2005), and an increased

recruitment of fast-twitch muscle fibres (Moritani et al., 1986; Takarada et al., 2000b).

It was previously assumed that all increases in voluntary muscle strength were the result of an increase in muscle hypertrophy, although, this is now known not to be the case, evidenced by a number of observations. Firstly, voluntary strength increases following resistance training have been shown to poorly correlate with changes in limb girth (Moritani and Devries, 1980; Jones and Rutherford, 1987), CSA (MacDougall et al., 1980), and muscle fibre type composition (Costill et al., 1979). This is especially true within the early stages of training where, although there are conflicting results (Staron et al., 1994; LaStayo et al., 2000; Lixandrao et al., 2016), the majority of studies suggest that muscle hypertrophy does not occur until roughly eight weeks into resistance training protocols (Narici et al., 1989; Akima et al., 1999). Secondly, force generation *via* electrically evoked muscle twitch and tetanic contractions is directly related to the muscle CSA, but electrically evoked force following training does not increase alongside voluntary strength changes (Davies and Young, 1983; McDonagh et al., 1983). Thirdly, strength changes are almost always greatest when the contraction mode utilised matches that used during training (concentric, eccentric or isometric), as well as when dynamic strength testing is used alongside dynamic training instead of isometric and vice versa (Morrissey et al., 1995). Together these findings suggest that there must be an increase in neural drive and/or a more efficient pattern of motor unit recruitment within the early stages of resistance training.

A number of neural adaptations have been hypothesised to allow for increases in voluntary strength without hypertrophy, including: 1) an increase in the amplitude and firing frequency of electrical muscle activity; 2) a reduction in antagonist co-activation; 3) an altered motor unit discharge pattern; and 4) an increase in synchronisation of motor units. Several studies have evidenced an increase in the amplitude of the integrated surface EMG signal prior to increases in leg girth and muscle CSA (Moritani, 1979; Häkkinen and Komi, 1983; Narici et al., 1989; Aagaard et al., 2002). For example, Narici et al. (1989) assessed changes in maximal isometric contraction force (knee extensors), muscle CSA

(measured *via* MRI), and surface EMG (peak integrated EMG averaged over five MVCs) of the vastus lateralis muscle prior to, and every 20 days throughout a 60 day (four times a week) knee extensor resistance training study. They found that maximal isometric force increased by 8.7%, coupled with a 24.8% increase in peak EMG but no increase in muscle CSA. As well as an increase in peak EMG, increases in motor unit firing rates may also occur and allow for further increases in voluntary strength (Patten et al., 2001; Kamen and Knight, 2004). Patten et al. (2001) tested the maximal voluntary force generation of the fifth finger abductors whilst measuring motor unit discharge activity *via* intramuscular needle electrodes over a 6-week training period. They demonstrated that the motor unit firing rate increased significantly alongside maximal force within the initial 48 hours both within young and older adults, but interestingly returned to baseline levels within older adults and remained elevated within the younger cohort whilst voluntary strength continued to increase. This return to baseline firing rates has previously been reported (Rich and Cafarelli, 2000), and suggests that the importance of firing rate to changes in voluntary strength may diminish with time, with contributions from other neural mechanisms such as increases in motor unit recruitment (Akima et al., 1999), and reductions in antagonist co-activation (Carolan and Cafarelli, 1992) playing a more important role.

It has been further hypothesised that a reduction in antagonistic muscle activity may occur, reducing the force opposing an activity, and allowing a greater agonist force to be produced for the same muscle CSA and activation (Kamen, 1983). For example, Carolan and Cafarelli (1992) showed a reduction in peak EMG activity (peak of the rectified and integrated raw signal) of the biceps femoris muscle from 14.9% to 11.5% following an eight week knee extension training study without a rise in vastus lateralis peak EMG. However, there is contradictory evidence with Gabriel and Kroll (1991) showing increased co-activation of the elbow extensors following elbow flexion strength training. This reduction in antagonist activation has also been shown following a four week eccentric training study of the plantar flexors (Pensini et al., 2002), however, comparisons between work-rate matched concentric and eccentric exercise has not been assessed, with further research required to elucidate the type and

magnitude of neural adaptations that occur between concentric and eccentric exercise.

Finally, the pattern of motor unit discharge activity may also be altered with resistance training, allowing for greater forces to be produced without muscle hypertrophy. Doublet firing, the paired discharge of a single motor unit with a short (between 2.5 and 20 ms) interspike interval (Simpson, 1969), has been shown to increase in frequency with resistance training (Van Cutsem et al., 1998) and fatigue (Binder-Macleod and Barker, 1991; Griffin et al., 1998), and likely results in an increased voluntary force, contraction speed, and maintenance of high forces (Mrówczyński et al., 2015). Increased synchronisation of motor unit firing may also occur, evidenced by Semmler and Nordstrom (1998) who directly measured motor unit synchronisation of the FDI muscle using intramuscular electrodes within musicians, resistance trained, and untrained individuals, finding that the synchronicity of motor unit firing, was lowest in untrained individuals and highest in resistance trained individuals.

1.7.2 Endurance training

Traditional resistance training applies high loads with low repetitions, aiming to increase voluntary muscle strength and mass, whereas traditional endurance training applies high repetitions with low loads to reduce muscle fatigability and increase cardiovascular and metabolic capacity. The predominant stress perceived by muscle cells is shifted from a mechanical disturbance to that of a metabolic one (Hoppeler, 2015). As highlighted in section 1.6.2, to match for concentric metabolic requirement, eccentric WRs are considerable larger, meaning as long as these high WRs remain tolerable for long durations, eccentric endurance exercise may be able to provide both a mechanical and metabolic stimulus for adaptations. Studies have, therefore, sought to determine whether eccentric endurance training can be performed at similar metabolic demands, and result in additional voluntary strength and muscle mass gains to those possible with traditional concentric or mixed endurance training (LaStayo et al., 2014; Hoppeler, 2015). Conversely, eccentric endurance training has potential as a therapeutic intervention for clinical populations presenting with exercise

intolerance, by presenting a high mechanical stimulus at a low cardiovascular and metabolic requirement not possible with other exercise modalities (see section 1.7.3). An important difference between traditional endurance exercise and eccentric endurance training is that the high loads required to achieve a greater metabolic demand limit the total duration of eccentric endurance training sessions, with the typical duration only lasting between five and twenty minutes (Hoppeler, 2015).

To our knowledge, only three studies have investigated the effects of eccentric endurance training on outcomes of voluntary muscle strength, mass, composition and function in healthy participants (LaStayo et al., 1999; LaStayo et al., 2000; Lewis et al., 2012; Leong et al., 2014). The first study by LaStayo et al. (1999) matched for metabolic demand (1 $L \cdot min^{-1}$) between concentric and eccentric cycling for six weeks in nine young healthy participants, with intensity and duration gradually increased to prevent the occurrence of severe DOMS (see 1.6.5). By the sixth week, the eccentric group was able to resist seven times more load than the concentric group, resulting in significantly greater increases in voluntary muscle strength (30%). However, they only measured isometric strength, which has been shown to be less sensitive to preferential eccentric strength gains (Roig et al., 2009), so may have seen greater voluntary strength changes if they had measured the modality-specific eccentric strength.

A later study by LaStayo et al. (2000) improved the study design by assessing changes in muscle ultrastructure and fibre area, as well as changes in $\dot{V}O_{2peak}$ and HR_{peak}. The intensity was again progressed throughout the eight-week training period showing approximately a four and seven-times increase in concentric and eccentric force, respectively (from week 1), and minimal development of DOMS (Figure 1.16). Intensity of exercise was matched by % of HR_{peak} achieved in a maximal concentric RIT, increasing from 55% in week one to 65% in week four. Matching for HR may have resulted in the eccentric metabolic demand being lower than concentric, as more recent studies have shown a lower oxygen pulse with acute bouts of eccentric exercise (see 1.6.3). By week eight there was a four-times greater eccentric WR, and a 36% increase

in voluntary strength from baseline assessment, whereas no increases in voluntary strength were seen within the concentric group. Vastus lateralis biopsies showed no differences between concentric and eccentric training in capillary density or mitochondrial density, however, significant increases in the CSA of muscle fibres (52% increase) and capillary-to-fibre ratio (47% increase, consistent with maintenance of aerobic capacity) were observed. This large increase in CSA was unexpected due to previous reports from traditional resistance training studies of a lack of muscle hypertrophy until roughly 8 weeks of training (Narici et al., 1989; Akima et al., 1999). These results may suggest that muscle hypertrophy can be stimulated earlier with eccentric training, but caution should be taken when interpreting these results due to the large regional variability that exists in muscle phenotypes taken with biopsies (Lexell et al., 1986). The lack of an increase in $\dot{V}O_{2peak}$, HR_{peak} and mitochondrial density suggests that the intensity of 65% of HR_{peak} was not sufficient enough to induce cardiovascular and metabolic adaptations. It remains unknown whether eccentric exercise performed at higher metabolic demands would be tolerable for long durations and allow both mechanical and metabolic adaptations to occur.

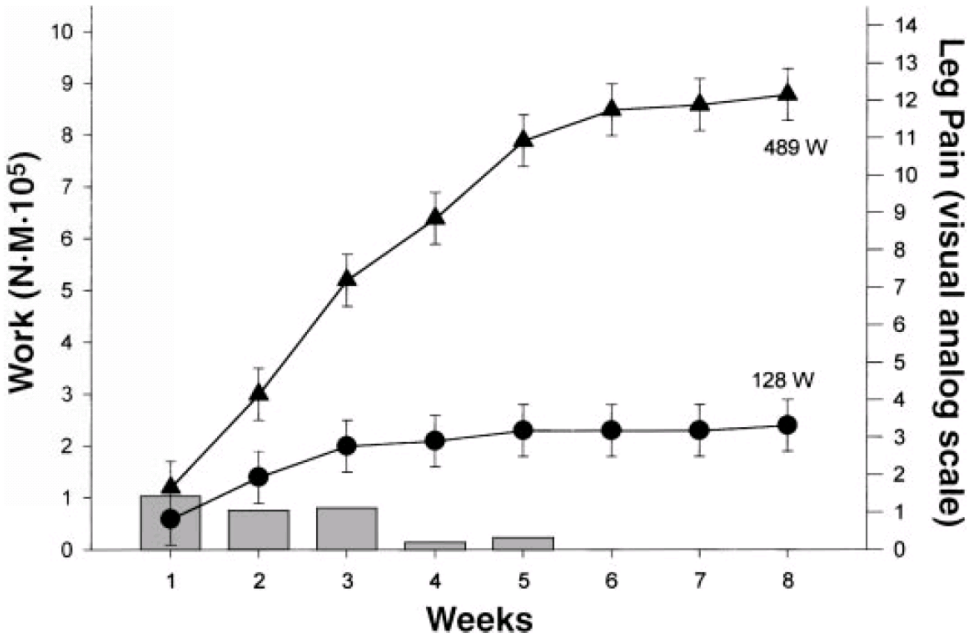

Figure 1.16. Concentric (●) and eccentric (▲) relative work, and eccentric perceived leg pain (□) during an 8-week training study matched for % of peak HR. No leg pain was reported for concentric group. Mean ± SE. (LaStayo et al., 2000).

Lewis (2012) investigated whether eccentric endurance exercise could be performed at lower loads than previously reported, whilst still placing a sufficient mechanical stimulus to induce voluntary strength gains. Eight weeks of concentric and eccentric cycling, both matched to 60% of peak concentric workload, resulted in a 59 beats·min^{-1} lower HR, lower perceived exertion, and a similar increase in voluntary strength (~12%) with eccentric compared to concentric training. In summary, eccentric endurance exercise has thus far not been investigated at high enough loads to induce cardiovascular and metabolic adaptations, likely due to the high mechanical forces required. Eccentric resistance training can, however, induce greater mechanical strain at a lower metabolic demand, translating into superior adaptations in muscle strength, mass, and composition. These data further highlight the potential use of eccentric endurance exercise within exercise-intolerant populations.

1.7.3 Eccentric training in an older adult population.

Eccentric exercise may provide a form of exercise for exercise-intolerant populations that reduce cardiorespiratory demand of the exercise, whilst maintaining or increasing the mechanical stimulus for muscular adaptations. Conditions such as cancer, COPD, CHF, and diabetes, as well as the asymptomatic ageing population may all stand to benefit. As ageing progresses, impaired function becomes evident within the neuromuscular (Vandervoort, 2002; Rolland et al., 2008; Verschueren et al., 2013), metabolic (Sharma and Goodwin, 2006), cardiovascular (Lakatta, 1990; Ferrari et al., 2003), respiratory (Sharma and Goodwin, 2006) and neurological systems (Levy, 1994). As the leading cause of accidental death worldwide (Vos et al., 2015), falls within the elderly population remain the primary concern, with the number of falls increasing year on year (Stevens et al., 2006). With the combination of osteoporosis (age-related decline in bone mineral density; BMD), falls are often traumatic and result in reduced mobility and independence, earlier admission to care homes, increased morbidity, frailty and mortality (Rubenstein, 2006). The causes of falls are often multifactorial with multiple risk factors making an older adult more at risk of falling. Rubenstein (2006) compiled data from 16 studies to determine the risk factors that are most predictive of falls within older adults

(>65y), showing that weakness was the leading predictor, followed by balance, gait, and visual deficits. As sarcopaenia, the age-related decline in muscle mass and quality (e.g. fibre type composition and oxidative capacity) is the primary cause of weakness within older adults (Goodpaster et al., 2006), an abundance of research has sought to investigate whether the effects of sarcopaenia can be halted or even reversed with the use of traditional resistance training.

Traditional resistance training within older adults has been shown to be extremely effective at increasing voluntary muscle strength, power and mass (Frontera et al., 1988; Fiatarone et al., 1990; Charette et al., 1991; Fielding, 1995; Schlicht et al., 2001; Reeves et al., 2004), with the relative rates of protein synthesis and voluntary strength changes being comparable to that of a healthy young population (Roth et al., 2001; Holviala et al., 2006; Chodzko-Zajko et al., 2009). Training programs incorporating two to three training sessions a week for a minimum of 9 weeks have reported at least a 10% increase in muscle fibre hypertrophy, with this increasing up to 62% with longer training durations (Fiatarone et al., 1990; Häkkinen et al., 2001; Trappe et al., 2001; Bamman et al., 2003). Important to note is that considerably lower muscle hypertrophy (up to five times lower; Bamman et al., 2003) is commonly reported within older female over males (Ivey et al., 2000; Hunter et al., 2004), potentially due to lower myostatin expression, as has been demonstrated in rodent models (McMahon et al., 2003). Coupled with improvements in muscle mass are improvement in muscle strength, with significant associations shown between resistance exercise and voluntary strength improvements (Peterson et al., 2010). Peterson's comprehensive meta-analysis assessed 47 studies with 1079 total participants, and showed considerable mean strength changes for leg press, chest press, knee extension, and lat pull down of 29 ± 2, 24 ± 2, 33 ± 3, and 25 ± 2 %, respectively.

Interestingly, it has been shown that with ageing, there is significantly less decline in eccentric voluntary strength compared to concentric or isometric strength (Frontera et al., 2000; Klass et al., 2005). For example, Klass et al. (2005) reported a ~20% and ~38% lower isometric and concentric ankle

dorsiflexor strength in old (mean age 76.7 ± 1.9 yr), vs young (mean age 25.7 ± 1.2 yr) males and females respectively, compared to just ~6.5% loss of eccentric strength; Hortobágyi et al. (1995) showed ~30 N strength decline per decade (age range 18-80 yr, 60m, 30f) with concentric and isometric quadriceps strength, but only a ~9 N per decade decline with eccentric strength. In fact, the eccentric force reductions with age are so minimal that Power et al. (2012) has shown no difference in eccentric force outputs between young and older adult males (26.1 ± 2.7 yr *vs.* 76.0 ± 6.5 yr). This maintenance of eccentric force is likely a result of the increased ratio of non-contractile passive tissue to contractile proteins that occurs with age, with Power et al. (2012) showing approximately 2.5 times greater residual force enhancement in older adult subjects, suggesting a non-contractile origin of the additional force enhancement (see section 1.6.1). This maintenance of eccentric voluntary strength with age may allow older adults to perform resistance or endurance training at far greater mechanical loads than traditional resistance training, increase the mechanical stimulus for muscle and bone growth, and ultimately reduce the risk of falls and severity of subsequent trauma. This natural ability to resist greater loads may also assist in preventing recurrent falls, as a greater increase in strength may improve confidence and reduce the fear of falling which usually results in a downward cycle of inactivity resulting in reduced muscle mass, strength, and gait abnormalities which further increase fall risk (Rubenstein, 2006).

A systematic review by Gluchowski et al. (2015) investigated the effectiveness of eccentric exercise training on outcomes of voluntary muscle strength, architecture, functional capacity, severity of DOMS, and difficulty of the exercise task within older adults. Twelve out of the thirteen studies reported significant improvements in voluntary strength, and the majority of studies comparing eccentric training to traditional (concentric or mixed) resistance training showed superior eccentric changes. The greatest increases in voluntary strength were seen in studies that isolated eccentric contractions and used the highest mechanical load. Despite these higher loads, participants generally perceived the eccentric exercise tasks to be easier to perform (lower RPE), which is potentially key to maintaining exercise compliance. In regards to changes in muscle mass, the results are conflicting, with ultrasound analysis showing similar

improvements between traditional and eccentric training (Mueller et al., 2009; Reeves et al., 2009) and MRI study (LaStayo et al., 2009) showing greater increases in mass with eccentric training. Similarly, muscle biopsies taken from the vastus lateralis reveal a non-significant increase in CSA with eccentric over concentric training (LaStayo et al., 2003).

Coupled with greater improvements in voluntary strength, were greater improvements in the ability to perform activities of daily living (ADLs), with only one out of eight studies reporting greater improvements with traditional resistance training. Improvements were seen with activities such as stair descent and balance (Berg balance scale) (LaStayo et al., 2003; LaStayo et al., 2009), as well as walking speed, timed up-and-go and sit-to-stand tests (Gault et al., 2012; Chen et al., 2013). The improvements reported with eccentric training are likely to reduce the risk of initial and recurrent falls, and therefore reduce hospital and care home admission, and ultimately the cost to the national health service. Studies investigating eccentric exercise within an older adult population have utilised a variety of modalities, from reverse cycle ergometers to resistance machines. These modalities do not often mirror everyday tasks and may, therefore, not transfer as effectively into improving ADL as a more ecologically valid modality would (Millet et al., 2002; Misic et al., 2009; Garber et al., 2011). In summary, eccentric training provides promising results within an older adult population. Older adults are able to apply greater mechanical loads at lower metabolic demands and perceive the exercise to be easier. Increases in voluntary strength translate into improvements in the ability to perform ADL, key to improving independence, quality of life (QoL), and reducing fall risk.

1.8 Aims and objectives

The primary aims of this thesis were to characterise the acute physiological and training responses of concentric and eccentric recumbent stepping exercise within a young and older adult population. To achieve this, we first aimed to adapt an eccentric recumbent stepping ergometer to allow tightly controlled comparisons between concentric and eccentric recumbent stepping and allow custom exercise protocols to be performed. This subsequently allowed the following three experimental studies to be undertaken:

1) Acute physiological responses of concentric and eccentric recumbent stepping matched for relative power and metabolic requirement within a young healthy population.

2) Chronic training adaptations to 8 weeks of concentric or eccentric recumbent stepping matched for relative power or metabolic requirement within a young healthy population.

3) Feasibility study assessing the tolerability and effectiveness of a short 4-week eccentric recumbent stepping program within an older adult population.

We hypothesised that at the same relative power as concentric, eccentric recumbent stepping would result in lower cardiovascular and metabolic responses, subsequently allowing for higher eccentric WRs at matched metabolic rates. We proposed that these high WRs will be tolerable for long durations and stimulate greater eccentric voluntary strength gains and reasoned that this exercise would remain tolerable within an older adult population, and result in functionally relevant strength and mobility changes.

Chapter 2 General methods

2.1 Participant recruitment and screening

All experimental interventions involved voluntary human participation. Ethical approval was obtained from the Faculty of Biological Sciences Ethical Committee for non-clinical research (University of Leeds) and complied with the latest version of the Declaration of Helsinki. Participants were recruited *via* approved routes such as posters, email and personal contact within the University of Leeds and surrounding areas. Once potential participants made contact, they were given participant information and given more than 24 hours to decide whether to participate. To reduce the physiological risks associated with the study, all participants completed a health and activity status questionnaire. This ensured participants met the inclusion and exclusion criteria, assessed their exercise activity levels and smoking history, confirmed no pertinent illnesses or injuries, documented their current medications, ensured no significant exercise related clinical symptoms, and documented any family history of sudden death. All studies aimed to recruit sedentary or moderately active individuals, and therefore excluded anyone undertaking more than two exercise sessions per week that may have limited their scope for response.

2.2 Participant involvement

Eligible participants were invited to attend the integrated exercise suite laboratories at the University of Leeds. An intermediate life support trained researcher was present at every session and a member of staff on call at all times. Two researchers were present in the laboratory for all sessions that involved an element of risk. All approved risk assessments were present within the laboratory being used. A log book was completed every session with the details of time, attendees, study, participant, session information and duration. Prior to all sessions (except during the exercise training period), participants

were asked to refrain from intense exercise and excessive alcohol consumption for 24 hours prior to testing, as well as caffeine and food consumption for three and two hours respectively. Laboratory conditions were kept constant throughout testing with the temperature being maintained at 19-20°C *via* an air conditioning unit with air diffuser to minimise air flow disturbance to calibration of the gas analyser. The study was explained in detail to the participant in person, and time was put aside for any further questions to be resolved. A consent form was signed at the first visit by the participant, person taking consent and the principal investigator for that study. Participants were free to withdraw at any point during the study without reason.

2.3 Use of a novel recumbent stepping ergometer

As discussed in chapter 1, eccentric modalities previously used within research settings have often lacked adequate safety precautions, and fine control of exercise parameters. Crucially, no current modality combines these aspects with natural eccentric activity that mirrors natural locomotion. Therefore, a commercially available eccentric recumbent stepping ergometer (Eccentron, BTE, Hanover, MD, USA, Figure 2.1) which more closely mirrors downhill walking or stair descent was adapted to minimise these limitations.

2.3.1 Eccentric recumbent stepping ergometer

Components of the eccentric recumbent stepping ergometer are presented in Figure 2.1. The user is seated in a recumbent position with their feet placed on the foot plates. A 1.5 HP motor (56T17V5331 B; Marathon Electric Motors, Wausau, USA) drives the foot plates forwards and backwards whilst the user resists the foot plates as they move towards them. The user fully relaxes as the foot plates move away, thereby isolating eccentric contractions. The load cells (RBV-500kg-BNP; Group Four Transducers Inc, Massachusetts, USA; reading error: ± 0.05 %, creep at 30 min: ± 0.06%, temperature effect: ±0.04%) are screwed directly into the left and right foot plates. These have a capacity of 500Kg, operate well within the laboratory temperature used (operating range: -

20 to +65°C), and are characterised as having high accuracy and linearity. This eccentric modality targets the major anti-gravity locomotor muscles such as the quadriceps (vastus lateralis, intermedius, medialis and rectus femoris) and gluteal muscles (gluteus maximus, medius and minimus). With modifications, this ergometer was identified as having the potential to apply a form of eccentric exercise that may more closely resemble normal activity, as well as being safer, and allowing tight control of a variety of exercise parameters.

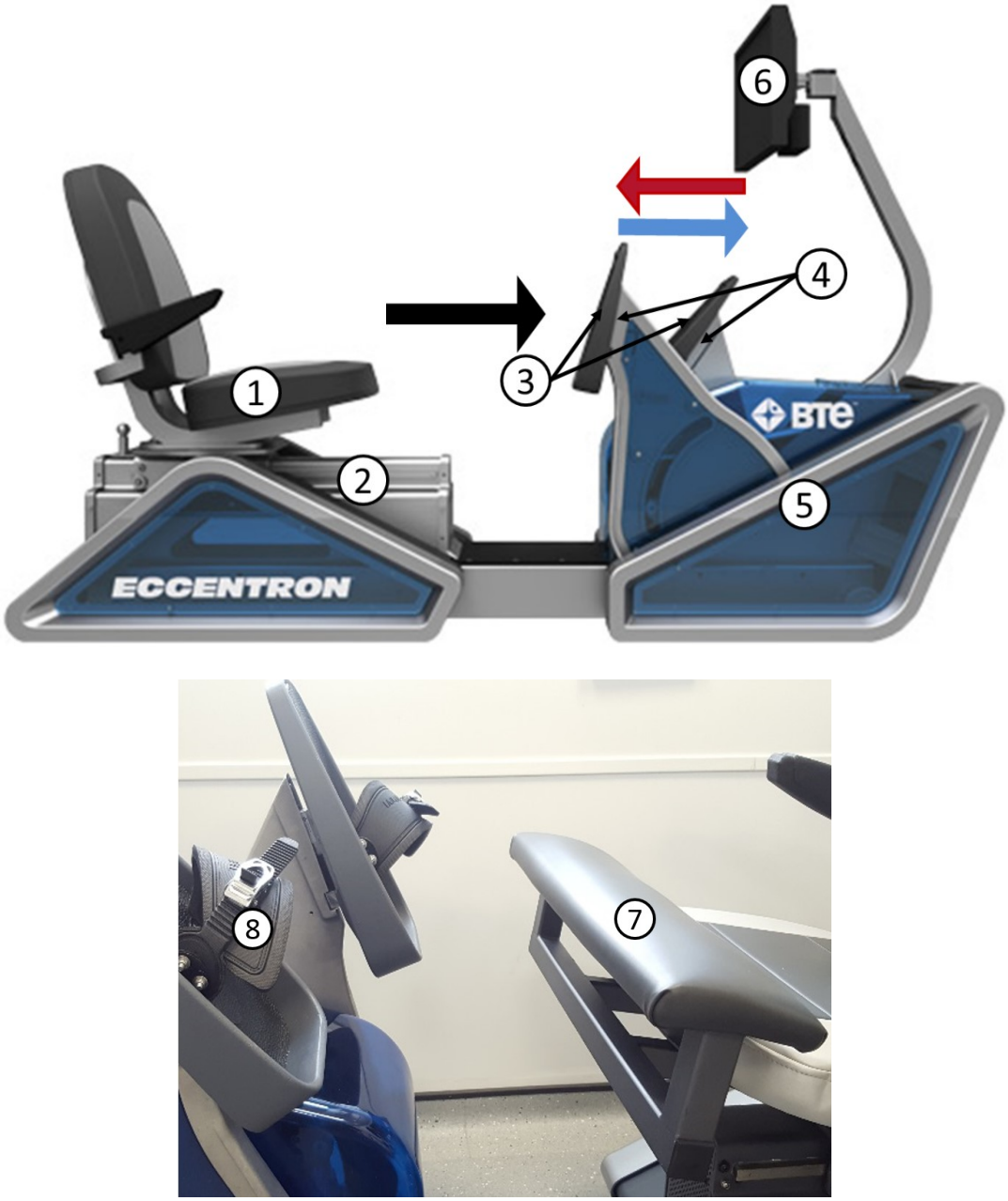

Figure 2.1: Eccentric recumbent stepping ergometer (BTE, Hanover, MD, USA; http://www.btetech.com/product/eccentron/). A reclined seat (1) can be moved forwards and backwards along rollers (2). The foot plates (3) are driven backwards and forwards by a motor housed within the main casing (5), and the load cells (4) are positioned behind each foot plate. A touch screen (6) is visible to the participant and can be connected to external computers to customise the display. Additionally, an adjustable under knee support (7) helps to further prevent knee hyperextension and foot straps were added (8) for additional participant comfort and stability. To produce concentric contractions, the participant was instructed to push the foot plates as they move away from them (black and blue arrows). To produce eccentric contractions, the participant was instructed to resist the foot plates as they moved towards them (black and red arrows).

The recumbent stepping ergometer has incorporated many features that ensure participant safety, presenting a more suitable form of exercise for use within less physically able populations, such as older adults. Firstly, unlike traditional resistance exercise were the user resists an applied load, this modality requires that the participant voluntarily produces the load to meet a target value. This reduces the chance of injury, as it is less likely that an excessive load will be placed on participants' joints. Secondly, the participant is positioned so that knee flexion is at 30°, acting to prevent hyper-extension of the knee and potential severe damage to associated structures. A cushioned bar positioned underneath the participants knees (see Figure 2.1) serves the same purpose. This is in contrast to reverse cycle ergometry where hyperextension is a major safety concern and limits the ability for unsupervised use within a commercial setting (Hoppeler, 2015). Thirdly, the participants are in a reclined position with handlebars either side for support. This almost completely eradicates the chance of the participant falling off the machine. Finally, the cadence on the eccentric recumbent stepping ergometer is set to 23 steps per minute (spm; ~46deg·s^{-1}), i.e. 2.6s per step per leg, a value lower than normal ambulatory values (Marshall et al., 2009), and a lower cadence than that normally used on a cycle ergometer (60-80rpm) (LaStayo et al., 2000; Dufour et al., 2004). This allows greater time for the participant to modulate their joint positions and applied force, potentially preventing un-natural loading through the joints.

The ergometers hardware (ability to change seat position) and software allow for individualised settings including stride length, cadence and joint angles. Importantly, all these settings can be maintained when performing concentric exercise, with only the timing of the applied load changing. This ensures that the only parameter that changes is the contraction type. As supplied, the ergometer has been developed to solely apply eccentric exercise, and to do so within a gym or rehabilitation setting. Therefore, the ergometer was modified to allow assessment of both concentric and eccentric exercise within a research setting.

Limitations that presented with the original recumbent stepping ergometer were 1) the inability to record load cell data during the concentric phase of foot plate

movement (i.e. when moving away from the participant); 2) the inability to change target WR during a session; 3) the inability to set an activity duration longer than 30 minutes, which is an appropriate safety feature for use in gyms, but is inadequate for our studies; 4) lastly, the inability to perform protocols such as a RIT or constant-power tests (CPTs) with altering loads. Therefore, we needed to alter the ergometer hardware, and develop software to improve flexibility of use.

2.3.2 Hardware changes

The original software was triggered to only record load cell data when the foot plate was moving towards the participant (i.e. during the eccentric phase). In order to measure concentric force, we bypassed this trigger by acquiring raw force output directly from the load cells. Each load cell was originally connected to an amplifier board located directly behind the foot plate, which converts resistance of the load cell into an amplified signal. To gain access to this signal, we utilised a breakout board to split the left and right signals *via* an RJ45 connector, with output to BNC connectors at the back of the ergometer. Splitting the signal allowed access to the raw data without impeding the function of the original ergometers software. The raw outputs (0-5 V) were then fed into a data acquisition system (ML870 PowerLab 8/30; ADInstruments Ltd, Oxford, UK) connected to a PC for processing. Additionally, foot straps were securely attached to the foot plates to prevent the feet lifting off before applying force, thereby reducing force spikes caused by acceleration.

2.3.3 Software changes

As previously indicated, the original ergometer software restricted the ability to change various exercise parameters, and most importantly did not allow the measurement of concentric force. Bespoke software therefore needed to be developed to combat these limitations. Duration and load limits were altered, to allow longer sessions at higher loads to be completed. The original software was still used to set the stride length, cadence, and to start and stop foot plate movement. All formation of participant views, and data collection, was

subsequently done using data acquisition software (LabChart 8; ADInstruments Ltd, Oxford, UK).

2.3.3.1 Unit conversion

To allow subsequent determination of power, load cell voltages were converted into Newtons (force that will accelerate 1kg of mass 1m.sec^2) following manufacturer instructions. Firstly, whilst in “calibration” mode, the “zero force voltage offset” (voltage output when the pedal is in the vertical position under no load) for each foot plate were determined within LabChart and used to convert V into N using the following equation:

$$F\ (N) = \ ((Vsignal - Vzero\_offset) \ * \ 4903.3) \ / \ 1.9937$$

Equation 2.

where “F” is force in Newtons (N), “Vsignal” is the current voltage value of the load cell, and “Vzero_offset” is the zero-force voltage previously determined.

A subsequent check against the original ergometer software was conducted, by a well familiarised subject matching a range of target forces as accurately as possible. Two assessors compared real-time force (N) outputs on the ergometer and LabChart which were found to be identical at all loads.

2.3.4 Choosing the appropriate exercise target

Validation testing identified that a target power, rather than a target mechanical force, was required to accurately control exercise intensity. Three well familiarised participants completed 200s of concentric and eccentric recumbent stepping at a target force of 500N. Raw force was collected from the right foot plate and integrated (with respect to 0N) to get cumulative work performed (integrated N). Firstly, the raw force traces of different participants (see Figure 2.2A) demonstrated variations in force curve characteristics, i.e. some participants would contract for shorter durations to meet the target force than

others. This translated to less cumulative work being performed in some individuals (see Figure 2.2B). For instance, in comparison to participant one, participant three had only produced 54% and 62% of the concentric and eccentric cumulative work respectively at 200s. Secondly, when comparing between contraction modalities (concentric *vs* eccentric) in single individuals (Figure 2.2B), a large discrepancy in cumulative work also existed. Thirdly, some participants naturally produced more force during the resting phase of the contraction (Figure 2.2A). This shifts the baseline force upwards, resulting in a higher integrated work value over time.

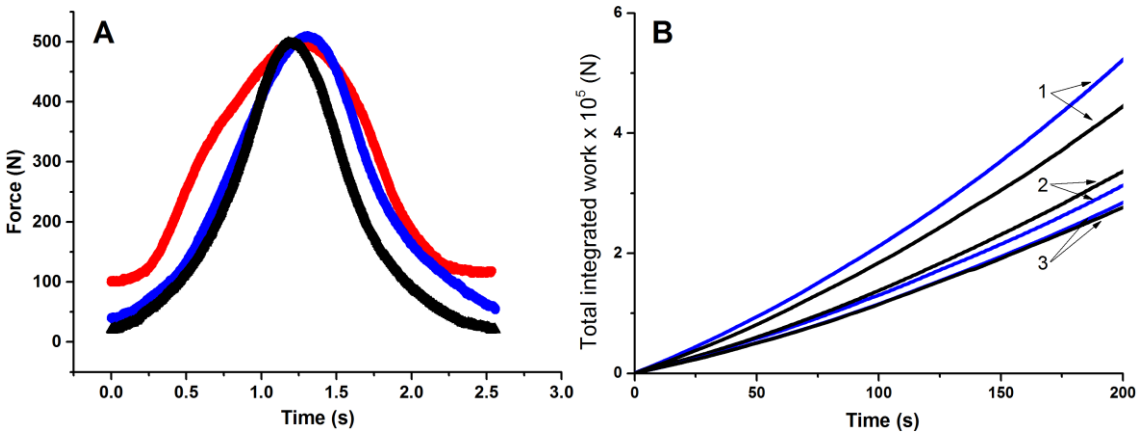

Figure 2.2: Example raw force (left) and integrated work (right) for three participants. Left trace shows raw force profiles for one eccentric contraction at a target of 500N. Right trace shows the Cumulative total mechanical work (integrated N) for three participants completing a concentric (blue) and eccentric (black) CPT (target force of 500N). Number represents participant number. Note the differences in force curve characteristics and the subsequent altered work.

To confirm that setting a target cumulative work would be an accomplishable and accurate method to use, a pilot RIT was conducted (see Figure 2.3). A familiarised participant performed an eccentric 4400N·min^{-1} (7W·min^{-1}) RIT to ~ 80% of their self-perceived LoT. They viewed their left and right cumulative work superimposed on a target band of ± 2% of the target (See Figure 2.4). Excluding the initial 10 seconds of force regulation, cumulative work remained within ± 0.5% (post-hoc analysis) of the target cumulative work for the entirety of the session (Figure 2.3B). When converting to instantaneous power (see section 2.6.3.1 for conversion), power fluctuated evenly above and below the target throughout the

session (Figure 2.3C/D). As expected, these fluctuations increase at greater targets, similar to that observed during cycle ergometry. This testing confirmed that setting a target cumulative work would be a more accurate method to use during testing.

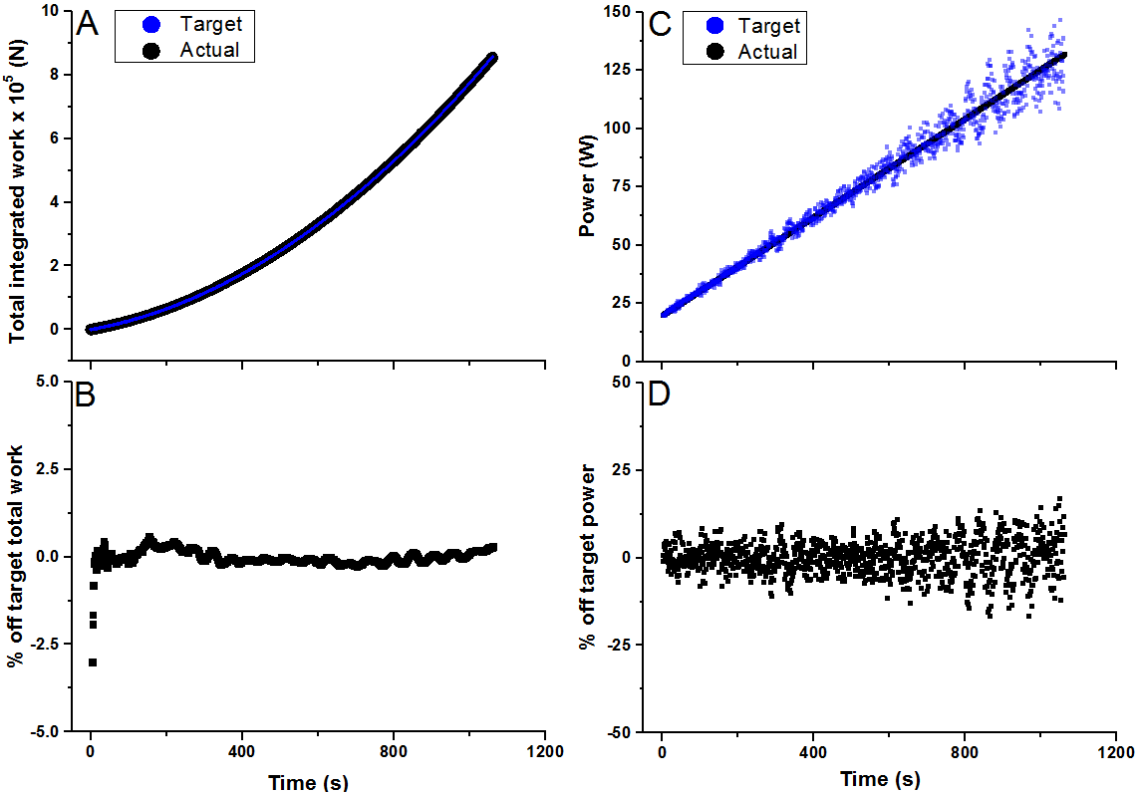

Figure 2.3: Actual vs. target cumulative work and instantaneous power completed during a ramp-incremental test (RIT)

, (Data exported at 1Hz (1s mean)).

Pilot testing was conducted to determine the optimal ramp rate that would enable accurate determination of key outcome measures such as LT and $\dot{V}O_{2peak}$. Within cycle ergometry, ramp durations between 8-12 minutes are commonly used to allow sufficient breath-by-breath data to be obtained, whilst retaining a tolerable duration for the participant (Davis et al., 1981). In a healthy population, cycle ergometer ramp rates of between 25 to 50W·min^{-1} are commonly used with rates greater than 50W·min^{-1} deemed too short to determine key outcome measures, and rates lower than 20W·min^{-1} not providing any further information (Davis et al., 1981). Breath-by-breath data obtained on the recumbent stepping ergometer is inherently noisier than during cycle ergometry (identified during pilot testing,

and likely due to core muscle bracing and subsequent breath holding), and therefore, the upper range of 10-12 minutes duration was chosen as the minimum duration. Concentric RITs are likely to be shorter than eccentric RITs due to a greater cardiovascular and metabolic demand to produce the same WR (see section 1.6.2). Pilot testing identified an optimal ramp rate of 2200N·min^{-1} (7W·min^{-1}) which was used for all subsequent RITs in all studies presented.

2.3.5 Setting target work

To allow the participant to attain a target cumulative work (integrated N), real time conversion of left and right raw forces (N) needed to be conducted. This was performed using data acquisition and processing software (LabChart 8; ADInstruments Ltd, Oxford, UK) at a sample rate of 1KHz. To simplify the view, left and right cumulative work were converted into a percentage of the target using the following equations for the unloaded and RIT phases:

$$Target\ total\ work = \ unloaded\ WR * t$$

Equation 3. Equation used to calculate target cumulative work during the unloaded (constant WR) stage.

$$Target\ total\ work = (unloaded\ WR * t) + \left(\frac{ramp\ rate}{2} * t^2\right)$$

Equation 4. Equation used to calculate target cumulative work during the incremental ramp stage.

where “unloaded WR” is the target work (integrated N) to be completed per minute, “ramp rate” is the increment in target work per minute, and “t” is time (minutes).

An unloaded WR of 6274N·min^{-1} and an incremental ramp rate of 2022N·min^{-1} were used throughout all incremental testing on the recumbent stepping ergometer. This converts to an unloaded power of 9.8W per leg and a ramp rate of 3.2W·min^{-1} (see section 2.6.3.1 for conversion). To calculate left and right

cumulative work performed, an integral was applied to the raw force (N), with a baseline of zero and no reset used (i.e. the integrated value was summed indefinitely). A further two channels were created within LabChart which converted cumulative work into a percentage of the target work:

$$\%\ of\ target\ work = \frac{100}{Target\ work} * Left\ or\ right\ total\ work$$

Equation 5.

To further guide the participants during testing, a participant view was created which displayed both the left and right % of target cumulative work on top of a green target band that identified ± 2% of the target cumulative work (Figure 2.4). This method has its benefits in that it provides necessary feedback for participants to accurately control the work done by both legs. Other modalities such as cycle ergometry often only control for the cumulative power being produced by both legs, therefore making it possible for one leg to perform more work than the other to achieve the target. Having this control allowed future flexibility to record data such as near-infrared spectroscopy (NIRS) and EMG from one leg, and accurately compare between tests. This method has limitations in that termination of testing at the LoT may occur due to greater weakness or fatigue in one leg, therefore, potentially limiting the peak WR's achieved and the peak cardiovascular and metabolic demand placed upon the user.

Figure 2.4: Participants' view during testing on the recumbent stepping ergometer. Green band identifies the target cumulative work (± 2 %). The horizontal black lines represent the left and right cumulative work as a percentage of the target. These values were also displayed in large numerical boxes which were coloured green when within the target range and red when outside the target range. Participants were asked to keep the lines as close to the middle of the green bar as possible, and the numbers as close to 100% as possible.

To simplify the switch between target WRs, macros were created to change the equation inputs within the LabChart channels quickly, and restart data collection. Using this method, data could be more accurately time aligned to other data being collected concurrently. It also allowed for multiple WRs to be programmed into the software, as used in chapter 4.

A separate screen allowed the assessor to visualise raw left and right forces, as well as HR and raw electrocardiogram (ECG) trace during testing to ensure participants were contracting at the right time, and to monitor for any abnormal ECG changes during exercise (Figure 2.5).

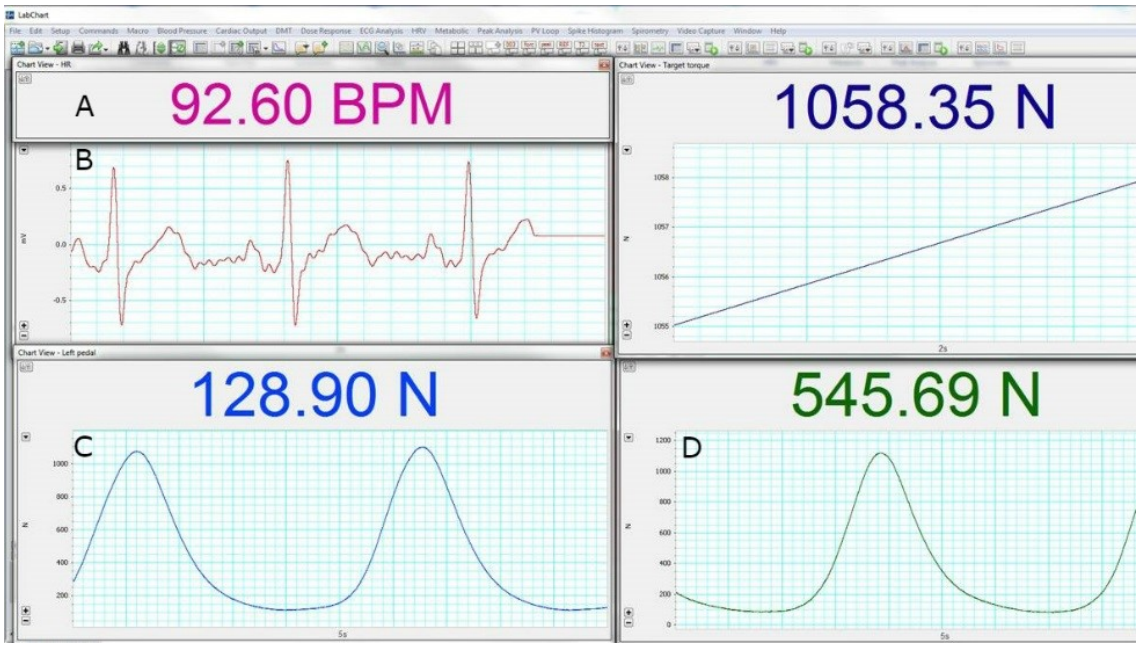

Figure 2.5: Assessors' view during testing on the recumbent stepping ergometer. HR (A), raw ECG trace (B), left (C) and right (D) raw force traces could be visualised during testing.

2.4 Experimental protocols

Study-specific protocols are expanded on within each experimental chapter. This section will focus on experimental protocols that have been used across multiple experiments.

2.4.1 Familiarisation

Eccentric exercise is an inherently un-natural and cognitively challenging activity to grasp, highlighted by the earlier activation, longer duration and magnitude of cortical signals compared to concentric (Perrey, 2017). Eccentric ergometers provide additional difficulty due to the need of the user to voluntarily produce a target force/power rather than the ergometer altering its resistance to meet a target demand. Studies utilising these modalities therefore require familiarisation sessions for participants to become fully accustomed to the exercise. It was hypothesised that participants would familiarise quicker to recumbent stepping than previously used modalities such as reverse cycle ergometry due to the fact it likely more closely resembles natural activities such as stair descent. These

sessions ensured the participants were able to contract correctly and accurately maintain the target cumulative work within the set boundaries defined. These sessions also acted to reduce the severity of DOMS experienced (section 1.6.5) as well as to reduce anxiety responses often reported with unaccustomed exercise (Pescatello, 2013).

The familiarisation protocol initially consisted of introducing participants to the eccentric recumbent stepping ergometer through verbal guidance, and the ergometer software introduction screens. Participants were positioned so that one leg was at 90º of knee flexion and the other at 30º. A note was made of the seat position, which was then used for all subsequent testing. The ergometer software initially guided the participant in performing eccentric contractions with each leg isolated, before combining them. Concentric and eccentric contractions were described as pushing the foot plates whilst they move away or resisting the foot plates as they move towards the subject respectively. The screen was then switched to the bespoke LabChart participant view (Figure 2.4) which was used throughout all further testing. At this point participants were instructed how to accurately meet the target cumulative work.

The rest of the familiarisation protocol consisted of RIT and CPT practice using the same protocols as during testing. To prevent severe DOMS presenting after familiarisation, and to reduce the severity of soreness following subsequent testing, participants were asked to continue up to only 80% of their self-perceived LoT during the RIT and a low CPT target was set. Up to three familiarisation sessions were completed, and subjects were deemed familiarised when they were able to maintain their cumulative work within ± 2% of the target for greater than two minutes.

2.4.2 Ramp Incremental Tests

RITs were conducted for two studies (chapters 3 and 4). This section will detail the methods used for the concentric and eccentric RITs conducted on the recumbent stepping ergometer, with any differences in protocol for upright cycle ergometry expanded on in the relevant chapter 4. By simultaneous measurement of breath-by-breath pulmonary gas exchange and HR during a RIT, a variety of important cardiovascular and metabolic responses could be determined. Most importantly, LT, $\dot{V}O_{2peak}$, HR_{peak} and peak relative power. Subsequent analysis of these data also allowed for determination of the relative power required for the subsequent moderate-intensity CPTs.

Concentric and eccentric RITs were completed in a random order greater than 48 hours apart. A schematic representation of the protocol is shown in Figure 2.6. Participants initially sat at rest on the ergometer in order to obtain resting measurements of pulmonary gas exchange. During this stage the respiratory exchange ratio (ratio of $\dot{V}CO_2$ to $\dot{V}O_2$; RER) breath-by-breath data was monitored. A value greater than 0.9 suggests the participant may be hyperventilating which invalidates estimation of LT (Ozcelik et al., 1999). In this instance, the start of the test would be delayed, or the test stopped, the participant removed from all equipment, and restarted after a short break. Once the RER had stabilised, and was between 0.7 to 0.9, the unloaded warm-up period commenced. This consisted of roughly three minutes of stepping at a target power of 20W. Once again, we would ensure that all values were within normal ranges and were stabilised before progressing testing (RER between 0.7 to 0.9 and $\dot{V}O_2$ stable). Target power then linearly increased at a rate of $7 W \cdot min^{-1}$ until the LoT was reached. Participants were instructed to keep as close to the target cumulative work as possible throughout the test. The LoT was identified as the point at which, despite strong verbal encouragement, the participant was no longer able to maintain the target power within the acceptable range (± 2% of the target power) for 5 consecutive contractions of either leg. At the LoT, the target power was reduced to 20W and the participant was instructed to continue stepping for a minimum of 2 minutes, to prevent blood pooling occurring and the participant feeling faint.

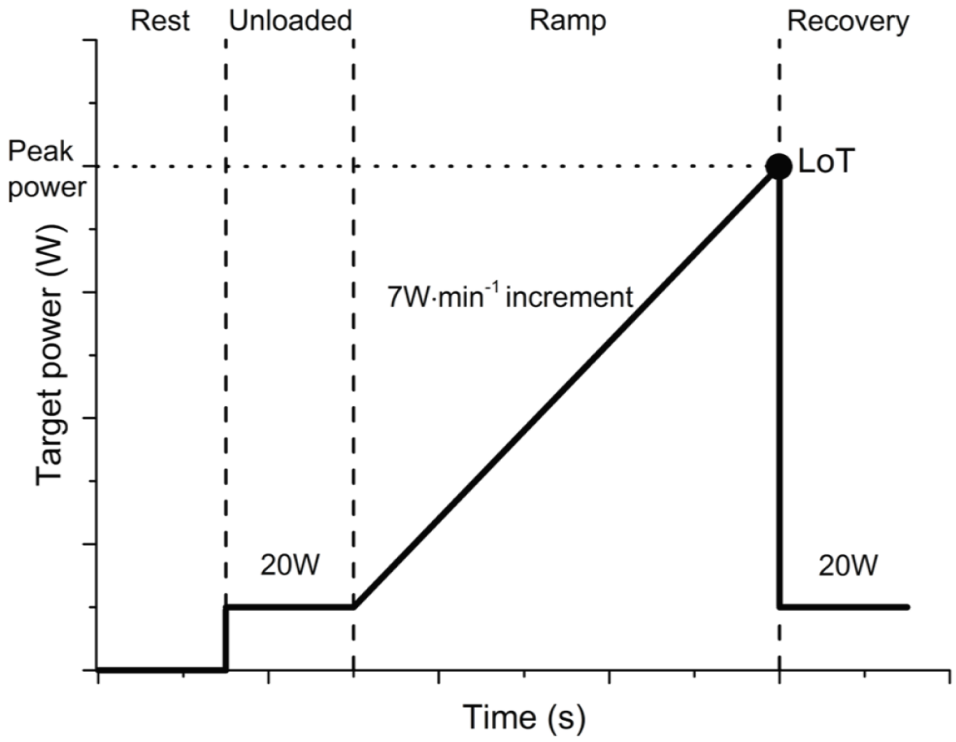

Figure 2.6: ***Schematic representation of the ramp-incremental tests (RITs) completed on the recumbent stepping ergometer. A rest period preceded a 20W constant-power phase. This was followed by a linear increase in target power of 7W·min^{-1}, culminating in the limit of tolerance (LoT, ●) at peak power (dotted line). At the LoT, the target power was reduced to 20W for a recovery period of ~3 minutes.***

2.4.3 Constant-power Tests

CPTs were conducted in studies described in chapters 3 and 4. The fundamental methods will be highlighted here, with additional detail provided within the relevant sections. A schematic of a typical CPT can be seen in Figure 2.7. The intensity of the constant-power exercise was set to be of moderate intensity, (below the concentric LT). Moderate intensity exercise was chosen based on the UK physical activity guidelines provided by the Department of Health (Bull et al., 2010). It was also predicted that, due to the expected reduction in cardiovascular and metabolic demand with eccentric exercise, exercise above moderate intensity may require WRs that would not be tolerable for the desired 15 minutes during eccentric exercise.

As with RITs, the CPTs were completed in a randomised order and were separated by more than 48 hours. The same cadence and stride lengths were used, and the rest, unloaded, and recovery periods were identical to that of the RIT protocol. The same methods were used to check for stability in pulmonary gas exchange measurements during the rest and unloaded stages of the protocol. Once this was achieved, the participants were given notice and a count-down was given for the relative target power to increase. This increased immediately and therefore required the participant to promptly increase the force they resisted. The participant was required, as with the RIT, to keep their left and right cumulative work within the green target band for the entire duration. At recovery, the participant was given notice and a countdown given before the relative target power was reduced to 20W.

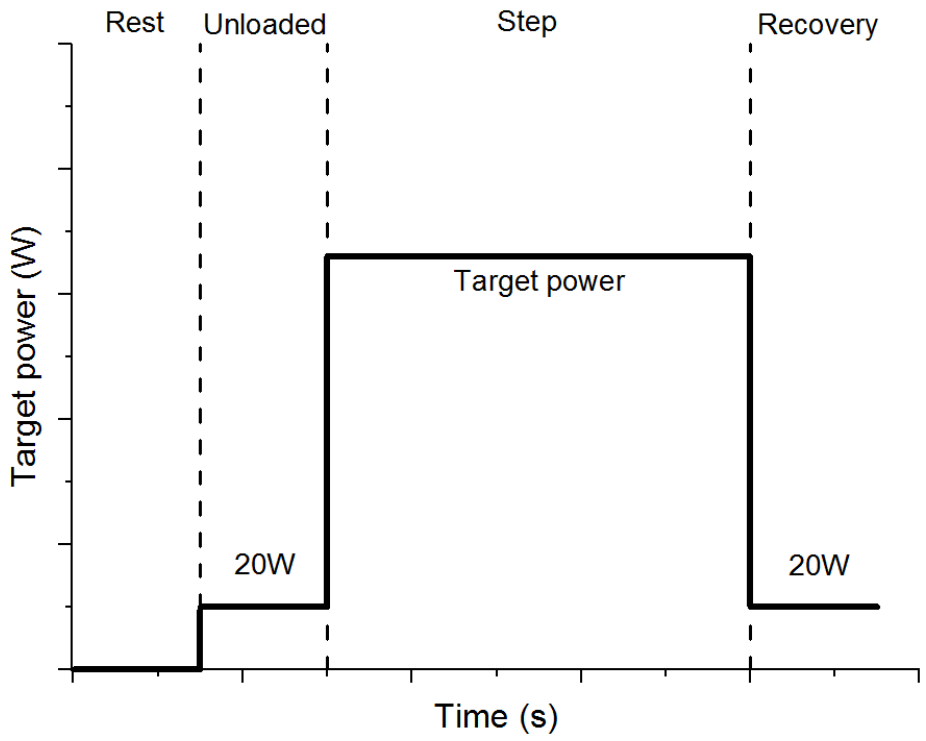

Figure 2.7: Schematic representation of the constant-power tests (CPTs) completed on the recumbent stepping ergometer. A rest period preceded a 20W constant-power phase. This was followed by an instantaneous step increase in relative target power for 15 to 30 minutes. Relative target power reduced to 20W post CPT phase for a recovery period of ~3 minutes.

2.4.4 Voluntary strength assessment

Isokinetic concentric and eccentric, as well as isometric voluntary strength were assessed in all studies using the modified eccentric recumbent stepping ergometer (Eccentron; BTE, Hanover, MD, USA). The common methods used will be highlighted in this section, with any specific alterations detailed as appropriate. All isokinetic assessments were conducted at the same cadence, stride length and seat position as the RITs and CPTs. Voluntary strength assessments were conducted once the participant was fully familiarised with the ergometer (section 2.4.1) and were always conducted on the participant's dominant leg. Following ergometer calibration, the participant performed a 2 minute warm-up where they were instructed to perform both concentric and eccentric exercise at gradually increasing intensities (up to 80% of their self-perceived maximum). For isometric contraction assessment, in order to remain stationary, the dominant leg foot plate was positioned furthest away from the participant. The seat was then moved forwards to elicit 90° of knee flexion, a knee angle commonly used for this assessment (LaStayo et al., 2003; Lastayo et al., 2010; Krishnan et al., 2011; Kim et al., 2014). A note was made of the seat position and used for all subsequent isometric testing. The order of contractions was randomised for all participants and the same order used for future tests. For concentric and eccentric assessment, the participants were instructed to push (concentric) or resist (eccentric) the foot plates at their maximal force for 3 consecutive pedal movements. For isometric assessments, the participant was instructed to push as hard as possible against the stationary foot plate for one to two seconds and asked to relax for three seconds. Each mode of contraction was repeated three times. A count down was provided for all assessments and strong verbal encouragement given throughout.

2.5 Outcome measures

The outcome measures common to experimental chapters will be highlighted within this section. The procedure for measuring work done (i.e. energy expenditure) and relative power output during testing has been detailed in section 2.3.

2.5.1 Anthropometric data

Participant height and mass were measured using a stadiometer (217; Seca, Birmingham, UK) and mechanical column scales (710; Seca, Birmingham, UK), respectively. Both measures were taken within the first participant visit within all studies.

2.5.2 Pulmonary gas exchange

Pulmonary gas exchange data were obtained using a breath-by-breath metabolic cart (MedGraphics D-Series; Medical Graphics Corporation, St Paul, MN, USA). The participant was connected to a preVent® bidirectional pitot tube flow sensor (range of ± 18 $L \cdot s^{-1}$, accuracy of ± 3% or 50 ml, resistance of < 1.5 cm H_20 at 14 $L \cdot s^{-1}$, and dead space of 39 ml) *via* a mouth piece with saliva trap, and wore a nose clip throughout to ensure all expired air was analysed. The flow sensor was connected to the gas analysis system *via* an umbilical which enclosed a gas drying sample circuit as well as a gas sample line that was sampled at a rate of 80-130 $ml \cdot min^{-1}$. Algorithms within the unit correct for the gas transit delay, and gas analysis time to accurately match with the flow signal. The O_2 concentration was analysed using a galvanic electrochemical sensor (range of 0-100%, accuracy of < 1%, response time (10-90%) of < 130 ms and a resolution of ± 0.1%) and the CO_2 levels analysed using a non-dispersive infrared sensor (range of 0-15%, response time of < 130 ms and a resolution of ± 0.1%). The on-board gas analysis software (Breeze suite V7.2, Medical Graphics Corporation, St Paul, MN, USA) sampled at a rate of 100Hz and returns one sample per breath, triggered by the change in pressure generated when switching from exhalation to inhalation.

Prior to each test, the flow and gas sensors of the breath-by-breath metabolic cart were calibrated. Firstly, to correct for between-test differences, the temperature (controlled to 19°C *via* a room air conditioning unit), barometric pressure, and humidity values were obtained *via* a thermometer, barometer and hair hygrometer respectively and entered into the on-board software. The flow sensor was zeroed and a 3 L syringe (Medical Graphics Corporation, St Paul, MN, USA) used to inject 10 x 3.0 L volumes at varied flow rates encompassing physiological flow rates between rest and exercise (acceptance criteria of 3.0 ± 0.02 L used). The O_2 and CO_2 gas sensors were calibrated using certified known gas concentrations (CO_2 0% and 5%, O_2 12% and 21%) which were sampled multiple times to eliminate inconsistent concentrations in sample lines. Calibration gasses were also sampled immediately post-testing to identify if any drift had occurred. A pre to post-test drift greater than 0.2 % identified an invalid test, warranting a repeat to be conducted greater than 48 hr later.

2.5.3 Heart rate

HR was measured using either a 12 (X12+; Mortara Instrument UK Ltd, Stirling, UK) or 3 (FE132 Bio Amp, ADInstruments Ltd, Oxford, UK) lead ECG, as appropriate. Prior to electrode placement, any obstructing hair was shaved, and alcohol wipes used to prepare the skin. Electrodes (BlueSensor R; Ambu Ltd, St. Ives, UK) were placed on relevant bony landmarks in a lead II configuration (3 lead ECG), or in the standard 12 lead configuration. The raw ECG trace was visualised during testing for any cardiac abnormalities *via* the LabChart or Breeze software interfaces. In both instances, the R-R interval of the ECG trace was used to determine HR.

2.5.4 Blood pressure

Resting and within-exercise BP were obtained using an automated sphygmomanometer (UA-787; A&D Medical, Abingdon, UK). A 5-minute resting period preceded cuff placement. The cuff was placed on the upper arm 1-2cm above the elbow crease with the air hose facing distally and in-line with the brachial artery. The arm was placed resting on a table, with the centre of the cuff

at the level of the heart. Three consistent readings were then taken, and the mean systolic and diastolic values calculated. MAP was calculated using the following equation.

$$MAP = SBP + \frac{1}{3}(SBP - DBP).$$

Equation 6.

where “DBP” is diastolic blood pressure and “SBP” is systolic blood pressure.

When BP was taken during exercise on the recumbent stepping ergometer, the left arm was placed on the arm rest and the participant asked to keep the arm as still as possible. Readings were taken every ~45s (30s duration, 15s rest).

2.5.5 Severity of muscle soreness

In order to reduce the severity of DOMS occurring following eccentric exercise, familiarisation sessions were incorporated, and the relative target power gradually increased during training. The severity of muscle soreness was assessed during both training studies (Chapters 4 and 5) *via* the use of a 0-100 visual analogue scale (VAS). Participants were instructed to place a mark on the VAS, with 0 being described as “no pain at all” and 100 being described as “the worst muscle pain you could imagine feeling after exercise”. This score was obtained for the previous session completed, and therefore the participant was asked to assess muscle pain experienced in the 48 hours following the previous session.

2.6 Data analysis

This section will focus on the data processing and analysis that was conducted on raw data obtained in more than one study. Data analysis specific to individual experiments will be expanded in the relevant chapter. LabChart (Labchart 8; ADInstruments Ltd, Oxford, UK), Microsoft Excel (Excel 2013; Microsoft, WA,

USA), and Origin Pro (OriginPro 9.1; OriginLab,MA, USA) were used for data analysis.

2.6.1 Lactate threshold estimation

The estimation of LT from a concentric RIT was crucial to the study design of chapters 3 and 4, enabling WR to be set within the desired exercise intensity domain (section 2.6.2). In the absence of blood La samples, LT can be estimated *via* analysis of the pulmonary gas exchange data. Of note, this intensity demarcator is also commonly referred to in the literature at the anaerobic threshold, gas exchange threshold, and ventilatory threshold, but in general terms refers to the onset of a metabolic acidosis. Blood La samples were subsequently collected in chapter 4 to confirm correct LT estimation.

LT was estimated using a variety of related methods, all time-aligned in an 8-panel plot (Beaver et al., 1986) (Figure 2.8). The V-slope identification of the LT is the most commonly used method, however, cross-checking against other pulmonary gas exchange variables greatly improves the accuracy of estimation (Rossiter, 2011whipp 1986|). The rate of carbon dioxide output ($\dot{V}CO_2$) (V-slope method), end-tidal fractional concentrations of O_2 and CO_2 ($F_{ET}O_2$ and $F_{ET}CO_2$), the ventilatory equivalents of O_2 and CO_2 ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$) and the respiratory exchange ratio (RER) were all plotted against the rate of $\dot{V}O_2$.

As exercise WR progresses from moderate to heavy intensity exercise, a resulting metabolic acidosis occurs. To combat the elevation in H^+, bicarbonate (HCO_3^-) is reduced to CO_2 and water (H_2O) as a major element of pH buffering in the blood:

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

Equation 7.

where "CO_2" is carbon dioxide, "H_2O" is water, "H_2CO_3" is carbonic acid, "HCO_3^-" is bicarbonate and "H^+" is hydrogen ions.

This results in a further increase in $\dot{V}CO_2$, and therefore the rate of increase in $\dot{V}CO_2$ now exceeds the rate of increase in $\dot{V}O_2$ levels, causing a break point in the V-slope graph (Figure 2.8, panel A).

At WRs greater than LT, a phase termed isocapnic buffering begins, which is essential to the estimation of LT as it confirms that the elevation in $\dot{V}CO_2$ is not due to hyperventilation. During this stage, although $\dot{V}E$ increases proportionally with $\dot{V}CO_2$, it increases disproportionally to $\dot{V}O_2$, bringing about an increase in $F_{ET}O_2$ and the ventilatory equivalent of O_2 ($\dot{V}E/ \dot{V}O_2$; Figure 2.8, panel B and C). The increase in $F_{ET}O_2$ and $\dot{V}E/ \dot{V}O_2$ with no change in $F_{ET}CO_2$ and $\dot{V}E/ \dot{V}CO_2$ can therefore be used to support the determination of LT from the V-slope method (Beaver et al., 1986; Whipp and Wasserman, 1986).

As exercise WR becomes progressively harder, bicarbonate stores become depleted and cannot constrain the rate of H^+ production. The pH therefore falls, causing a metabolic acidosis. This increased acidity likely stimulates peripheral chemoreceptors which increase activity of the respiratory centre in the medulla oblongata (see section 1.4) resulting in hyperventilation, a phase termed respiratory compensation. This respiratory compensation then results in a further increase in $\dot{V}E$ that is above that of $\dot{V}CO_2$, thus, the ratio of $\dot{V}E$ to $\dot{V}CO_2$ increases and a break point emerges (Figure 2.8, panel E). Hyperventilation with respect to $\dot{V}CO_2$ causes more CO_2 to be eliminated at the lungs, also resulting in a reduction in $F_{ET}CO_2$ (Figure 2.8, panel B).

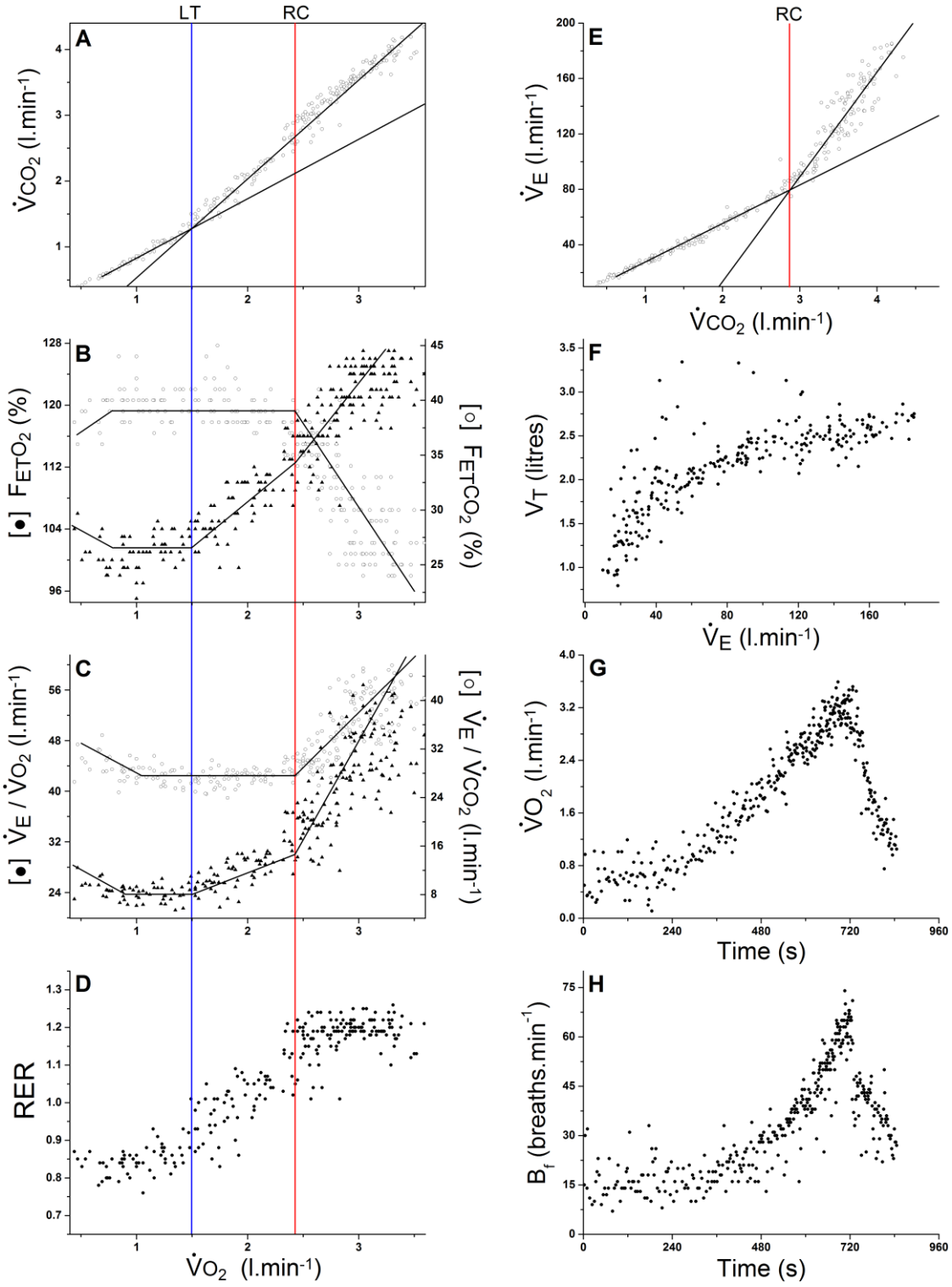

Figure 2.8: Example 8 panel plot of raw breath-by-breath pulmonary gas exchange RIT data. Blue line represents the estimation of lactate threshold (LT) and red line the estimation of respiratory compensation (RC). Panel (A). "V-slope" graph (pulmonary CO_2 output/pulmonary O_2 uptake; $\dot{V}CO_2/\dot{V}O_2$). Panel (B). End-tidal fraction of O_2 ($F_{ET}O_2$) and CO_2 ($F_{ET}CO_2$) relative to $\dot{V}O_2$. Panel (C). Ventilatory equivalents for O_2 and CO_2 ($\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$) relative to $\dot{V}O_2$. Panel (D). Ratio of $\dot{V}CO_2$ to $\dot{V}O_2$ (Respiratory Exchange Ratio; RER) relative to $\dot{V}O_2$. Panel (E). Ventilation ($\dot{V}_E$) relative to $\dot{V}CO_2$. Panel (F). Tidal volume (V_T) relative to $\dot{V}_E$. Panel (G). $\dot{V}O_2$ against time. Panel (H). Breaths per minute (B_f) against time.

Although these methods of determining LT have a sound physiological basis, it is important to emphasise that it involves estimations which can introduce error. One example is when hyperventilation exists prior to the ramp-incremental stage of exercise (see Figure 2.9). Hyperventilation causes more CO_2 to be breathed out resulting in a drop in end tidal CO_2 ($F_{ET}CO_2$), an indirect measure of the arterial partial pressure of CO_2 (PCO_2). During incremental exercise, extra CO_2 produced at the muscle is diverted to replenish the low PCO_2 stores. Therefore, less CO_2 is breathed out and results in a reduced $\dot{V}CO_2$ slope. Once stores are replenished, extra CO_2 is once again breathed out and results in a kick up in the V-slope graph (Figure 2.9, panel D). This culminates in a pseudo-threshold and an underestimated LT. As detailed in section 2.4.2, we ensured that the RER was stable and between 0.7 to 0.9 before progressing with the test (i.e. no hyperventilation). This ensured that a pseudo-threshold did not exist, allowing for accurate estimation of LT.

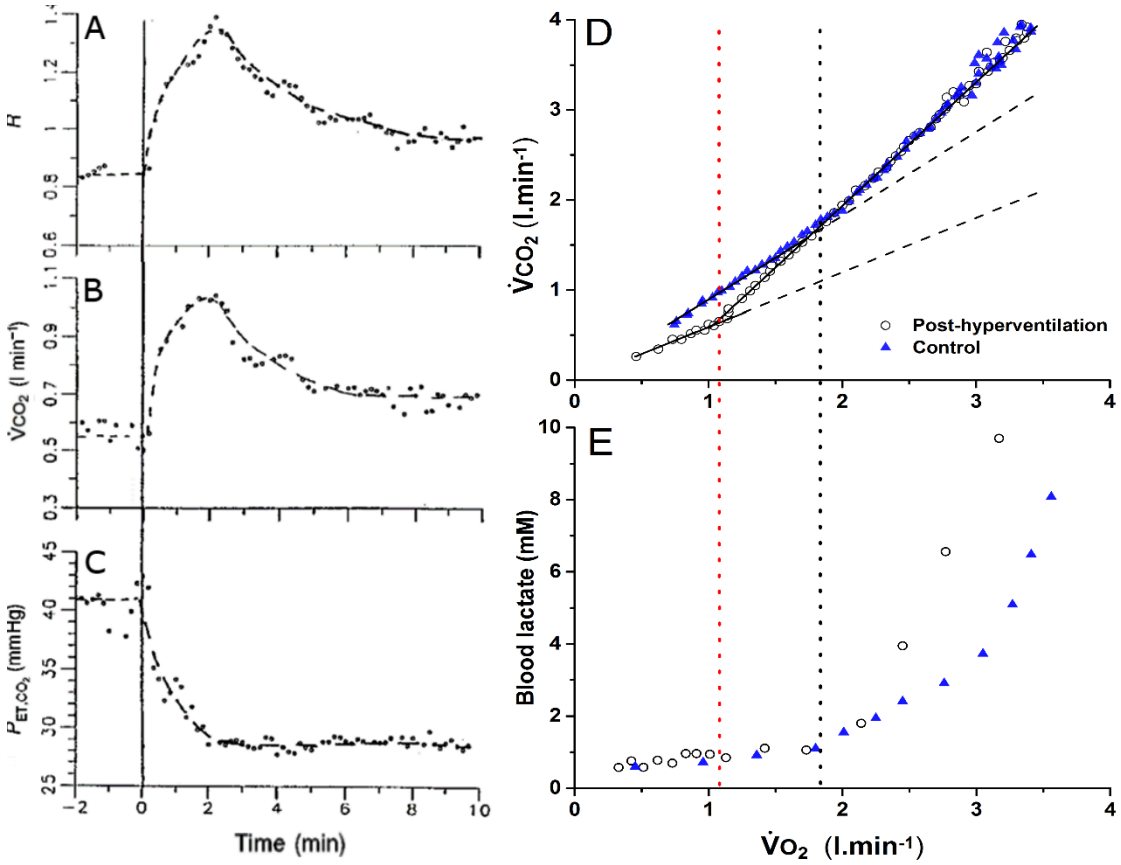

Figure 2.9: Effect of hyperventilation on LT estimation. Panel A, B and C show the effect of voluntary hyperventilation (onset at vertical solid black line) on respiratory exchange ratio ($\dot{V}CO_2$:$\dot{V}O_2$; R), CO_2 output ($\dot{V}CO_2$) and end tidal CO_2 tension ($P_{ET.CO2}$). Panel D shows the estimation of a representative subjects' LT from incremental tests both with (open circles) and without (blue triangles) hyperventilation. Red dotted line represents V-slope estimation post-hyperventilation, whilst the black dotted line represents V-slope estimation without hyperventilation (control). Panel E shows the measured blood lactate levels at the corresponding $\dot{V}O_2$. Adapted from (Ozcelik et al., 1999).

2.6.2 Determination of target WR from LT

The collection of breath-by-breath data during RITs allowed the subsequent setting of CPT relative power to match for a target metabolic demand. All constant-power exercise was matched to be within the moderate intensity domain, i.e. below LT. The target metabolic demand was chosen to be 90% of the LT. The LT determined from exercise conducted on the recumbent stepping ergometer was used as constant-power exercise would be performed on this ergometer, and because LTs determined during cycle ergometry are significantly higher (determined from pilot testing, likely due to a larger active muscle mass in upright *vs.* recumbent sitting). As the same $\dot{V}O_2$ would be used to match for

metabolic demand between concentric and eccentric recumbent stepping exercise, the LT from only one of the RITs needed to be used. The data was considerably less noisy in the concentric RIT and was therefore adopted for standard use.

The methods used to determine the relative power required to achieve 90% of the concentric LT are detailed in Figure 2.10. 60s of work was subtracted from the relative power identified due to the time delay that occurs in $\dot{V}O_2$ recorded at the mouth compared to its production in muscle during incremental exercise. This delay is termed the mean response time and comprises the delay for blood to travel from the exercising muscles to the lungs (cardio-dynamic phase) as well at the time constant for the phase II response of pulmonary $\dot{V}O_2$ kinetics (Jones and Poole, 2005).

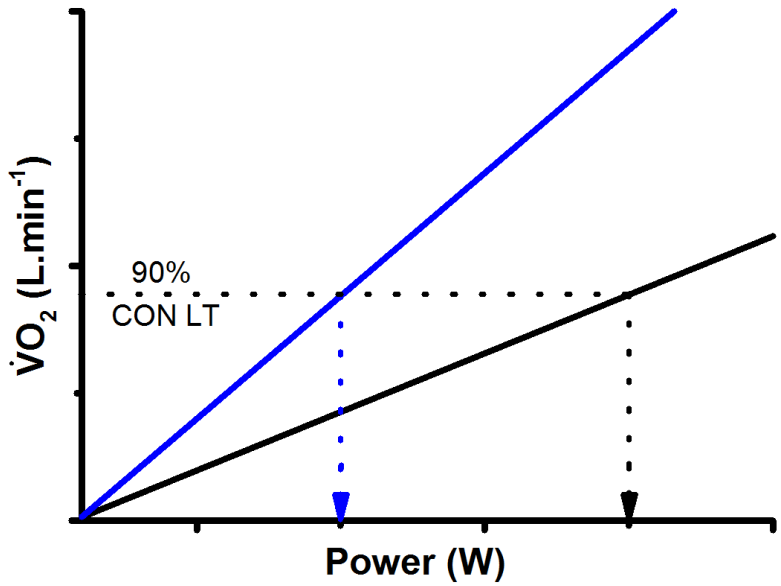

Figure 2.10: Schematic of the typical $\dot{V}O_2$ responses during ramp-incremental recumbent stepping exercise, and the methods used to determine the relative target power for CPTs. The intercept of the target $\dot{V}O_2$ (90% CON LT; horizontal dashed line) with the concentric (solid blue line) or eccentric (solid black line) RIT $\dot{V}O_2$ responses, identifies the target relative power. 60s of power (7W) was further subtracted to account for the time delay present with incremental exercise.

2.6.3 Force/power

The majority of data exported from the LabChart software that ran the protocols on the recumbent stepping ergometer required no further processing. The process of converting work performed on the recumbent stepping ergometer into relative power, as well as the determination of peak relative power will be described here. Concentric and eccentric muscle contractions are not mechanically equivalent with eccentric muscle contractions involving energy absorption (negative power) and concentric contractions energy liberation (positive power) (Abbott and Aubert, 1951; Asmussen, 1953). However, to simplify presentation of results and provide consistency with recent studies reporting the mechanical requirements of concentric and eccentric exercise (LaStayo et al., 2000; Dufour et al., 2004; Elmer et al., 2017), all eccentric relative powers will be reported as positive.

2.6.3.1 Conversion to Watts (W)

To convert the raw force (N) obtained from the ergometer into power (W), the following equation was used:

$$P = F * V,$$

Equation 8.

where “P” is mechanical power (W), “F” is mechanical force (N) and “V” is velocity of the ergometer foot plates ($m.s^{-1}$).

To determine the velocity of the foot plates, the equation needs to be expanded:

$$V = \left(C * \left(R * rad_{spm}\right)\right)/60,$$

Equation 9.

where “V” is velocity of the ergometer foot plates ($m.s^{-1}$), “C” is the cadence of the foot plates (steps per minute; spm), “R” is the radius of the arc (metres, m), and “rad_{spm}” is the displacement of the foot plate in one step (radians; rad) (see Figure 2.11).

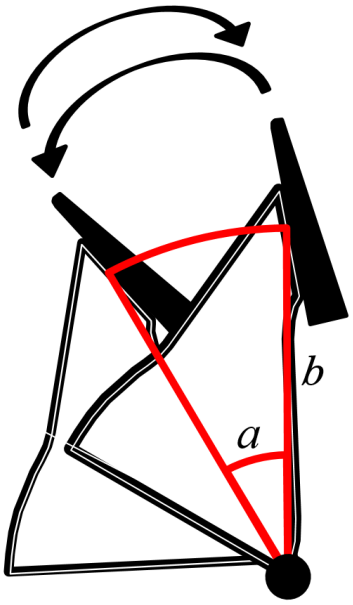

Figure 2.11: Measurement obtained from the recumbent stepping ergometer to enable the conversion of force (N) into mechanical power (W).

Arrows represent the forward and backward movement of the foot plates during stepping exercise, "● " represents the axis of rotation of the foot plates, "a" represents the degree of displacement of the foot plate relative to the axis of rotation, and "b" represent the radius of the arc (distance from the axis of rotation to the point where force is applied to the load cell).

The cadence and stride length of the foot plates can be changed within the ergometer settings, however a constant cadence of 23 spm and a stride length of 2 (13° displacement) were used throughout all experiments. The radius of the arc, measured as the distance from the axis of rotation of the ergometer pedal to the loading point of the load cell (see Figure 2.11) remained a constant of 0.54m throughout all testing. The angular displacement per pedal cycle (radians) was converted from degrees of displacement using the following equation:

$$rad_{spm} = (deg_{dis} * 2) * \frac{\pi}{180},$$

Equation 10.

Where "rad_{spm}" is the foot plate displacement completed in one step (rad), and "deg_{dis}" is the degree of displacement of the foot plate (°).

The degree of displacement of the foot plates were measured whilst the foot plates were stationary at the point of maximal displacement. The fulcrum of a

long arm goniometer was placed at the axis of rotation, the stationary arm positioned in line with the centre of one foot plate, and the moveable arm at the centre of the other (Figure 2.11). One step involves both the forward and backward movement of the foot plate, and therefore the degree of displacement was doubled to obtain the displacement completed in one step.

Based on the settings used throughout all studies (cadence = 23 spm, stride length = 2, radius of the arc = 0.54m), the following equation could be used to calculate average power:

$$P = F * 0.094,$$

Equation 11.

where “P” is power (W), “F” is force measured at the foot plates (N), and 0.094 is the velocity of the ergometer foot plates ($m.s^{-1}$). For example, if the force applied to the load cell was 500N, this would be equivalent to a power of 47W.

2.6.3.2 Determination of RIT peak power

The use of a consistent criteria to determine the LoT within the RITs (see section 2.4.2) allowed the use of ramp duration to determine peak power achieved. The following equation was used:

$$Peak\ RIT\ power = (t * ramp\ rate) + unloaded\ intesity$$

Equation 12.

where “t” is time in minutes, “ramp rate” is the increment in target power per minute ($W \cdot min^{-1}$) and “unloaded intensity” is the target power during unloaded recumbent stepping/cycling (W).

2.6.3.3 Peak force (voluntary strength assessment)

To determine the peak force produced during the voluntary strength assessments, the peak analysis module within the LabChart software (Labchart 8; ADInstruments Ltd, Oxford, UK) was used. This analyses the raw left or right force plate data and determines the peak force value for each contraction. A visual check was conducted to ensure the peak of each force profile was accurately detected. In the case of incorrect detection, the peak value was manually determined within LabChart.

2.6.4 Pulmonary gas exchange

2.6.4.1 Filtering

Raw pulmonary gas exchange data were filtered within Origin pro to remove abnormal breaths such as coughs or sighs, a common occurrence when breathing with a mouth piece and nose clip (Lamarra et al., 1987; Rossiter et al., 2000). It has also been observed that the signal to noise ratio is higher during concentric and eccentric stepping exercise conducted on the recumbent stepping ergometer. Algorithms within the breath-by-breath software detect the start and end of each breath, and return the mid breath value. However, during these abnormal breaths, the signal to noise ratio can be too high for the algorithms to return a correct value.

To avoid elimination of true data, a conservative approach was used during filtering which yields less than a 0.005% chance of deleting true physiological data. A linear regression with 99% prediction bands (four standard deviations about the local mean) was fit to both the unloaded/warm up period, as well as the CPT/RIT $\dot{V}O_2$ data. Data points lying outside of these bands were manually deleted. Removal of these breaths are justified by the fact that they do not represent the underlying metabolic response.

2.6.4.2 Interpolation

To time align the pulmonary gas exchange data with other data collected, and to allow accurate minute-by-minute binning of data, the breath-by-breath data were linearly interpolated using Origin Pro. This method involved a linear fit between 2 known points and interim data being extrapolated to fit on this regression line.

2.6.4.3 RIT gas analysis

Following filtering and linear interpolation, RIT $\dot{V}O_2$ data were analysed to determine unloaded $\dot{V}O_2$, functional gain ($\dot{V}O_{2gain}$) and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$). Unloaded $\dot{V}O_2$ was determined by taking a mean of the final minute of unloaded data, and functional gain ($\Delta\dot{V}O_2/\Delta WR$; $ml \cdot min^{-1} \cdot W^{-1}$) determined by fitting a linear regression line within Origin Pro to the linear region of the $\dot{V}O_2$/power relationship (Ferguson et al., 2007).

$\dot{V}O_{2max}$ and $\dot{V}O_{2peak}$ are often incorrectly used interchangeably (Whipp, 2010). $\dot{V}O_{2max}$ is defined as the highest attainable $\dot{V}O_2$ achieved during an incremental exercise test and is primarily characterised by the presence of a plateau in the $\dot{V}O_2$ response. On the other hand, $\dot{V}O_{2peak}$ refers to the maximal $\dot{V}O_2$ achieved during an incremental test regardless of a plateau occurring (Day et al., 2003). As highlighted by Day *et al.,* (2003), only ~17% of incremental tests present with a plateau, resulting in incorrect presentation of $\dot{V}O_{2max}$ results. To accurately verify $\dot{V}O_{2max}$ attainment, a secondary supramaximal CPT at a WR of ~90% of peak WR is often utilised (Rossiter et al., 2006). Based on the difficulty of reporting $\dot{V}O_{2max}$, the increased participant demand, and the inherently noisier breath-by-breath data obtained during recumbent stepping exercise, $\dot{V}O_{2peak}$ will be reported throughout all results. $\dot{V}O_{2peak}$ was identified as the maximum 12-breath moving average during the last 20 breaths of the RIT. This method ensures that breath-by-breath noise has minimal impact on $\dot{V}O_{2peak}$, whilst not including too many breaths that could underestimate the rapid change in $\dot{V}O_2$ and reduce the $\dot{V}O_{2peak}$ estimation (Rossiter et al., 2006).

2.6.4.4 CPT gas analysis

Following filtering and linear interpolation, CPT $\dot{V}O_2$ data were analysed to determine unloaded $\dot{V}O_2$, minute $\dot{V}O_2$ means, and the $\Delta\dot{V}O_2$ increase from predicted steady state. Unloaded $\dot{V}O_2$ was identified as explained above, and minute $\dot{V}O_2$ means determined automatically within OriginPro by fitting a linear regression line with slope set to "0". Based on the CPT exercise being matched within the moderate-intensity exercise domain, it would be expected that a steady-state would be reached by 3 minutes into the test (see section 1.2). Therefore, the $\Delta\dot{V}O_2$ was calculated as the change in $\dot{V}O_2$ between the third and last minute of CPT exercise.

2.6.5 Heart rate

2.6.5.1 Processing/filtering

In the case of noisy HR data, R wave determination parameters were altered within the LabChart cyclic calculation options. The "minimum peak height", "QRS width" and "minimum period" were adjusted, and the raw ECG trace monitored to ensure the R waves were being accurately detected. When LabChart was unable to accurately detect all R waves, the data was filtered post exportation. A conservative approach was used, with 99% prediction bands (four standard deviations about the local mean) fit to the unloaded and RIT linear portions of the HR trace. Data points lying outside of these bands were manually deleted.

2.6.5.2 Ramp-incremental test heart rate

Following ECG processing and filtering, unloaded HR, increment in HR with relative power (HR_{gain}), and peak HR (HR_{peak}) were determined. Unloaded HR was determined by taking a mean of the final minute of unloaded data. HR_{gain} was determined by fitting a linear regression line to the linear region of the HR/power response (generally at values above 100bpm). Only data obtained from the recumbent stepping ergometer RITs displayed a consistent linear increase in HR and therefore, determination of the HR_{gain} from cycle ergometer

RITs was not possible. HR_{peak} was manually determined as the maximum value attained during the RIT phase.

2.6.5.3 Constant-power test heart rate

Following ECG processing and filtering, unloaded HR, minute HR means and ΔHR from predicted steady state were determined as described with $\dot{V}O_2$ data above.

2.7 Statistics

Specific statistical analysis methods are detailed within the relevant study chapter. Briefly, all data is presented as means ± standard error of the mean (SE). Statistical analysis was conducted using GraphPad Prism (GraphPad v6.0, Graphpad Software Inc, CA, USA) and significance determined when $P < 0.05$. All data was tested for normal distribution and relevant nonparametric tests used when parametric test assumptions were not met. Adjusted Bonferroni *P* values are presented when further post-hoc analysis was conducted.

Chapter 3 Acute responses of concentric *vs.* eccentric recumbent stepping.

3.1 Introduction

Eccentric exercise lowers both the cardiovascular (up to 35% reduction in HR) and metabolic (up to 70% reduction in $\dot{V}O_2$) requirement of concentric exercise at the same relative power (Dufour et al., 2004). Therefore, when exercise is matched for the same metabolic demand as concentric, eccentric exercise allows for up to a 500% greater relative power to be performed (Dufour et al., 2004), which likely translates into greater voluntary strength and muscle mass increases when incorporated within training programs (Roig et al., 2009; Isner-Horobeti et al., 2013). Crucially, the ability to resist higher forces at lower cardiovascular and metabolic requirements may be beneficial for patient groups exhibiting exercise intolerance such as COPD, CHF or older adults. Providing a higher training stimulus is likely to translate into greater increases in voluntary muscle strength and mass, increase mobility, and ultimately QoL than seen with traditional (concentric) exercise regimes. Importantly, voluntary strength increases are consistently shown in functional muscle groups such as the knee flexors and extensors, with increases of 77% compared with 37% with concentric training (Miller et al., 2006). Exercise rehabilitation currently advocated for exercise-intolerant populations consists predominantly of concentric or mixed modes of contractions, potentially limiting recovery potential. Adherence to group classes such as pulmonary rehabilitation is poor (Hayton et al., 2013), likely due to the slow progression of noticeable improvements in mobility and strength, and the high cardiovascular and metabolic requirements of the tasks. Although rehabilitation leads to significant functional, psychological and economical cost benefits (Almeida and Rodrigues, 2014), utilising eccentric exercise may provide a superior exercise modality to further enhance these.

Although we have a greater understanding of the central pulmonary $\dot{V}O_2$ demand of eccentric exercise, intramuscular oxygenation responses during eccentric exercise remains under investigated, with only one study assessing changes during constant-power exercise (Penailillo et al., 2017). Responses during concentric ramp-incremental cycling have shown that, despite previous assumptions of a hyperbolic profile, a sigmoidal response of desaturation with mechanical power increment occurs (Ferreira et al., 2007). It remains unclear whether eccentric RIT changes in muscle oxygenation would be similar, and NIRS may provide an effective, non-invasive estimation of changes in muscle O_2 extraction during eccentric exercise (DeLorey et al., 2003). A study by Penailillo et al. (2017) utilised NIRS to compare % changes in vastus lateralis muscle oxygen saturation during power-matched concentric and eccentric constant-power cycling. Based on the assumption that the majority of pulmonary $\dot{V}O_2$ changes are a result of exercising muscle oxygen utilisation (Poole and Richardson, 1998), and that whole body pulmonary $\dot{V}O_2$ is lower during eccentric compared with concentric exercise, it would be expected that peripheral muscle oxygen utilisation would mirror this reduction. They confirmed this hypothesis, showing a 16% higher eccentric tissue saturation in comparison to power-matched concentric exercise. This reduction, however, was less than expected, likely due to differential muscle group recruitment and hence contributions to total pulmonary $\dot{V}O_2$.

As detailed previously, the majority of eccentric exercise research has utilised reverse cycle ergometry (Isner-Horobeti et al., 2013; Hoppeler, 2015), were a motor drives the bike cranks in reverse whilst the participant resists the pedal movement. Unlike previous interventions, cycle ergometry allows tighter control of exercise parameters such as cadence, joint angles and power, and therefore provides an effective modality to utilise within a research setting (Hoppeler, 2015). However, reverse cycle ergometry likely provides an un-natural movement that doesn't represent activities perform on a daily basis. It has previously been shown that training that mirrors the intended task to be performed, especially using the same muscle groups, joint angles and velocities, translates into greater physiological and performance gains (Morrissey et al., 1995; Stone et al., 2000; Millet et al., 2002; Misic et al., 2009; Garber et al.,

2011). Consequently, there is a need for an eccentric modality that mirrors natural locomotion, whilst maintaining tight control of exercise parameters. These combined will hopefully translate into a more effectively research tool, and provide better improvements in everyday mobility and QoL within exercise-intolerant clinical populations.

We have therefore utilised an eccentric recumbent stepping ergometer which may more closely mirror natural activities like downhill walking or stair descent than other eccentric modalities (see section 2.3 for details). This ergometer retains fine control of exercise parameters present in eccentric cycle ergometry, whilst reducing the safety issues previously identified (Hoppeler, 2015). It is unknown, however, if the reduction in cardiovascular and metabolic demand, present with reverse cycle ergometry, also applies to this modality, or if increasing relative power during eccentric recumbent stepping exercise to match for concentric metabolic demand can be sustained for long periods of time.

The primary aims of this study were: 1) to compare the cardiovascular and metabolic responses that occur during concentric and eccentric ramp-incremental stepping exercise; 2) to compare the concentric and eccentric cardiovascular and metabolic responses during constant-power stepping exercise matched for either the same relative power, or same metabolic ($\dot{V}O_2$) demand.

We hypothesised that: 1) cardiovascular and metabolic responses will increase at a slower rate during the eccentric RIT; 2) peak relative power will be higher during the eccentric RIT; 3) peak cardiovascular and metabolic responses will be lower during the eccentric RIT; 4) cardiovascular and metabolic responses will be lower during eccentric exercise matched for the same relative power; 5) matching for $\dot{V}O_2$ requirement will be tolerable and allow a greater relative power to be performed eccentrically.

3.2 Methods

This study was approved by the Faculty of Biological Sciences Ethical Committee for non-clinical research (University of Leeds, BIOSCI 13-028), and complied with the latest version of the Declaration of Helsinki. Common protocols, measurements and equipment are detailed in Chapter 2 and will therefore only briefly be described here.

3.2.1 Participants

Eight healthy, recreationally active (5 male, 3 female) volunteers took part (mean ± SE; 22 ± 1 yr, 70 ± 3 kg, 172 ± 2 cm). Participants were screened using a health and activity status questionnaire, as well as inclusion and exclusion criteria to determine their suitability for participation and any contraindications to exercise. Consent forms were signed by participant, lead researcher, and assessor prior to commencement of testing. The inclusion and exclusion criteria were as follows:

Inclusion criteria: Healthy male or females between the ages of 18-45 years.

Exclusion criteria: 1) Any active medical disorder which may alter physiological responses; 2) use of prescription medication or over the counter preparations that may influence exercise performance; 3) a recent illness or viral infection (within the last two weeks); 4) use of recreational or performance enhancing drugs; 5) ingestion of alcohol in the previous 24 hours; 6) history of anaemia, asthma, diabetes, epilepsy, family history of sudden death, fainting, heart disease, high BP, respiratory disease, muscle or joint injury; 7) unable to provide informed consent or understand English; 8) current or suspected pregnancy; 9) recent intense exercise <48 hours pre testing.

3.2.2 Stepping ergometer

All testing was performed on a modified eccentric recumbent stepping ergometer (Eccentron; BTE, Hanover, MD, USA). Details of all modifications, calculations and calibrations are detailed in section 2.3. Raw left and right force (N) data were obtained from the load cells positioned beneath each foot plate and fed into data acquisition software (Labchart 8; ADInstruments Ltd, Oxford, UK). Load cells were calibrated under no load before every test. All participants were set up with identical criteria for all tests: a goniometer was used to determine the seat position which resulted in knee range of movement between 90° and 30° of flexion, cadence was permanently set at 23 spm, and stride length at setting 2 of 3 (13° foot place displacement). Throughout all RITs and CPTs, participants viewed the same custom screen developed within the data acquisition software (LabChart 8; ADInstruments Ltd, Oxford, UK) (see section 2.3.5), consisting of two lines representing left and right cumulative work (integrated N), overlaid onto a green band that identified ± 2% of the target. This tight band ensured a high accuracy of WR control, particularly important as eccentric exercise requires the participant to actively meet the target work, rather than the ergometer altering its resistance. This also ensured that the participants produced the same cumulative work for each leg, not often achieved with traditional cycle ergometry, enabling the collection of muscle oxygenation from one leg during testing.

3.2.3 Familiarisation

Prior to the commencement of testing, participants attended up to three familiarisation sessions to ensure accurate control of WR, to reduce the severity of muscle soreness post-testing, and to reduce the anxiety response that often occurs with exercise testing (Pescatello, 2013). Participants attended a minimum of two sessions on separate days (both concentric and eccentric familiarisation completed) and were deemed familiarised when they were able to maintain their left and right cumulative work within the target band (± 2% of target) for 2 minutes. A 3-lead ECG was recorded and the trace visualised throughout testing to screen for any untoward cardiovascular events.

3.2.4 Experimental design

Following familiarisation, five exercise testing sessions were completed in a random order (two RITs followed by three CPTs) ≥ 48 hours apart. ECG, breath-by-breath pulmonary gas exchange, NIRS, and raw force data were collected throughout all protocols. Participants were required to refrain from intense exercise and alcohol consumption in the 24 hours preceding, as well as food and caffeine ingestion in the 3 hours preceding.

3.2.4.1 Ramp-incremental tests

The RIT protocol followed is identical to that detailed within section 2.4.2, (*Figure 2.6*). Briefly, each RIT protocol consisted of: 1) a 2 minute rest period. 2) ~3 minutes of unloaded stepping at 20W. 3) 7W·min^{-1} RIT to the LoT. 4) ~3 min of recovery at 20W. Participants were required to maintain their left and right cumulative work within the target band (± 2 %) throughout.

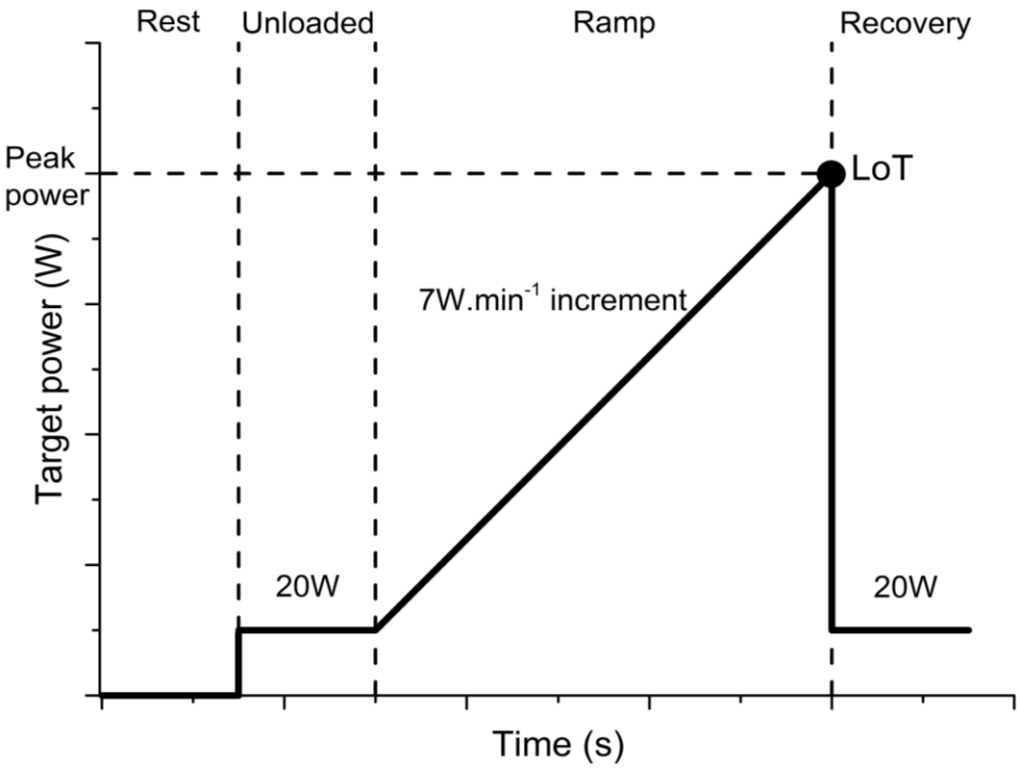

Figure 3.1. Schematic representation of the ramp-incremental tests (RITs) completed on the stepping ergometer. A rest period preceded a 20W constant-power phase. This was followed by a linear increase in target power of 7W·min^{-1}, culminating in the limit of tolerance (LoT, ●). At the LoT, the target power reduced to 20W for a recovery period of ~3 minutes.

3.2.4.2 Constant power tests (CPTs)

Following the RITs, three CPTs were completed in a randomised order matched for both relative power and metabolic response ($\dot{V}O_2$) between concentric and eccentric exercise. The LT of the concentric RIT was first estimated by three experienced assessors (see section 2.6.1). 90% LT was chosen as the metabolic target (government guidelines for exercise prescription; Bull et al. (2010)) placing participants within the upper limit of moderate intensity exercise. The methods to determine target CPT relative power from the concentric and eccentric RIT results are detailed in section 2.6.2). The power required to achieve this metabolic target during the concentric RIT was used for a concentric (concentric moderate intensity (CON_{MI})) and eccentric (eccentric power matched (ECC_{PM})) CPT. The relative power required to achieve the same metabolic target during the eccentric RIT was used for the final eccentric CPT (eccentric $\dot{V}O2$ matched ($ECC_{\dot{V}O2}$). The protocol followed was identical to that detailed in section 2.4.3. Briefly, each CPT comprised of: 1) A 2 minute rest period; 2) ~3 minutes of

unloaded stepping at 20W; 3) 15 minutes of concentric or eccentric recumbent stepping at the target relative power; 4) ~3 min of recovery at 20W.

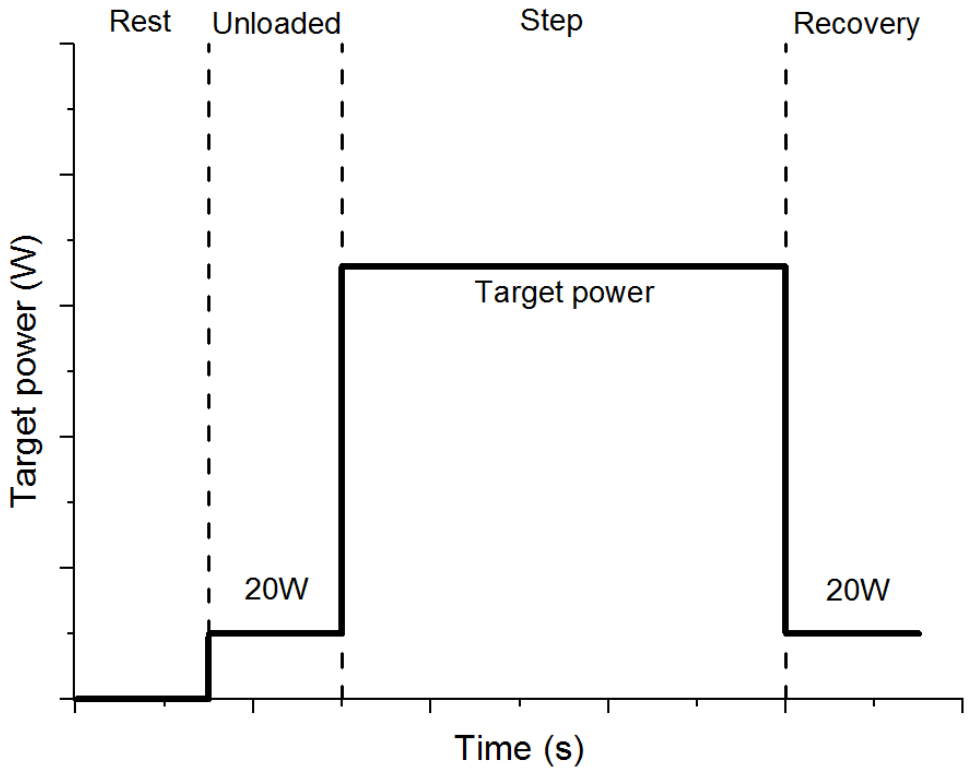

Figure 3.2. Schematic representation of the constant-power tests (CPTs) completed on the stepping ergometer. A rest period preceded a 20W constant-power phase. This was followed by a step increase in target power for 15 to 30 minutes. Target power reduced to 20W post step phase for a recovery period of ~3 minutes.

3.2.5 Outcome measures

3.2.5.1 Mechanical force and power

Force acquisition and unit conversion is detailed in section 2.3. In brief, raw force data were obtained from calibrated load cells positioned behind the left and right foot plates of the stepping ergometer and were fed into a data acquisition system (ML870 PowerLab 8/30; ADInstruments Ltd, Oxford, UK) connected to a PC for processing (Labchart 8; ADInstruments Ltd, Oxford, UK). Mechanical force (N) was post-hoc converted into power (W) *via* the equations previously detailed.

3.2.5.2 Pulmonary gas exchange

Pulmonary gas exchange data were collected with a breath-by-breath gas analysis system (MedGraphics D-series; Medical Graphics Corporation, St Paul, MN, USA; see section 2.5.2 for details). Briefly, the pneumotach and gas sensors were calibrated using flow rages and gas concentrations that encompass normal physiological values between rest and peak exercise. Gases were sampled immediately after testing to assess if any drift had occurred. Participants were connected to the gas analysis system *via* an umbilical line and mouthpiece, and wore a nose clip to ensure all expired air was analysed. Data collection was started at the same time as the master clock to ensure all data could be subsequently time aligned.

3.2.5.3 Heart rate

ECG data were obtained at a rate of 1kHz using a 3-lead bio amplifier unit (FE132 Bio Amp, ADInstruments Ltd, Oxford, UK) positioned in a lead II configuration. The raw ECG traces were visualised during testing to screen for any untoward cardiovascular events, and the R-R interval used to calculate heart (HR) in real time.

3.2.5.4 Blood pressure

Details of BP collection are detailed in section 2.5.4. In short, systolic (SBP) and diastolic (DBP) BP were obtained three times at rest and every ~45 seconds during testing using a portable automatic digital brachial cuff device (UA-787, A&D Medical, Abingdon, UK). The cuff was positioned at the level of the heart with the sample line aligned at the point of the brachial artery. The participant was encouraged to keep the arm as still as possible during testing to avoid movement artefacts.

3.2.5.5 Muscle oxygenation

The relative changes in the concentrations of oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb) and total haemoglobin (tHb) of the rectus femoris (RF) muscle were assessed using continuous wave NIRS (PortaMon Mk II; Artinis medical systems, Elst, Netherlands). This small device consists of three transmitters which all emit two wavelengths of infrared light (760 and 850 nm). Infrared light passes through the skin and muscle and scatters back to the receiver. The distance between the transmitters and receivers was 30, 35, and 40 mm.

NIRS data was collected at a rate of 10Hz from the RF muscle of the participants' right leg and was fed into a real-time data collection and graphing program (Oxysoft, Artinis medical systems, Elst, Netherlands). Anatomical landmarks were used to standardise the position of the device between participants and subsequent visits (European recommendations for surface electromyography), (Hermens et al., 1999). The sensor was wrapped in one layer of cling film to prevent moisture damage to the device, and was securely wrapped in black opaque cloth to prevent external light interference. The film over the transmitters and receivers were checked to ensure they lay flush with no obscuring folds.

3.2.6 Data analysis

3.2.6.1 Pulmonary gas exchange

$\dot{V}O_2$ processing and analysis is detailed in section 2.6.4. To summarise, $\dot{V}O_2$ data were filtered (99% prediction bands) to exclude erroneous breaths lying outside of the expected physiological response. The LT was estimated by three experienced assessors and a mean value determined. All data were linearly interpolated to provide second by second data, allowing time alignment with other outcome measures and calculation of global means. Unloaded $\dot{V}O_2$ was identified by taking a mean of the last minute of unloaded stepping. $\dot{V}O_{2gain}$ was identified by fitting a linear regression to the linear phase of the RIT $\dot{V}O_2$/power relationship. $\dot{V}O_{2peak}$ was identified as the maximum 12 breath moving average during the last 20 breaths of the RIT. For the CPTs, data is presented as second

by second data and minute means calculated for statistical analysis. The differences between the 3rd (sufficient time to allow attainment of a steady state) and 15th minute means were calculated to quantify the change in $\dot{V}O_2$ from the predicted steady state value.

3.2.6.2 Heart rate

ECG processing and analysis of HR is detailed in section 2.6.5. Briefly, unloaded HR was determined as the mean of the last minute of unloaded stepping, and HR_{gain} by fitting a linear regression to the linear phase of the RIT HR/power relationship. HR_{peak} was determined as the highest HR recorded during the RIT phase.

3.2.6.3 Blood pressure

MAP was calculated as $DBP + {}^{1}/_{3} * (SBP - DBP)$. Due to the low time resolution present with these BP recordings, a mean of the last 10 minutes of the CPT is presented. Recording BP responses during RIT exercise was not possible due to excessive movement of the arm at high target powers.

3.2.6.4 Near-infrared spectroscopy

All NIRS data was exported at 1Hz and a blood volume correction factor applied to the raw HHb values as described by Ryan et al. (2012). This method eliminates the contribution of additional HHb due to increased blood volume. A blood volume correction factor (β) was initially calculated for each data point using the following equation:

$$\beta = \frac{O_2Hb}{O_2Hb + HHb}$$

Equation 13.

where "β" is the blood volume correction factor, "O_2Hb" is the raw oxyhaemoglobin signal and "HHb" is the raw deoxyhaemoglobin signal.

This was then used to correct the corresponding time points HHb using the following equation:

$$HHb_c = HHb - (tHb\ x\ \beta)$$

Equation 14.

where “HHbc*” is* the blood volume corrected deoxyhaemoglobin signal, “HHb” *is* the raw deoxyhaemoglobin signal, “tHb” *is* the raw total haemoglobin signal and “β” *is* the blood volume correction factor.

All RIT HHb_c NIRS data was normalised to the highest and lowest 10 second moving average and converted to a percentage of maximum value attained. Due to the variation in ramp durations, time was converted into a % of RIT duration. To allow the calculation of a mean concentric and eccentric response for all participants, all tests needed to have the same number of data points and be time aligned. Therefore all of the concentric and eccentric tests were linearly interpolated to the longest test. As all CPTs were performed for identical durations, we were able to take a mean of all raw NIRS data for the 3 minutes of unloaded exercise and 15 minutes of target relative power.

3.2.7 Statistical analysis

All results are expressed as means ± standard error of the mean (SE). GraphPad prism statistical software (GraphPad v6.0, Graphpad Software Inc, California, USA) was used to conduct all statistical analyses and significance determined when $P < 0.05$. All data was tested for normality prior to choosing the appropriate statistical test. To reduce the risk of type I error, Bonferroni corrections were used for all post-hoc multiple comparisons. Paired Students t-tests were used to compare concentric and eccentric RIT responses. For the CPTs, two-way repeated measures analysis of variance (ANOVA) were conducted to compare the main effects of time and exercise session on the $\dot{V}O_2$ and HR responses, whilst a one-way ANOVA was used to compare the 10-minute mean MAP response between exercise groups.

3.3 Results

3.3.1 Familiarisation

A mean of two familiarisation sessions were required (1.7 ± 0.3, range = 1-3) before participants were able to maintain their total cumulative relative work within ± 2% of the target for greater than two minutes.

3.3.2 Ramp incremental tests

Example RIT data from one participant is shown in Figure 3.3. Mean concentric and eccentric cardiovascular and metabolic responses during the RITs are summarised in Table 3.1.

Table 3.1. Cardiovascular and metabolic responses during concentric and eccentric ramp-incremental stepping exercise.

	CON	ECC	ECC (% of CON)
Unloaded $\dot{V}O_2$ ($L \cdot min^{-1}$)	0.51 ± 0.04	0.42 ± 0.03	85 ± 7
Unloaded HR ($beats \cdot min^{-1}$)	81 ± 5	78 ± 4	96 ± 3
$\dot{V}O_{2gain}$ ($ml \cdot min^{-1} \cdot W^{-1}$)	68 ± 5	34 ± 3 *	51 ± 5
HR_{gain} ($beats \cdot min^{-1} \cdot W^{-1}$)	3.3 ± 0.3	2.0 ± 0.3*	60 ± 4
Peak power (W)	68 ± 4	87 ± 8 *	128 ± 6
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.21 ± 0.09	1.75 ± 0.11 *	79 ± 3
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	31.9 ± 1.5	25.1 ± 1.5 *	79 ± 3
HR_{peak} ($beats \cdot min^{-1}$)	159 ± 5	150 ± 7	94 ± 3

Concentric (CON), eccentric (ECC). * Significantly different ($P < 0.05$) *vs.* concentric exercise. Results presented as means ± SE.

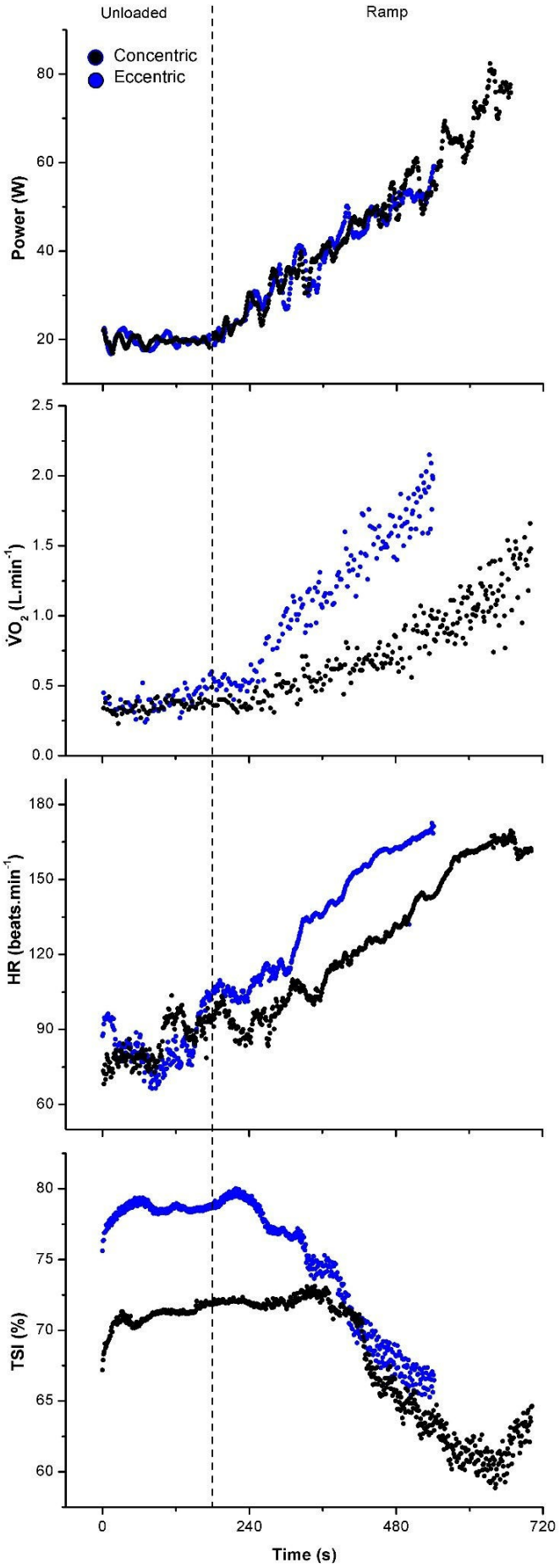

Figure 3.3. Example participants RIT responses. concentric (black) and eccentric (blue) relative power (W) performed, oxygen uptake ($\dot{V}O_2$), heart rate (HR), and tissue saturation index (TSI %) are shown. Vertical dashed line represents the onset of the ramp-incremental phase.

3.3.2.1 Mechanical power

All participants maintained their cumulative work within the target boundaries (± 2% of the target) for the duration of the RITs. As relative target power increased, W fluctuation increased both within the concentric and eccentric RIT (see Figure 3.3 for example). Peak relative power achieved during eccentric RITs was 28 ± 6 % higher than the concentric RIT (Table 3.1; $P < 0.05$).

3.3.2.2 Oxygen uptake

There was no difference between the concentric and eccentric RIT unloaded $\dot{V}O_2$ (Table 3.1; *n.s.*). All participants had a lower $\dot{V}O_{2gain}$ ($\Delta\dot{V}O_2/\Delta W$) during the eccentric RIT compared to the concentric RIT, with a mean gain reduction of 49 ± 5 % ($P < 0.05$). The mean concentric LT was 1.28 ± 0.05 $L \cdot min^{-1}$, 58 ± 1 % of the concentric $\dot{V}O_2$ max. We were unable to be determined eccentric LT due to excessive breath-by-breath noise (see Figure 3.3). All participants achieved a lower absolute and relative $\dot{V}O_{2peak}$ during the eccentric RIT, with a mean reduction of 21 ± 3 % ($P < 0.05$).

3.3.2.3 Heart rate

There was no difference in unloaded HRs between concentric and eccentric RITs (Table 3.1; *n.s.*). All participants had a lower HR gain ($\Delta HR/\Delta W$) during the eccentric RIT compared to the concentric RIT, however this reduction was less pronounced than with $\dot{V}O_2$ with a mean gain reduction of 40 ± 4 % ($P < 0.05$). All but one participant achieved a lower HR_{peak} during the eccentric RIT, however, however the mean reduction was not significant.

3.3.2.4 Oxygen pulse

Oxygen pulse ($\dot{V}O_2/HR$) during unloaded exercise were not different between concentric (6.3 ± 0.5 $ml \cdot min^{-1} \cdot bpm^{-1}$) and eccentric (5.4 ± 0.4 $ml \cdot min^{-1} \cdot bpm^{-1}$) RITs (*n.s.*). Concentric and eccentric oxygen pulses derived from the RIT $\dot{V}O_2$ and HR gains were similar (21.7 ± 2.5 (concentric) *vs.* 18.2 ± 2.0 (eccentric);

n.s.), however the peak oxygen pulses attained were higher during the concentric RIT (14.1 ± 0.9 *vs.* 11.7 ± 0.8 ml·min^{-1}.bpm$^{-1;}$ $P < 0.05$).

3.3.2.5 Muscle oxygenation

Figure 3.4 shows three participants' muscle oxygen desaturation responses during the concentric and eccentric RITs, illustrating intra-group variability. Figure 3.5 shows the variability in pattern of desaturation that occurred between concentric and eccentric RITs within subjects, emphasising inconsistency in response among individuals. Figure 3.6 shows the mean normalised HHb_c and tissue saturation index (TSI%) responses to RIT stepping normalised to % of RIT duration.

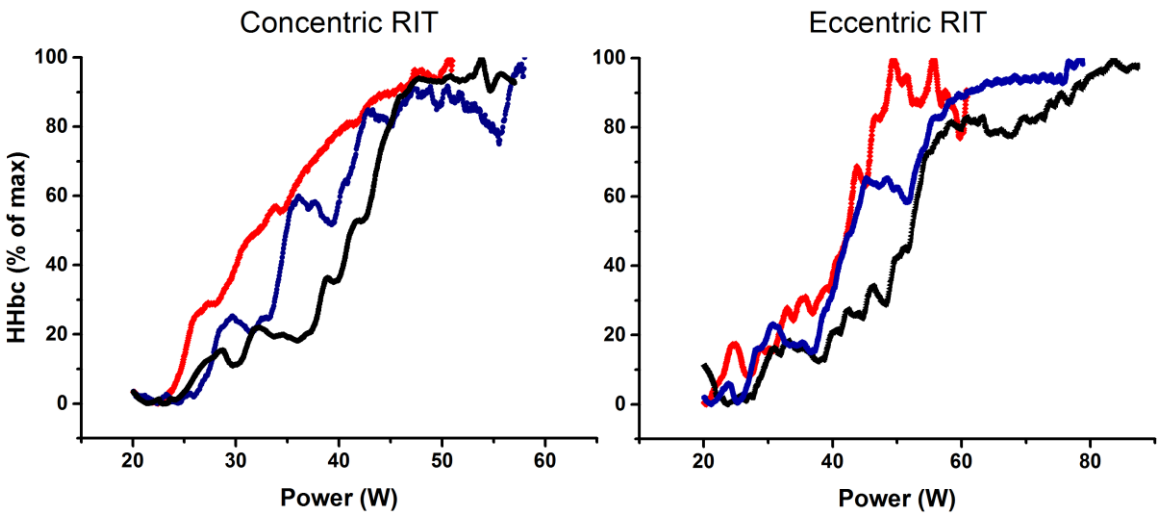

Figure 3.4: Example RIT NIRS traces for three participants (different coloured lines). Blood volume corrected deoxyhaemoglobin (HHbc, % of maximum value attained) levels during the concentric (left) and eccentric (right) ramp-incremental tests (RIT).

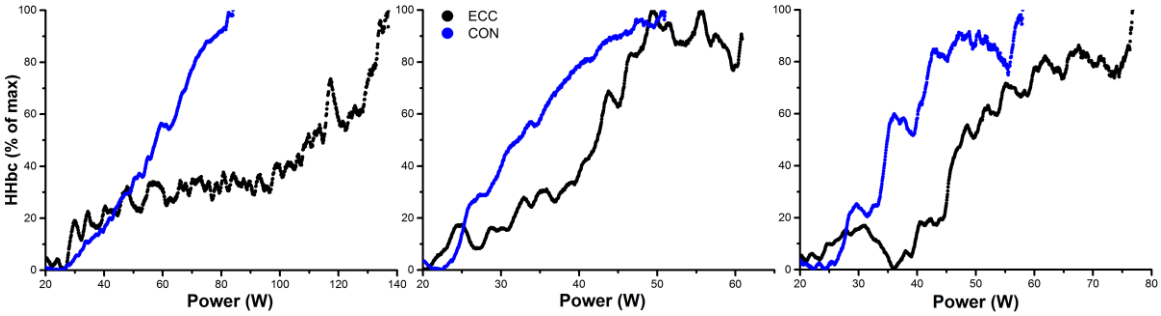

Figure 3.5: Comparison between concentric (CON, blue) and eccentric (ECC, black) NIRS RIT responses for three participants. Blood volume corrected deoxyhaemoglobin (HHb, (% of max)) levels displayed.

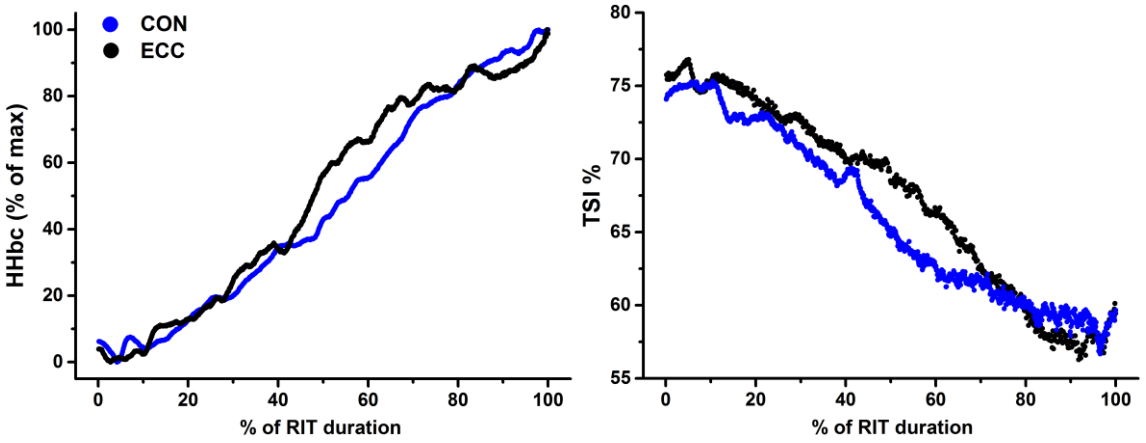

Figure 3.6: mean (n = 8) NIRS responses during the ramp-incremental tests (RITs). Blood volume corrected deoxyhaemoglobin (HHbc, left) normalised to % of maximum value attained, and tissue saturation index (TSI, right. Concentric (CON), eccentric (ECC). Values expressed as a percentage of RIT duration.

3.3.3 Constant power tests

3.3.3.1 Power

The relative power predicted to achieve the target $\dot{V}O_2$ (90% of the concentric LT) was 36 ± 2 W for the CON_{MI} and ECC_{WM} tests, and 60 ± 5 W for the $ECC_{\dot{V}O2}$ test ($P < 0.05$). There was less than a 0.4% difference between the predicted and actual relative power performed during all tests (0.28 ± 0.16 % (CON_{MI}), 0.39 ± 0.12 % (ECC_{WM}), 0.34 ± 0.19 % ($ECC_{\dot{V}O2}$); all *n.s.*). All participants tolerated the full 15 minute CPT duration throughout all tests.

3.3.3.2 Oxygen uptake

There was no difference in unloaded $\dot{V}O_2$ between all groups (*n.s.*). When matching for the same relative power between concentric (CON_{MI}) and eccentric (ECC_{PM}) exercise, a steady state was attained within 3 minutes (*n.s.* after 3 min; Figure 3.8). In the final minute, ECC_{PM} $\dot{V}O_2$ was 47 ± 3 % lower (0.52 ± 0.06 $L \cdot min^{-1}$) compared to CON_{MI} ($P < 0.05$; Figure 3.8; Table 3.2). Post-hoc power analysis determined that our study has a power of 1.0 to detect a change between CON_{MI} and $ECC_{\dot{V}O2}$ $\dot{V}O_2$. When matching for the same metabolic requirement, concentric (CON_{MI}) and eccentric ($ECC_{\dot{V}O2}$) $\dot{V}O_2$ were initially similar (*n.s.*). However, comparison of minute means showed a higher $\dot{V}O_2$ during the $ECC_{\dot{V}O2}$ test from 10 minutes onwards ($P < 0.05$). This resulted in a Δ $\dot{V}O_2$ of 0.33 ± 0.05 $L \cdot min^{-1}$ between 3 and 15 minutes in the $ECC_{\dot{V}O2}$ test ($P < 0.05$), and a 0.23 ± 0.1 $L \cdot min^{-1}$ higher final minute $\dot{V}O_2$ in comparison to CON_{MI} (Table 3.2; $P < 0.05$).

Table 3.2. Final minute mean responses during the three constant-power tests.

	CON_{MI}	**ECC_{PM}**	**$ECC_{\dot{V}O2}$**
$\dot{V}O_2$ ($L \cdot min^{-1}$)	1.10 ± 0.07	0.58 ± 0.04*	1.34 ± 0.06*
HR ($beats \cdot min^{-1}$)	112 ± 4	93 ± 3*	133 ± 6*
O_2 pulse ($ml \cdot min^{-1} \cdot bpm^{-1}$	10.0 ± 0.8	6.3 ± 0.4*	10.2 ± 0.5
TSI %	70 ± 2	74 ± 1*	62 ± 4*

Oxygen uptake ($\dot{V}O_2$), heart rate (HR), oxygen pulse (O_2 pulse) and rectus femoris tissue oxygen saturation (TSI %). * Significantly different ($P < 0.05$) *vs.*. CON_{MI}. Results expressed as means ± SE.

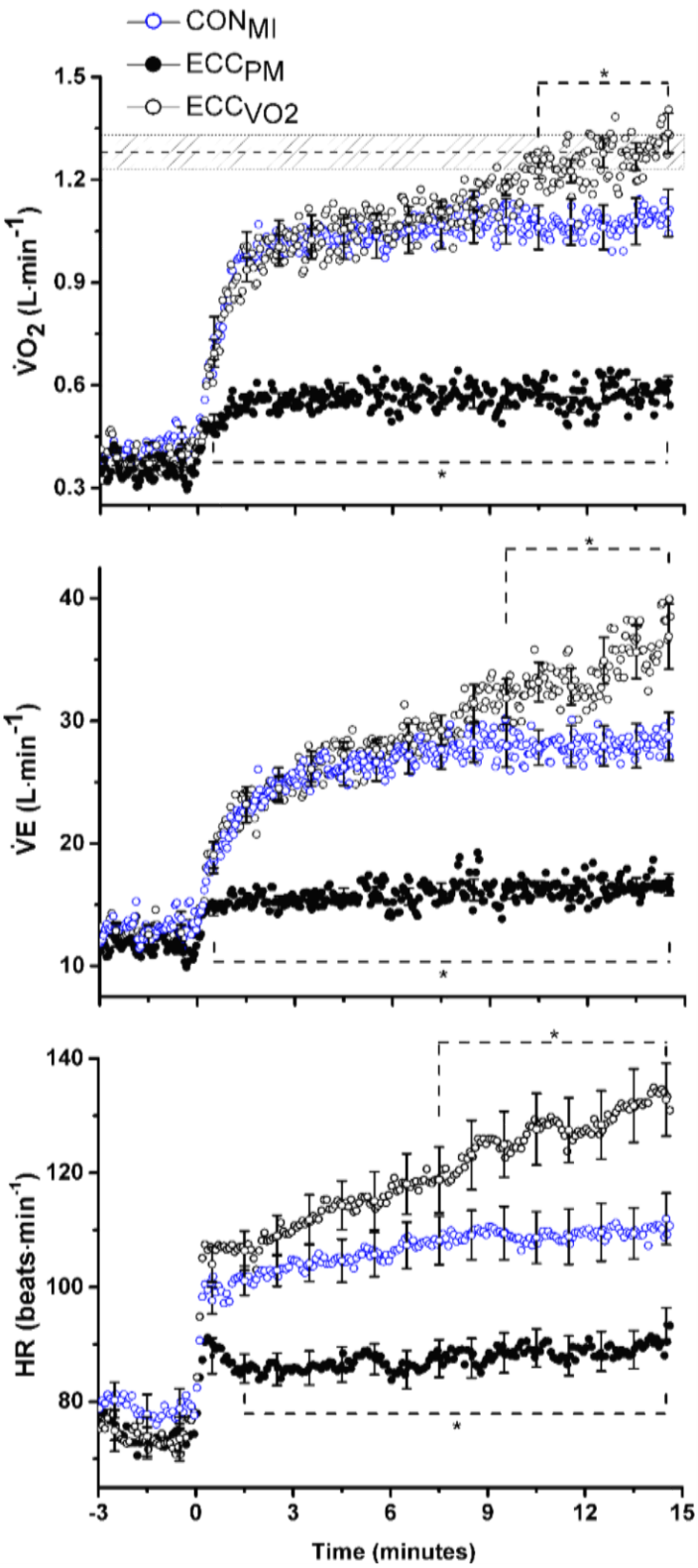

*Figure 3.7: Cardiovascular and metabolic responses to constant-power stepping exercise. Mean (n = 8) A) interpolated oxygen uptake ($\dot{V}O_2$), B) ventilation ($\dot{V}E$) and C) heart rate (HR) (5s smoothing applied) during the 15 minute CPTs. Error bars represent 1 min means ± SE. Hashed box represents mean concentric LT ± SE. * Significantly different (P < 0.05) vs. CON_{MI}.*

3.3.3.3 Heart rate

There was no difference in the unloaded HR between all groups (*n.s.*). When matched for the same relative power, HR also attained a steady state after 3 minutes within both the concentric (CON_{MI}) and eccentric (ECC_{PM}) tests (*n.s.* after 3 min; Figure 3.8). Within the final minute, $ECC_{\dot{V}O2}$ HR was 16 ± 2 % lower (19 ± 3 beats·min^{-1}) in comparison to CON_{MI} (both $P < 0.05$; Figure 3.8, Table 3.2). When matching for the same metabolic requirement, concentric (CON_{MI}) and eccentric ($ECC_{\dot{V}O2}$) HR were also initially similar (*n.s.*; Figure 3.8), however, when comparing minute means, $ECC_{\dot{V}O2}$ HR was significantly greater after 7 minutes, resulting in a Δ HR of 20 ± 3 beats·min^{-1} between 3 and 15 minutes ($P < 0.05$), and a 21 ± 6 beats·min^{-1} higher final minute eccentric HR in comparison to CON_{MI} (*n.s.*; Figure 3.8).

3.3.3.4 Oxygen pulse

When matching for relative power between concentric and eccentric recumbent stepping, there was a lower eccentric O_2 pulse (10.0 ± 0.9 (CON_{MI}) *vs.* 6.5 ± 0.3 (ECC_{PM}) ml·min^{-1}·bpm^{-1}; $P < 0.05$; Figure 3.8). When matching for the metabolic requirement, there was no difference in O_2 pulses (10.0 ± 0.9 (CON_{MI}) *vs.* 9.7 ± 0.5 ($ECC_{\dot{V}O2}$) ml·min^{-1}·bpm^{-1}; *n.s.*).

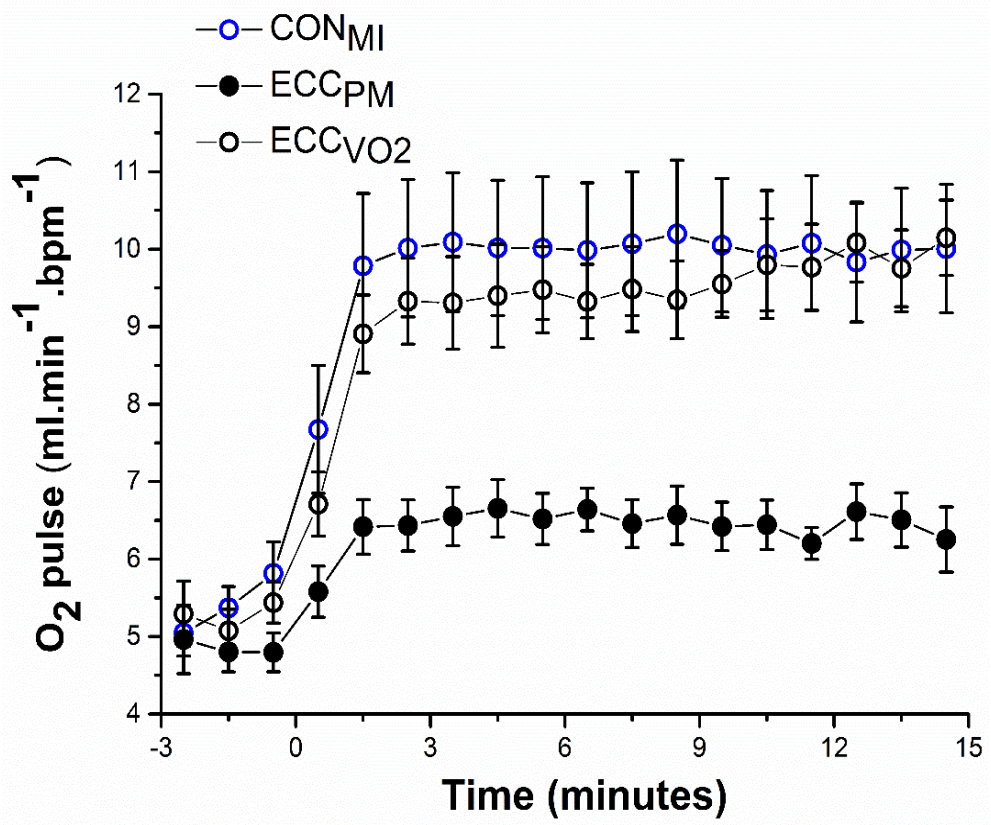

Figure 3.8: Mean ± SE minute O_2 pulses during the three CPTs.

3.3.3.5 Blood pressure

There was no difference in resting MAP between the three CPTs (*n.s.*). When matching for relative power, ECC_{PM} MAP was 11 ± 2 mmHg lower than CON_{MI} (103 ± 2 *vs*. 92 ± 2 mmHg; $P < 0.05$). in spite of the increased relative power performed, when matching for metabolic requirement, $ECC_{\dot{V}O2}$ MAP was not different to CON_{MI} (103 ± 2 (CON_{MI}) *vs*. 95 ± 5 ($ECC_{\dot{V}O2}$) mmHg; *n.s.*).

3.3.3.6 Muscle oxygenation

TSI changes during the three CPTs are shown in Figure 3.9 and Table 3.2. Unloaded TSI was the same between all CPTs (*n.s.*). When matching for relative power, there was no difference between concentric (CON_{MI}) and eccentric (ECC_{WM}) TSI within the first 13 minutes (*n.s.*). There was, however, a lower CON_{MI} TSI from 14 minutes onwards, resulting in a 4.0 ± 1.5 % lower final minute value (70.2 ± 2.1 *vs.* 74.2 1.0; $P < 0.05$; Table 3.2). When matching for metabolic response, there was a lower eccentric ($ECC_{\dot{V}O2}$) TSI throughout the entire CPT

duration compared to concentric (CON_{MI}) ($P < 0.05$), resulting in an 8.2 ± 1.5 % lower TSI within the final minute (70.2 ± 2.1 *vs.* 62.1 ± 3.7 %; $P < 0.05$; Table 3.2).

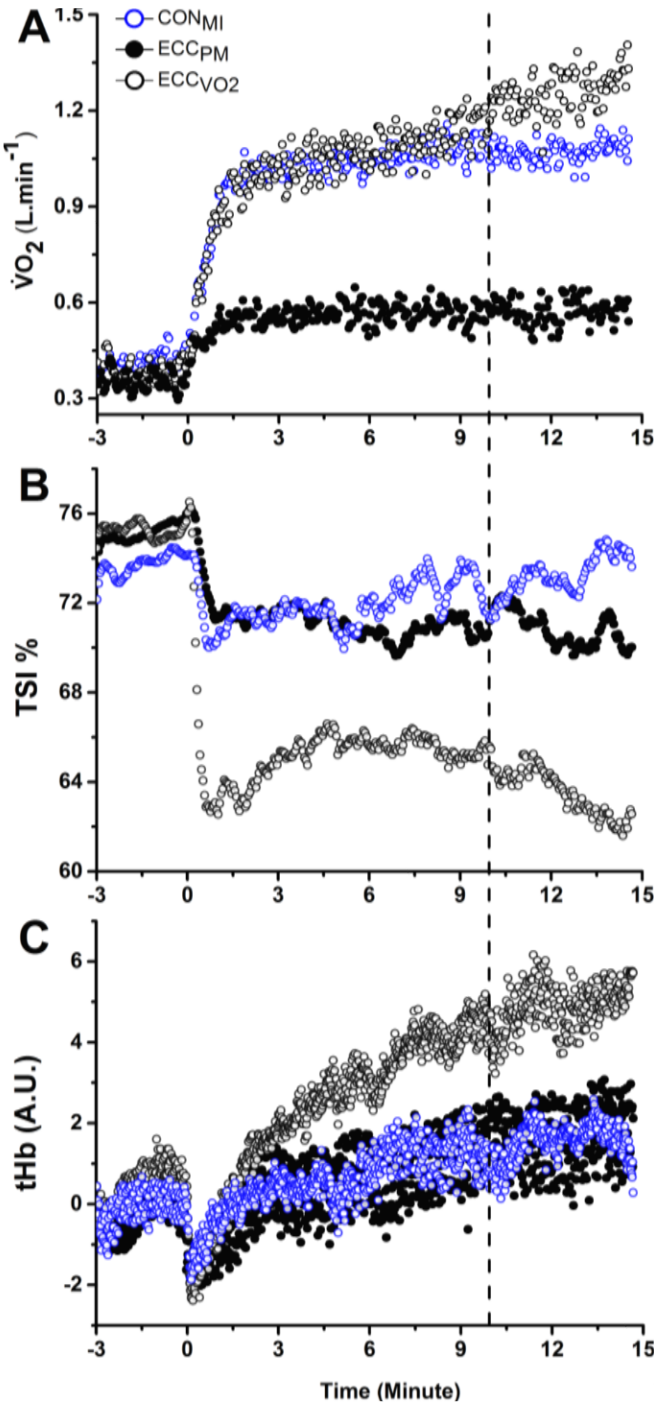

***Figure 3.9:** Mean (n=8) oxygen uptake ($\dot{V}O_2$, panel A), tissue saturation index (TSI %, panel B) and total haemoglobin (tHb, panel C) during the three constant-power tests. Vertical dashed line represents the point at which the $ECC_{\dot{V}O2}$ $\dot{V}O_2$ becomes significantly higher than the CON_{MI} $\dot{V}O_2$.*

3.4 Discussion

By comparing concentric and eccentric ramp-incremental and constant-power recumbent stepping, this study showed that, in comparison to concentric stepping: 1) eccentric RIT $\dot{V}O_2$ and HR gain was lower; 2) at the same relative power, eccentric CPT $\dot{V}O_2$, HR and MAP were lower; 3) eccentric RF tissue saturation was not reduced during constant-power exercise at the same relative power; 4) eccentric recumbent stepping matched for metabolic response resulted in a 65% increase in relative power; 5) at the same predicted metabolic response, $\dot{V}O_2$ and HR were initially matched, however, there was a delayed increase suggesting a higher eccentric exercise intensity; 6) MAP was similar and TSI lower eccentrically when matching for metabolic requirement.

Our data demonstrates that eccentric recumbent stepping exercise is a valid alternative exercise modality to use within a research setting. All subjects became familiarised to this modality in less than three sessions, a comparable, or shorter familiarisation duration than seen with other modalities (Dufour et al., 2004; Beaven et al., 2014). This may be due to the more natural form of exercise that this modality may present, as well as the lower cadenced used. The potential increased ecological validity of this task, coupled with the similar joint angles, stride lengths and cadences of stair ascent and decline (Costigan et al., 2002) may provide a superior training stimulus to previous modalities, and potentially translate more effectively into improving everyday mobility in exercise intolerant populations. This ergometer is also safer than previous modalities, with the risk of knee hyperextension and severe damage to associated structures minimised by tight control of joint angles *via*, seat positions and knee cushions (Hoppeler, 2015).

3.4.1 Acute responses to ramp-incremental stepping

To our knowledge, this is the first study to compare concentric and eccentric RIT responses, with previous studies utilising step increments in target power. This enables better comparison of eccentric responses to that of the gold standard cycle ergometry testing, for which there is abundant research, and allowed the subsequent matching of relative power and metabolic response with fewer visits required. The lower eccentric $\dot{V}O_2$ and HR gain observed is consistent with previous finding utilising cycle ergometry (Isner-Horobeti et al., 2013). We did, however, observe a smaller disparity between concentric and eccentric gains than previous CPT studies have shown. E.g. Dufour et al. (2004) showed $\dot{V}O_2$ and HR gain to be just ~25% and ~45% of the concentric response, compared to our 51% and 60% difference. This lower cardiovascular and metabolic demand likely allowed the participants to reach higher eccentric peak relative powers (28% greater), however, participants could not tolerate high enough relative powers to achieve similar $\dot{V}O_{2peak}$ responses, showing a 22% lower $\dot{V}O_{2peak}$ during the eccentric RIT. This suggests that the eccentric LoT was likely due to a peripheral inability to produce the required relative power, rather than an inability to meet the metabolic requirements of the exercise.

Despite the lower eccentric $\dot{V}O_{2peak}$, HR_{peak} was not different, resulting in a lower eccentric O_2 pulse, in agreement with previously studies (Hesser et al., 1977; Dufour et al., 2004). The cause of this elevated cardiovascular demand at the same metabolic requirement remains unclear, however, increased muscle tension and temperatures that occur with higher relative powers, are the most probable theories (Thomson, 1971) (see section 1.6.2). The higher peak tension developed during eccentric exercise is thought to increase type III and IV muscle afferents firing rate, increasing HR *via* autonomic activation of cardiovascular centres in the medulla (Hayward et al., 1991; Craig, 1995). Higher muscle temperatures during eccentric exercise have been reported by Sargeant and Dolan (1987) and are similarly thought to increase firing of group III and IV muscle afferents *via* thermoregulation activation (Hertel et al., 1976). Increases in eccentric HR can likely not be explained by disruptions to muscle blood flow and venous return due to the fact that cadence was matched between

contraction modes, duration of contraction was likely similar, and force of contraction within both modes was likely above the threshold for blood flow occlusion (Barcroft and Dornhorst, 1949; Sjøgaard et al., 1988) (see section 1.6.3).

The profile of the muscle oxygen desaturation (TSI%) during concentric and eccentric ramp-incremental stepping exercise displayed a sigmoidal shape representing a slow rate of desaturation at low relative powers, a progressive and mostly linear increase in desaturation with increased relative powers, and a slowing of desaturation towards the LoT. This profile mirrors previous cycle ergometry results, and implies a nonlinear relationship between muscle blood flow and muscle O_2 utilisation at the start and end of the RIT. Muscle blood flow likely exceeds O_2 uptake at low relative powers and significantly slows at relative powers close to the LoT. This rapid increase in muscle blood flow at the start of exercise is a well-established phenomenon (Sarelius and Pohl, 2010) and is predominantly due to vasodilation of local vasculature, involving both neural (sympathetic withdrawal) and humoral (metabolic dilators) components. The similarity in pattern of tissue saturation changes during concentric and eccentric exercise suggests a similar matching of muscle blood flow and muscle oxygen utilisation at intensities relative to maximum power output. However, as peak concentric RIT power was lower in all participants (mean reduction of 22%), eccentric TSI at the same relative power output was likely lower.

At the LoT, despite a 21% lower eccentric pulmonary $\dot{V}O_2$, there was no difference between concentric and eccentric TSI. The slowing desaturation rate towards the LoT suggests that the RF muscle may have been approaching maximal desaturation limits during both RITs. Cannon et al. (2013) has shown *via* ^{31}P chemical shift and T_2 imaging that individual muscles, and even specific regions within one muscle, exhibit heterogeneity in phosphocreatine depletion during incremental exercise. Just 5% of muscle mass reached critical metabolic disturbance at the LoT, suggesting that regional metabolic insufficiency may be the cause of task failure, rather than the mean response of all muscle groups. It may therefore be possible that the RF muscle reached critical desaturation

during the eccentric RIT and was the primary contributor to task failure. Other contributing muscle groups may not have reached the same level of desaturation and therefore may help explain the reduction in pulmonary $\dot{V}O_2$ responses observed.

It is important to highlight that there are limitations that present with the measurement and analysis of NIRS data, and therefore caution should be taken with the interpretation of results. Firstly, the NIRS device used is only able to assess a small (estimated to be ~ 3 to 6 cm^3; Grassi and Quaresima (2016)) sample of tissue directly underneath the probe which may not be representative of the whole muscle phenotype and physiological response. For example, it has been demonstrated using histochemical analysis of young and old cadaveric tissue, that the proportion of type I to II muscle fibres increases at greater depths of muscle (Lexell et al., 1983; Dahmane et al., 2005), and also that blood perfusion, and therefore O_2 delivery, can vary greatly within different regions (Kalliokoski et al., 2000). Secondly, the overlying skin and adipose tissue can significantly alter the scattering of light, with an increased melanin content (Adami et al., 2017), and adipose tissue thickness (Van Beekvelt et al., 2001) reducing the magnitude of light received at the infrared detector. Skin fold thickness corrections are therefore essential when comparing absolute values, but less important when assessing the rate of change of NIRS parameters. Assessment of TSI is also limited based on the fact that tissue saturation is merely a balance between O_2 delivery and utilisation. Changes in blood flow, and therefore O_2 delivery, that commonly occur during exercise (Ryan et al., 2013) can therefore influence TSI without a true change in $\dot{V}O_{2m}$.

3.4.2 Acute responses to constant-power stepping

When matching for relative power, eccentric cardiovascular and metabolic responses were reduced, presenting with a 46%, 18% and 11% lower $\dot{V}O_2$, HR and MAP compared to concentric repectively. This lower demand is consistent with previous studies (Hesser et al., 1977; LaStayo et al., 1999; Perrey et al., 2001; Dufour et al., 2004), however, as with our RIT results, discrepency between concentric and eccentric responses are lower than previously reported.

The exercise was set to be of moderate intensity to match government guidelines for exercise (Whipp and Wasserman, 1972; Bull et al., 2010), and to allow the metabolic matching of eccentric exercise for long durations. This metabolic intensity is far lower than previous studies have shown, with our study achieving a mean concentric $\dot{V}O_2$ of just 1.1 $L \cdot min^{-1}$ in comparison to, e.g., Dufour et al. (2004) achieveing a concentric metabolic demand of ~3.8 $L \cdot min^{-1}$. The 23 spm cadence we used is also considerably lower than the cadences used wtihin cycle ergometry (~60 rpm; LaStayo *et al.,* 1999 & 2000). This lower $\dot{V}O_2$ and cadence, coupled with the difference in exercise modality used, makes comparing the magnitude of differences between studies difficult, with the $\dot{V}O_2$/power relationship lilkely being very different (Ozyener et al., 2001).

Due to the differences in $\dot{V}O_2$ gains during the concentric and eccentric RITs, a 65% greater relative power was required to match for concentric metabolic response during the $ECC_{\dot{V}O2}$ test. This relative power increment was also lower than previous studies that showed increases of 412% (Hesser et al., 1977) and 500% (Dufour et al., 2004) required. These significant differences cannot solely be explained by the exercise intensity under which comparisons were made, and therefore suggest that there are important differences in either ergometer mechanics or calculations used to determine the relative power performed. Within the stepper ergometer, a motor drives both the forwards (concentric) and reverse (eccentric) foot plate movement, making comparisons between concentric and eccentric $\dot{V}O_2$/power relationships valid. Cycle ergometry studies, however, often only use a motor driven ergometer for the eccentric cycling, and a normal flywheel ergometer for concentric cycling. They may, therefore, not be be making fair comparisons, e.g. not accounting for the contribution of inertia present in concentric cycling, or incorrectly assuming that power produced by the motor during eccentric cycling was the same power performed by the participant.

When matching for metabolic demand, as expected, concentric and eccentric $\dot{V}O_2$ and HR were initially matched. However, a delayed rise in eccentric ($ECC_{\dot{V}O2}$) $\dot{V}O_2$ and HR presented suggesting this test was performed at a higher

exercise intensity (Rossiter, 2011). This rise in $\dot{V}O_2$ from a predicted steady state may be explained by the addition of a $\dot{V}O_2$ slow component (Whipp and Wasserman, 1972). The $\dot{V}O_2$ slow component is a progressive reduction in skeletal muscle contractile efficiency when exercising at a constant-power above LT (Jones et al., 2011),and can culminate in the attainment of $\dot{V}O_2$ max when exercise is perfomed above critical power (Whipp, 1994) (See section 1.2). This reduction in muscle efficiency was previously hypothesised to originate from increased muscle temperature (Ferguson et al., 2006) or reduced pH (Bailey et al., 2009b), however, most conclusive evidence supports the theory of additional recruitment of higher threshold motor units (those with larger motor neurons), resulting in a greater proportion of type II fibres and a higher O_2 cost to produce the same mechanical work (Krustrup et al., 2004b; Krustrup et al., 2004a; Krustrup et al., 2008). As can be seen in Figure 3.8, the delayed rise in $\dot{V}O_2$ during the $ECC_{\dot{V}O2}$ test correlates with a fall in TSI at a similar time point. This relationship between this presumed $\dot{V}O_2$ slow component and muscle oxygenation has previously been reported (Belardinelli et al., 1995; Grassi et al., 2003), and further supports the hypothesis that the slow component originates at the muscle level (Gaesser and Poole, 1996; Rossiter, 2011).

The mean $\dot{V}O_2$ response had not reached steady state after the 15 minute CPT duration, and therefore it cannot be ascertained whether the exercise was performed within the heavy, or very heavy intesity domain (see section 1.2). The sampling of blood La during testing may have been able to confirm that, although the $\dot{V}O_2$ was similar between concentric and eccentric exercise, the eccentric exercise intesity was infact above the LT. Due to this intensity elevating $\dot{V}O_2$ and HR above expected values, care would need to be taken when prescribing eccentric exercise based on RIT results. Cardiovascular and metabolic demand would be expected to rise above the predicted value and may result in a higher exercise intensity than prescribed.

Eccentric MAP was lower when matching for relative power and the same when matching for metabolic response. This finding agrees with previous studies showing a reduction in eccentric BP despite a higher relative power output

(Horstmann et al., 1994; Overend et al., 2000; Vallejo et al., 2006). This reduced BP response is likely due to barostatic mechanisms that are more closely coupled to the percieved exertion of the task and cardiovascular and metabolic demand, rather than the relative power output (MacDougall et al., 1992). MacDougall et al. (1992) showed that the concentric and eccentric BP responses were similar at the same perceived effort of intensity, despite eccentric force being ~56% higher. Exercising within the moderate intesity domain is advised for hypertensive patients (Ghadieh and Saab, 2015), and having a lower BP response eccentrically may allow hypertesive patients to exercise safer, work at higher intensities, and therefore increase the training stimulus.

RF tissue oxygen saturation was the same when matching for relative power and was greater during eccentric recumbent stepping matched for the same metabolic demand. This doesn't agree with previous findings (Penailillo et al., 2017) that show a ~16.3% reduction in tissue saturation during relative power-matched eccentric exercise. The lack of a lower concentric TSI in the presence of a 47% lower eccentric pulmonary $\dot{V}O_2$, suggests that the RF muscle may not be a good representation of the total active muscle mass response, and may be more active during eccentric *vs.* concentric recumbent stepping exercise. During low intensity exercise, utilisation of oxygen within the exercising muscles contributes to the majority of whole body pulmonary $\dot{V}O_2$ (Jones and Poole, 2005). Therefore, it would be expected that, if we were able to measure the TSI of all active muscle groups, a similar % difference in TSI compared to pulmonary $\dot{V}O_2$ would be present. The relatively higher metabolic intensity with which Penailillo et al. (2017) performed concentric exercise (mean concentric $\dot{V}O_2$ of 2.81 $L \cdot min^{-1}$ *vs.* our concentric $\dot{V}O_2$ of 1.1 $L \cdot min^{-1}$), as well as the use of a different exercise modality (reverse cycle ergometer) may also help explain these differences.

tHb can be used as an indirect measure of blood flow within skeletal muscle, however, care needs to be taken with its interpretation as, coupled with the limitation of NIRS stated above, many inferences are used within its calculation. Our results agree with previous studies (DeLorey et al., 2003; Clifford and

Hellsten, 2004; Penailillo et al., 2017) that show an initial reduction in concentric and eccentric tHb at CPT exercise onset, followed by a progressive increase back to, or above baseline. This initial reduction is likely due to mechanical compression and occlusion of vasculature within the active skeletal muscle. The similarity in tHb between concentric and eccentric exercise matched for relative power agrees with Penailillo et al. (2017). They, however, showed a higher TSI for the same blood volume eccentrically, therefore concluding that a reduction in ATP utilisation within the muscle may be present. With our data presenting with the same TSI between concentric and eccentric recumbent stepping matched for relative power, this suggests that there may be a reduced efficiency of ATP utilisation with this modality or within the RF in comparison the vastus lateralis.

3.4.3 Mechanisms for reduced eccentric cardiovascular and metabolic requirement

The reduced cardiovascular and metabolic demand that we have shown with, both eccentric RIT and CPT stepping, is likely due to a reduced ATP requirement for the same relative power. As detailed In section 1.6, there are a variety of theories that attempt to explain this reduced energy demand, with the most plausible being a non-ATP dependent contribution to force *via* the mechanical breaking of cross-bridges (Stauber, 1988) and additional contribution from the protein titin (Herzog et al., 2016; Nishikawa, 2016). Briefly, the pulling apart of cross-bridges that occurs during eccentric contractions is thought to eliminate the need for ATP to bind to the myosin head and trigger its release from an adjacent actin filament. This pulling apart is thought to produce additional passive tension and leave the myosin head in it's active state allowing faster reattachment to actin and subsequent force production. Titin is thought to produce additional tension *via* the three filament (Herzog) or winding filament (Nishikawa) hypotheses. Within both of these, titin is thought to bind to actin during active (Ca^{2+} dependent) lengthening and produce additional tension *via* it's extensible region.

3.4.4 Conclusions

The findings from this study of acute responses suggest that eccentric recumbent stepping may be a viable alternative training modality for use within athletic and exercise intolerant populations. Results suggest that eccentric recumbent stepping could be performed at similar or higher relative powers than concentric exercise, whilst minimising the cardiovascular and metabolic requirement. This is likely to increase the mechanical training stimulus above what is currently possible with concentric training and result in superior changes to mobility and athletic function. Within exercise intolerant populations, working at a lower metabolic demand will reduce the restrictive sensations such as dyspnoea, allowing users to exercise safely and comfortably. At present, eccentric exercise is not routinely used within populations that may benefit, likely due to the small number of studies investigating it's benefits (Isner-Horobeti et al., 2013), as well as the safety concerns, cost, availability and occurrence of DOMS (Hoppeler, 2015). Further research is therefore required to investigate training responses to alternative modalities, and strategies to prevent severe muscle soreness occurring.

Despite showing a reduction in cardiovascular and metabolic demand, our results also demonstrate a progressive increase in eccentric $\dot{V}O_2$ and HR when matching for a relatively low concentric metabolic requirement. This suggests that exercise intensity may be higher eccentrically and that care needs to be taken with exercise prescription within at risk populations. Whether eccentric recumbent stepping training at this intensity would be tolerable remains unknown, as do the training responses in comparison to other modalities. By utilising the same methods as used within this chapter, accurate comparisons between concentric and eccentric recumbent stepping training at similar mechanical and metabolic demands would be possible.

Chapter 4 Chronic effects of concentric *vs.* eccentric recumbent stepping

4.1 Introduction

The acute findings presented within the previous study, as well as earlier eccentric literature (see section 1.6.2), have demonstrated that at the same relative power, compared to traditional (concentric) exercise, cardiovascular and metabolic responses during eccentric exercise are significantly lower. Thus, a greater eccentric relative power is required to achieve the same concentric metabolic response, and greater peak forces are attainable in comparison to concentric activity (Westing and Seger, 1989; Leonard et al., 2010). As increases in voluntary muscle strength and mass are strongly correlated to the mechanical demand placed upon the exercising skeletal muscle (Goldberg et al., 1975; Roig et al., 2009), eccentric training may provide a greater stimulus to induce positive neuromuscular adaptations.

Superior voluntary strength and muscle mass increases have been consistently demonstrated with eccentric *vs.* concentric resistance training (Roig et al., 2009), however, these studies lack fine control of exercise WR or cardio-metabolic demand, and utilise high intensities (80-100% of maximal force generating capacity, Roig *et al*., 2009), likely not tolerable during endurance exercise of longer durations. Eccentric endurance training studies have shown that, even at lower relative intensities, similar voluntary strength changes are attainable within young healthy (LaStayo et al., 1999; LaStayo et al., 2000), and elderly (LaStayo et al., 2003; Gluchowski et al., 2015) populations. Although achieving high mechanical strains at low cardiovascular (~65% of peak WR, LaStayo *et al*., 2000) and metabolic (~1 $L \cdot min^{-1}$ LaStayo *et al*., 1999) demands, these protocols do not accurately control for individual exercise intensity or make comparisons to similar concentric exercise (Gerber et al., 2007a; Marcus et al., 2008; LaStayo et al., 2009; Elmer et al., 2017). Eccentric endurance exercise performed within

the moderate-intensity exercise domain should ensure activity remains tolerable (see section 1.2), however, to our knowledge, no studies have matched individuals into this exercise intensity domain and assessed voluntary muscle strength and mass changes between concentric and eccentric training.

Previous training studies suggest that eccentric endurance exercise is not able generate a sufficient cardiovascular or metabolic demand to increase outcomes from a standard cardio-pulmonary exercise test (LaStayo et al., 2000). Results presented within Chapter 3, suggest that the LoT during stepping RITs occurs at lower $\dot{V}O_2$'s than predicted for cycle ergometry, and is lower during eccentric recumbent stepping at higher WRs. This suggests that the LoT within the stepping RITs is likely determined by a peripheral (skeletal muscle) inability to produce the required power, rather than an inability to meet the metabolic requirements of the exercise. Thus, although the metabolic demands of the training may not be adequate enough to increase peak outcomes within the cycle ergometer RITs, voluntary strength improvements following training may allow for increases in peak WR or $\dot{V}O_{2peak}$ within the stepping RITs.

The majority of research investigating eccentric endurance training programmes have so far utilised reverse cycle ergometry, allowing tight control of exercise parameters but at the cost of a task that is rarely performed in nature (see section 1.5). Eccentric recumbent stepping may provide a modality that more closely matches natural activity, and therefore may enable a greater transferability of voluntary muscle strength changes to functional everyday mobility (Morrissey et al., 1995; Millet et al., 2002; Misic et al., 2009; Garber et al., 2011). However, it remains unknown whether moderate-intensity eccentric recumbent stepping remains tolerable for long durations, and whether the extent of muscle soreness at this intensity can be constrained by gradual WR progression methods previously implemented (LaStayo et al., 1999; Nosaka et al., 2001; Lastayo et al., 2010).

The primary aims of this study were: 1) to compare voluntary strength and muscle mass changes between concentric and eccentric training matched for

either metabolic ($\dot{V}O_2$) or relative power requirement; 2) to assess if gradual progression of WR attenuates eccentric induced muscle soreness; 3) to compare peak relative power and cardio-metabolic outcomes for stepping and cycling RITs.

We hypothesised that: 1) voluntary strength increases will be greater following eccentric *vs.* concentric recumbent stepping matched for metabolic requirement; 2) voluntary strength changes will be similar following concentric and eccentric recumbent stepping matched for relative power; 3) gradual progression of WR at the start of training will minimise the muscle soreness experienced; 4) increases in voluntary muscle strength will allow participants to achieve higher peak relative powers and metabolic limits within the stepping RITs.

4.2 Methods

This study was approved by the Faculty of Biological Sciences Ethical Committee for non-clinical research (University of Leeds, BIOSCI 15-001, Appendix B), and complied with the latest version of the Declaration of Helsinki. Common protocols, measurements and equipment are detailed in Chapter 2 and will therefore only briefly be described here.

4.2.1 Participants

21 healthy, recreationally active males took part (mean ± SE; 25 ± 1 yr, 76 ± 2 kg, 178 ± 1 cm). Participants were screened using a health and activity status questionnaire, as well as inclusion and exclusion criteria to determine contraindications for exercise. Consent forms were signed by participant, lead researcher and assessor prior to commencement of testing. The inclusion and exclusion criteria for the study were as follows:

Inclusion criteria: Healthy males between the ages of 18-45 years.

Exclusion criteria: 1) any relevant medical disorder which may alter physiological responses; 2) use of prescription medication or over the counter preparations that may influence their performance; 3) a recent illness or viral infection (within the last two weeks); 4) use of recreational or performance enhancing drugs; 5) ingestion of alcohol in the previous 24 hours (pre and post assessments); 6) history of anaemia, asthma, diabetes, epilepsy, family history of sudden death, fainting, heart disease, high BP, respiratory disease, muscle or joint injury; 7) unable to provide informed consent or understand English; 8) recent intense exercise <48 hours before testing (pre and post assessments).

4.2.2 Stepping ergometer

As within the previous experimental chapter, all concentric and eccentric exercise was performed on a modified eccentric recumbent stepping ergometer (Eccentron; BTE, Hanover, MD, USA; Details of all modifications, calculations and calibrations are detailed in section 2.3). Load cells positioned behind the foot

plates were calibrated under no load before every session. Participant set up was identical to the previous study (section 3.2.2), and was maintained for every session. Briefly, knee range of movement was set between 90° and 30° of flexion, cadence was set at 23 spm, and stride length set at machine position 2 (13° displacement). Participants viewed the same custom LabChart screen (section 2.3.5) throughout all RITs, CPTs and training sessions. This custom view required participants to match their total work within ± 2% of the target throughout all sessions, and ensured the same total work was performed for each leg.

4.2.3 Cycle ergometer

An upright cycle ergometer (Lode Excalibur Sport PFM, Lode BV, Groningen, NL) was used to perform the cycle ergometer RITs. Target power was set *via* associated software and maintained irrespective of the cadence at which the participant was pedalling. A feedback system controls the electromagnet braking force with reductions in cadence resulting in an increased braking force to maintain the desired power output (torque x angular velocity) and vice versa. To ensure accuracy of power, calibration is performed yearly by the manufacturers. As supplied, the ergometer has a load range of between 8 and 2500 W, a cadence range of between 25 and 180 rpm, is capable of minimum load increments of 1 W, and has an accuracy of ± 2% of the target power (within the range of 100 to 1500W). Participants were able to adjust the saddle height and angle, as well as the handlebars vertical and horizontal position for optimal comfort. The ergometer software allowed all of these positions to be saved so that the exact bike setup can be used for post testing.

4.2.4 Familiarisation

Up to three familiarisation sessions (minimum of two), were completed prior to testing, as detailed in section 2.4.1. Participants performed both concentric and eccentric recumbent stepping during each session, and were familiarised to the RIT, CPT and voluntary strength assessments during every session. The duration of the RITs and CPTs were shorter and participants only continued up

to a maximum of 80% of their self-perceived maximum effort during the RIT. Participants were deemed familiarised when they were able to maintain their left and right cumulative work within ± 2% of the target for > 2 minutes.

4.2.5 Experimental design

An overview of the experimental design is shown in Figure 4.1. Following familiarisation, baseline assessment was completed comprising: measurement of leg girth, height and weight; assessment of concentric, eccentric and isometric voluntary strength; completion of a concentric and eccentric recumbent stepping RIT; and completion of a cycle ergometer RIT. Participants were then randomised into one of three training groups to complete 8 weeks (twice a week) of concentric or eccentric recumbent stepping exercise. 1) concentric recumbent stepping performed at an estimated power to achieve 90% of the concentric LT (concentric moderate-intensity, CON_{MI}); 2) eccentric recumbent stepping performed at the same estimated relative power (eccentric power-matched, ECC_{PM}); and 3) eccentric recumbent stepping performed at an estimated relative power to achieve the same $\dot{V}O_2$ demand as concentric recumbent stepping (90% of concentric LT; eccentric $\dot{V}O_2$ matched, $ECC_{\dot{V}O2}$). Training WR and duration progressed between sessions (Figure 4.4) and measurements of HR, RPE and muscle soreness were taken every session. To assess the cardiovascular and metabolic demand of the exercise, measures of $\dot{V}O_2$, HR, and blood La were taken within the final training session. Following training, all participants repeated the baseline assessments. Participants were required to refrain from intense exercise and alcohol consumption within the last 24 hours, as well as food and caffeine ingestion within the last 3 hours before baseline, final training session, or post training assessment.

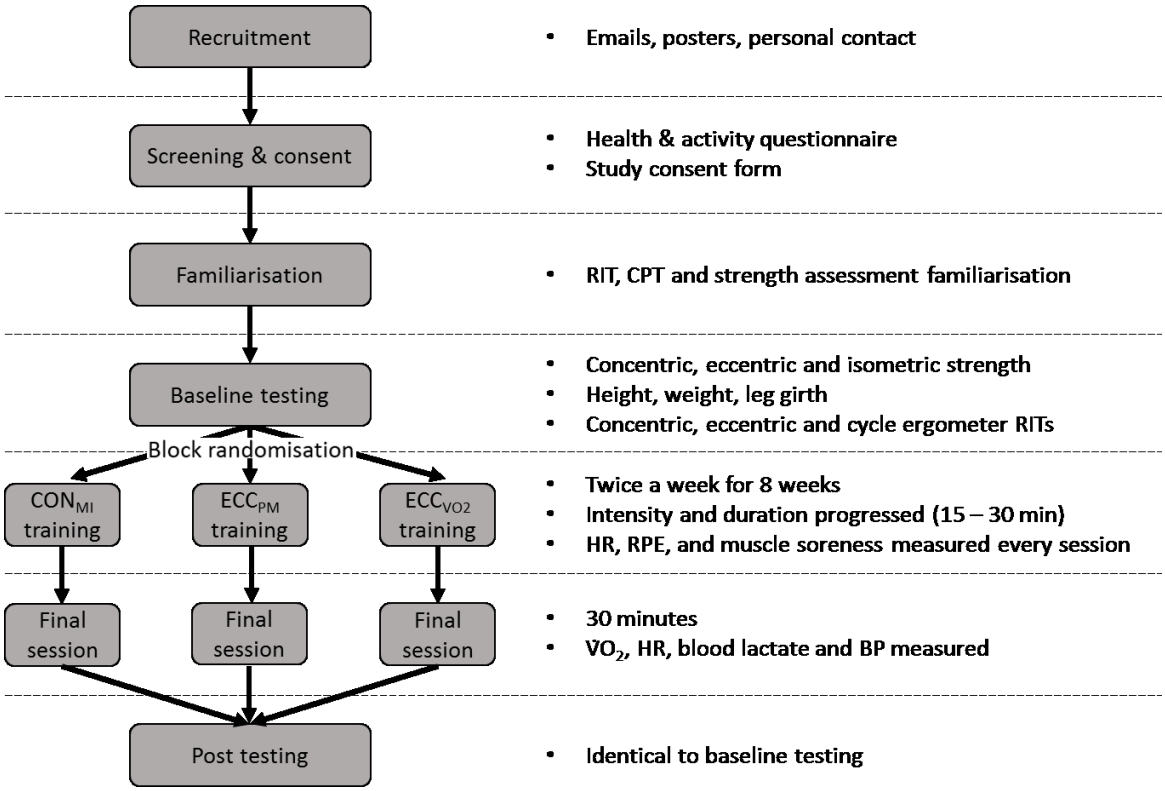

Figure 4.1. Study protocol flow chart.

4.2.5.1 Pre & post training assessment

4.2.5.1.1 Anthropometric data

Prior to other baseline assessments, and within the first post training session, measurements, of height, weight and leg girth were taken (see section 2.5.1). To measure leg girth, a flexible tape measure was used to take a mid-thigh measurement on the participant's dominant leg. The anterior superior iliac spine and knee joint line were palpated, the distance between them measured, and a pen mark placed at the midpoint between them. The tape measure was then wrapped around the thigh in a horizontal position at the pen mark, and three readings were taken to obtain a mean thigh circumference.

4.2.5.1.2 Voluntary strength assessment

Assessment of concentric, eccentric and isometric voluntary strength were performed on the modified stepping ergometer as detailed in section 2.4.4. Assessment was repeated twice for each contraction type (total of 6 contractions for each contraction type) and the peak force value determined using the peak

analysis module within LabChart. The order of contraction type was randomised, and kept the same for post-training assessment.

4.2.5.1.3 Ramp incremental tests

Maximal concentric stepping, eccentric stepping and cycle ergometer RITs were conducted in a randomised order more than 48hrs apart. $\dot{V}O_2$, HR (3-lead ECG for stepping ergometer, 12-lead ECG for cycle ergometer) and power were measured throughout. Concentric and eccentric RIT stepping protocols were performed on the modified stepping ergometer (Figure 4.2; details within section 2.4.2). The cycle ergometer RITs were conducted on an upright cycle ergometer within a climate controlled laboratory (set to 19°C). The fundamentals of the test were identical to that of the stepping ergometer RITs, with only the modality, measurements, and ramp rates different. Participants initially sat at rest on the ergometer for ~2 min, followed by ~3 min of unloaded pedalling. Pulmonary gas exchange data were monitored during these phases to ensure a steady state was achieved with normative respiratory exchange ratio values (see section 2.5.2). The target power then linearly increased at a rate of 25 $W \cdot min^{-1}$ until the LoT was reached. This ramp rate within a young healthy population often yields a duration of 8-12 minutes, allowing sufficient breath-by-breath data to be obtained, whilst retaining a tolerable duration for the participant (Davis et al., 1981). The participant was instructed to gradually increase their cadence so that they were pedalling at 90-100 rpm towards the end of the test. Verbal encouragement was given throughout the test. The LoT was identified as the point at which cadence dropped below 50 rpm. At the LoT, the power was reduced to 20W, and the participant was instructed to continue pedalling for a minimum of 2 minutes to prevent blood pooling and feeling faint.

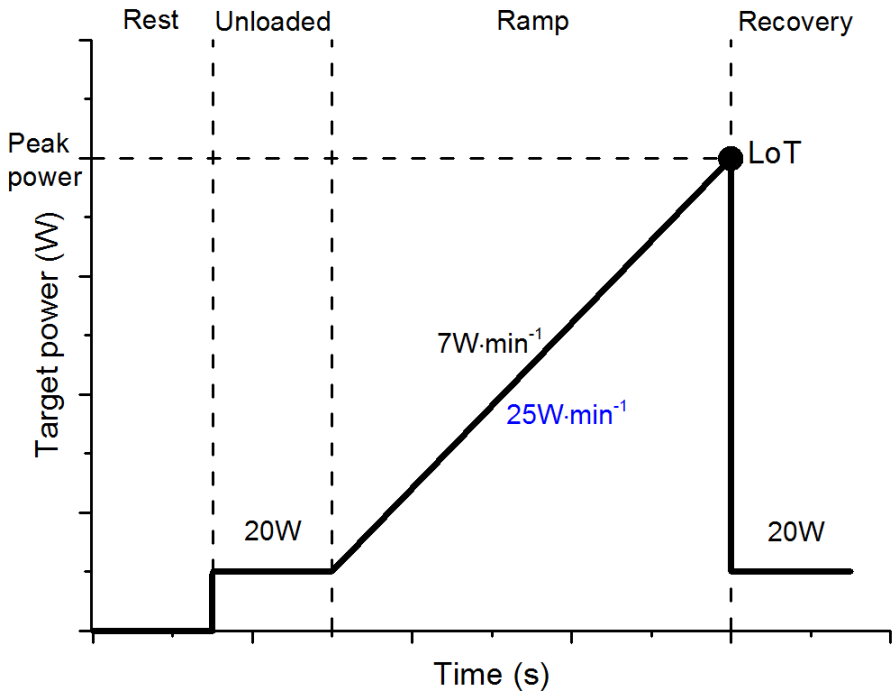

Figure 4.2. Schematic representation of the ramp-incremental tests (RITs) protocol. A rest period preceded an unloaded 20W constant-power phase. This was followed by a linear increase in target power (7W·min-1 for stepping RITs and 25W·min-1 for the cycle ergometer RIT), culminating in the limit of tolerance (LoT, ●). At the LoT, the target power reduced to 20W for a recovery period of ~3 minutes.

4.2.5.2 Training

Following baseline assessment, participants were block randomised (www.randomizer.org) into one of three training groups. In order to match concentric and eccentric recumbent stepping exercise for relative power and $\dot{V}O_2$, the same methods were used as in chapter 3 (see section 2.6.2). All exercise was matched to be below LT (within the moderate intensity domain). Briefly, the concentric LT was first determined by three experienced assessors, and the concentric and eccentric RITs analysed to obtain the $\dot{V}O_2$/power relationship. These relationships were then used to determine the concentric power required to achieve 90% of the concentric LT (CON_{MI} training group & eccentric (relative) power matched group (ECC_{PM})), and the eccentric relative power required to achieve the same $\dot{V}O_2$ within the eccentric RIT ($ECC_{\dot{V}O2}$ training group).

All participants completed eight weeks of training, attending two sessions a week (total of 16 session) separated by at least one day. The seat position, stride length and cadence used were the same as those used during baseline assessment and were kept constant throughout training. Each session comprised of a 1 minute warm-up, 15-30 minutes at the target power, and a 1 minute recovery period (Figure 4.3). During the warmup, participants were instructed to maintain their cumulative work within 60 to 70% of the target. The data acquisition software was then refreshed, and the participant was required to remain within the green target band (± 2% of the target cumulative work) throughout. During recovery, the participants were instructed to gradually reduce their applied force and were free to cool down for longer than 1 minute if required.

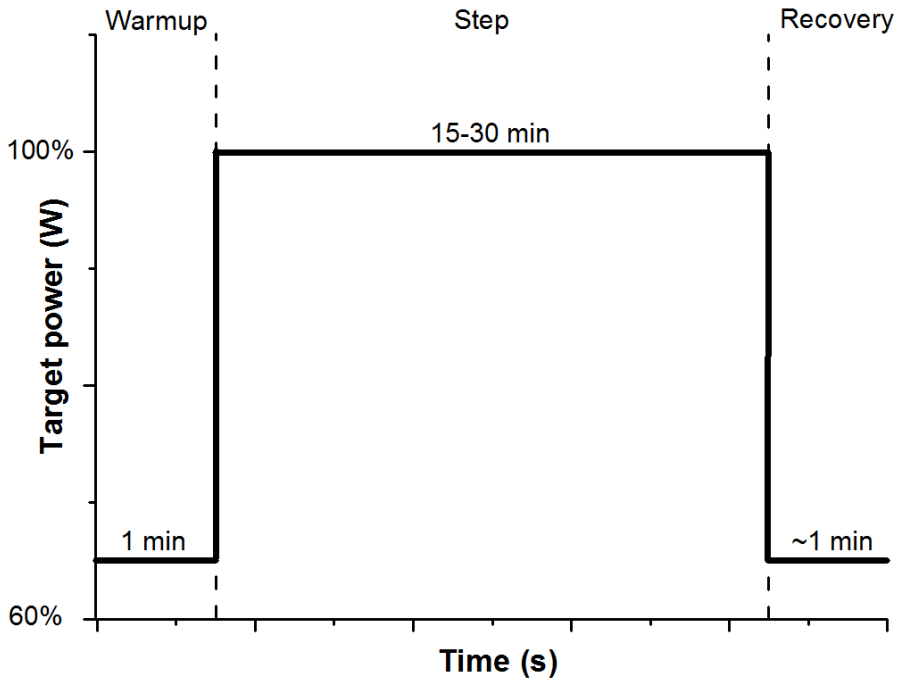

Figure 4.3. Schematic representation of the training session protocol. A warmup period at 60-70% of the target training power preceded a 15-30 minute step increase to target training power. A recovery period followed, in which the participant gradually reduced their applied force.

To reduce the severity of muscle soreness experienced, target power and duration increased throughout the training period (see section 1.6.5 for rationale). CPT duration was initially set at 15 minutes, and increased by 2.5 minutes per session from the 6th week onwards, culminating in a final training session duration of 30 minutes. Target power was set as a % Δ between the

unloaded intensity (20W) and target intensity (90 % of LT). Target power increased by 10 % per session, starting at 60%, and reaching the target power by the fifth session. A measures of RPE was taken at the end of every session (or at 15 minutes for sessions that were longer) and a rating of muscle soreness related to the 48 hours following the previous session obtained.

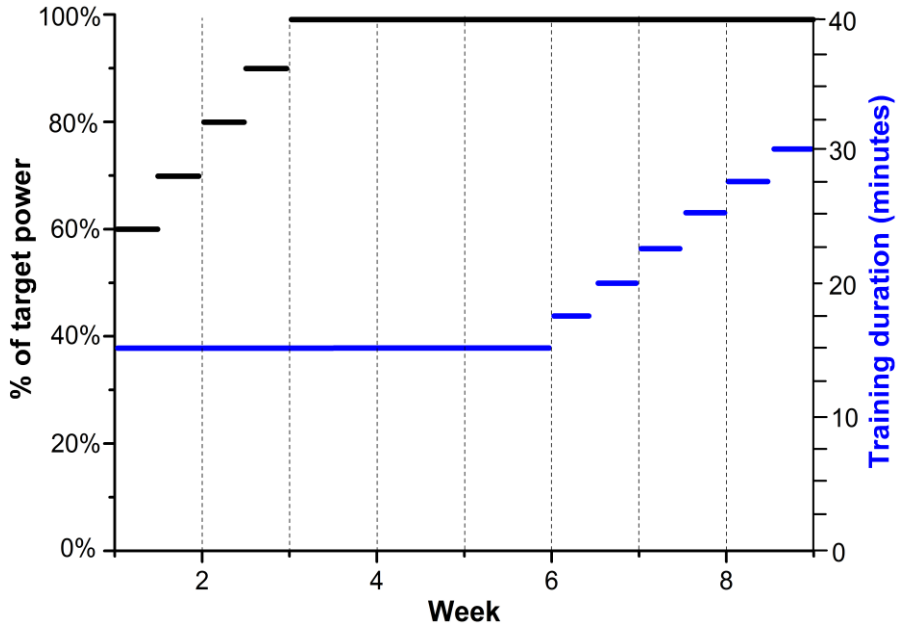

Figure 4.4. Progression of target power and session duration during training. Target power (black) increased in increments of 10%, from 60% between unloaded power (20W) and target power at week 1, to 100% at week 3. Session duration (blue), increased in increments of 2.5 minutes from 15 minutes at week 6 to 30 minutes at the final session.

4.2.5.3 Final training session

Within the final training session, additional measures of $\dot{V}O_2$, HR, and blood La were taken. The protocol used was identical to that detailed in section 2.4.3, with the unloaded/warm-up intensity set to 20W to allow stable pulmonary gas exchange values to be obtained prior to increasing target power. A rest period of ~ 2 min was completed followed by ~3 minutes of unloaded stepping at 20W. Following this, the target power was increased to the target training power and maintained for 30 minutes. Finally, a ~2 minute recovery period was completed at a target power of 20W. Blood La samples were taken during the CON_{MI} and ECC_{VO2} training sessions at rest, 5, 10, 20 and 30 minutes.

4.2.6 Outcome measures

4.2.6.1 Mechanical force and power

Force acquisition and unit conversion is detailed in section 2.3. In brief, raw force data were obtained at a rate of 1kHz from calibrated load cells positioned behind the left and right foot plates of the stepping ergometer and were fed into a data acquisition system (ML870 PowerLab 8/30; ADInstruments Ltd, Oxford, UK) connected to a PC for processing. Mechanical force (N) was post-hoc converted into power (W) *via* the equations previously detailed.

4.2.6.2 Pulmonary gas exchange

Pulmonary gas exchange data were collected with a breath-by-breath gas analysis system (MedGraphics D-series; Medical Graphics Corporation, St Paul, MN, USA; see section 2.5.2 for details). Briefly, the pneumotach and gas sensors were calibrated using flow rates and gas concentrations that encompass normal physiological values between rest and peak exercise. Calibration gases were sampled immediately after testing to assess if any drift had occurred.

4.2.6.3 Heart rate

ECG traces were obtained and HR determined in real-time using the R-R interval (see section 2.5.3) during baseline testing, the final training session, and post training assessment,. A 3-lead, lead II ECG configuration was used during stepping exercise (FE132 Bio Amp, ADInstruments Ltd, Oxford, UK), and a 12-lead configuration during cycle ergometry (X12+; Mortara Instrument UK Ltd, Stirling, UK) due to the higher cardiovascular and metabolic limits reached (identified from pilot testing). Raw ECG traces were visualised during testing to screen for any untoward cardiovascular events. HR during training was recorded at a frequency of 0.2Hz using a HR belt (RS800CX; Polar, Broxell Cl, Warwick, UK). The belt was moistened with water to increase conductance and positioned level with the xiphoid process.

4.2.6.4 Perceived exertion and muscle soreness

To monitor the difficulty of the exercise prescribed, a RPE (visual analogue scale (VAS), 0-100) was taken at the end of every session, with 0 representing no effort required, i.e. the pedals moving their legs for them, and 100 representing the hardest exercise they could imagine. A score was also obtained from each participant for the perceived level of muscle soreness experienced in the 48 hr period following the previous training session (VAS, 0-100, See section 2.5.5).

4.2.6.5 Blood lactate concentration

Finger capillary La samples were obtained from the left hand during testing. The sampling area was cleaned using an alcohol wipe and a push button safety lancet (safe-T-Pro Plus; Accu-chek, Roche, Germany) used to puncture the skin close to the nail bed of each finger. After discarding the first droplet, blood was collected into capillary tubes (GMRD-054; Analox instruments, London, UK), pre-treated with fluoride, nitrite and heparin to prevent coagulation and to stabilise the La content. When blood flow was inadequate, light pressure was applied to the finger to aid sample collection. The capillary tubes were inverted five times, sealed at each end with capillary closures, and placed on ice until analysis could be completed (~30 minutes).

4.2.7 Data analysis

4.2.7.1 Oxygen uptake

The methods by which $\dot{V}O_2$ data were filtered to remove erroneous breaths, interpolated to provide second by second data, as well as those used to determine unloaded $\dot{V}O_2$, $\dot{V}O_{2gain}$ and $\dot{V}O_{2peak}$ are detailed in section 2.6.4. Interpolation allowed $\dot{V}O_2$ data to be time aligned to power and HR data. The concentric LT was estimated by three experienced assessors using the methods described in section 2.6.1, and a mean value determined.

4.2.7.2 Force and power

The methods used to convert force applied to the foot plates (N) into power (W) are detailed in section 2.3.3, and the methods used to determine peak power and force are detailed in section 2.6.3. Following concentric LT determination, the $\dot{V}O_2$/power relationship of the concentric and eccentric RIT were plotted, and used to estimate the relative power required to achieve the target metabolic requirement (90% of concentric LT; see section 2.6.2).

4.2.7.3 Heart rate

HR filtering and processing were performed using LabChart and Origin Pro as detailed in section 2.6.5. Unloaded HR was determined as the mean HR within the final minute unloaded stepping or cycling. HR_{gain} was determined for the concentric and eccentric recumbent stepping RITs using the methods outlined in section 2.6.5.2. HR_{peak} was the highest HR attained during the RIT phase of the protocol.

4.2.7.4 Blood lactate

Analysis was conducted using an enzymatic metabolite analyser (Analox GM7; Analox instruments, London, UK) with an accuracy of 97% (88-104 mmol/L), and precision of 1.6% (manufacturer specifications). La concentration is determined *via* an oxidoreductase enzyme reaction, with the rate of O_2 release being directly related to La concentration:

$$La + O_2 \xrightarrow{Lactate\ oxidoreductase} pyruvate + H_2O_2$$

Equation 15.

where “La” is blood La, “O_2” is oxygen and “H_2O_2” is hydrogen peroxide.

The system was calibrated using an 8 mmol/L reference sample with an acceptance criteria of 7.8 – 8.2 mmol/L. 5 µL samples were then pipetted (Microman M25; Gilson, Middleton, USA) into the system for analysis. Analysis

continued until three consistent readings (± 0.1 mmol/L) were obtained for each sample. The 8 mmol/L reference was re-sampled every ~10 blood samples to ensure no drift had occurred. A mean of the three consistent readings determined the La value for each sample.

4.2.8 Statistical analysis

All results are expressed as means ± standard error of the mean (SE). GraphPad Prism statistical software (GraphPad v6.0, GraphPad Software Inc, California, USA) was used to conduct all analyses and estimate statistical significance ($P < 0.05$). All data was tested for normality prior to choosing the appropriate statistical test, and Bonferroni corrections were applied to all post-hoc comparisons to reduce the risk of type I error. Two way repeated measures ANOVA's were used to compare pre to post changes for the RITs, as well as changes throughout the training period or final training session. One way ANOVA's were used to compare baseline characteristics between groups, mean responses within the final training session, and Δ changes pre to post training.

4.3 Results

All participants familiarised to the exercise (able to maintain their cumulative mechanical work within ± 2% of the target work for > than 2 minutes) within three familiarisation sessions (range: 1 to 3). The methods used to control for the power produced by the participant during training (see section 2.3.4) ensured that the actual power remained within ± 2% of the target power throughout training.

4.3.1 Acute comparison: concentric *vs.* eccentric

At baseline assessment, comparison of concentric, eccentric and isometric voluntary strength for all participants revealed that eccentric strength was 29 ± 4 % higher than concentric strength (2028 ± 119 N *vs.* 1578 ± 77 N) and 48 ± 4 % higher than isometric strength (2028 ± 119 N *vs.* 1395 ± 95 N), (both $P < 0.05$ (Table 4.3; Figure 4.5). Baseline concentric strength was 17 ± 4 % higher than isometric strength ($P < 0.05$).

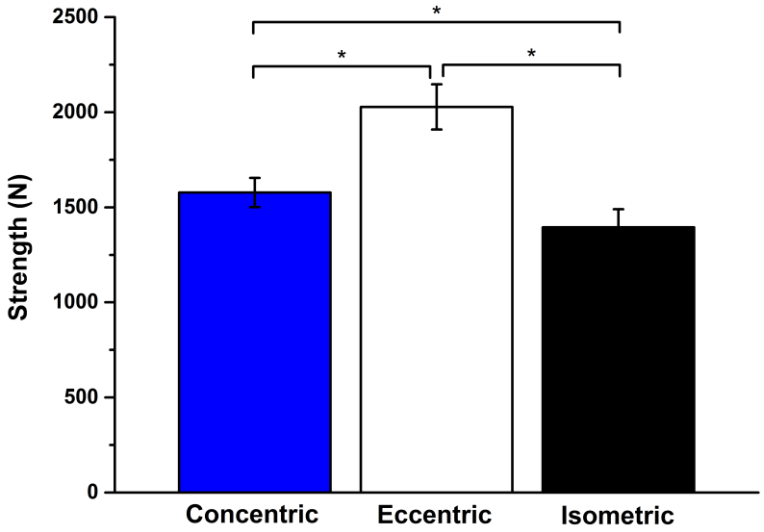

Figure 4.5. Dynamic (Concentric and eccentric) and static (isometric) knee extension voluntary strength (N) at baseline. Data presented as means ± SE. * Significant difference between groups (P < 0.05).

4.3.1.1 Ramp-incremental stepping: Acute responses

Similar to the previous study, a comparison of baseline cardiovascular and metabolic responses between concentric and eccentric ramp-incremental stepping for all participants was conducted (Table 4.1). There were no differences observed in regards to unloaded $\dot{V}O_2$ or HR_{peak}. There was a lower eccentric unloaded HR, $\dot{V}O_{2gain}$, HR_{gain}, absolute $\dot{V}O_{2peak}$, and relative $\dot{V}O_{2peak}$, and a higher eccentric peak relative power compared to concentric (all $P < 0.05$).

Table 4.1: Acute cardiovascular and metabolic responses to concentric (CON) and eccentric (ECC) ramp-incremental stepping exercise.

	CON	ECC	ECC (% of CON)
Unloaded $\dot{V}O_2$ ($L \cdot min^{-1}$)	0.51 ± 0.03	0.46 ± 0.02	94 ± 4
Unloaded HR ($beats \cdot min^{-1}$)	80 ± 3	76 ± 3*	96 ± 2
$\dot{V}O_{2gain}$ ($ml \cdot min^{-1} \cdot W^{-1}$)	31.4 ± 1.3	16.6 ± 0.9*	53 ± 2
HR_{gain} ($beats \cdot min^{-1} \cdot W^{-1}$)	1.54 ± 0.06	0.96 ± 0.06*	64 ± 4
Peak power (W)	86 ± 3	119 ± 4*	140 ± 3
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.62 ± 0.09	2.27 ± 0.07*	87 ± 2
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	34.6 ± 0.9	30.0 ± 0.9*	87 ± 2
HR_{peak} ($beats \cdot min^{-1}$)	170 ± 3	169 ± 3	100 ± 2

* Significantly different ($P < 0.05$) *vs.* concentric exercise. Results presented as means ± SE, n=22.

4.3.2 Baseline assessment

4.3.2.1 Physical characteristics

At baseline assessment, comparison between the three training groups (CON_{MI}, ECC_{PM} and $ECC_{\dot{V}O2}$) revealed no differences in age, height, weight, leg girth, or

concentric LT (all *n.s.* Table 4.2). Similarly, there were no differences in concentric, eccentric, isometric or mean voluntary strength between groups (all n.s. Table 4.3).

Table 4.2: Baseline physical characteristics.

	CON_{MI}	ECC_{PM}	$ECC_{\dot{V}O2}$
Age (yr)	24.6 ± 0.5	25.7 ± 1.7	25.1 ± 1.2
Height (cm)	179 ± 3	177 ± 2	179 ± 3
Mass (kg)	77 ± 3	74 ± 3	76 ± 3
Leg girth (cm)	53.8 ± 1.1	53.0 ± 1.7	53.2 ± 1.1
Concentric LT ($L \cdot min^{-1}$)	1.50 ± 0.08	1.47 ± 0.10	1.45 ± 0.06

Concentric moderate intensity (CON_{MI}), eccentric relative power-matched (ECC_{PM}) and eccentric $\dot{V}O_2$-matched ($ECC_{\dot{V}O2}$) training groups. Data presented as means ± SE.

4.3.2.2 Ramp-incremental tests

At baseline assessment, all responses measured during the cycle ergometer RIT were similar between training groups (absolute $\dot{V}O_{2peak}$, relative $\dot{V}O_{2peak}$, peak HR, and peak power (all *n.s.* Table 4.4). Similarly, all responses from the concentric recumbent stepping RIT were similar (HR_{gain}, absolute $\dot{V}O_{2peak}$, relative $\dot{V}O_{2peak}$, peak HR and peak power (all *n.s.* Table 4.5). Within the eccentric RITs, baseline responses were similar for HR_{gain}, HR_{peak}, and peak relative power (all *n.s.*), however, there was a higher absolute (P = 0.019) and relative (P = 0.033) $\dot{V}O_{2peak}$ within the $ECC_{\dot{V}O2}$ group compared to the ECC_{PM} group (Table 4.6).

4.3.3 Training period

4.3.3.1 Target WRs

The mean target relative powers within the three training groups were 44.2 ± 2.1 W in the CON_{MI} group, 41.4 ± 5.1 W in the ECC_{PM} group and 62.4 ± 3.8 W in the $ECC_{\dot{V}O2}$ group (Figure 4.6). CON_{MI} and ECC_{PM} target relative powers were similar (*n.s.*), and the $ECC_{\dot{V}O2}$ target relative powers were 41% and 51% higher than CON_{MI} and ECC_{PM} respectively (both $P < 0.05$). All participants tolerated the target relative powers for the full duration of every training session.

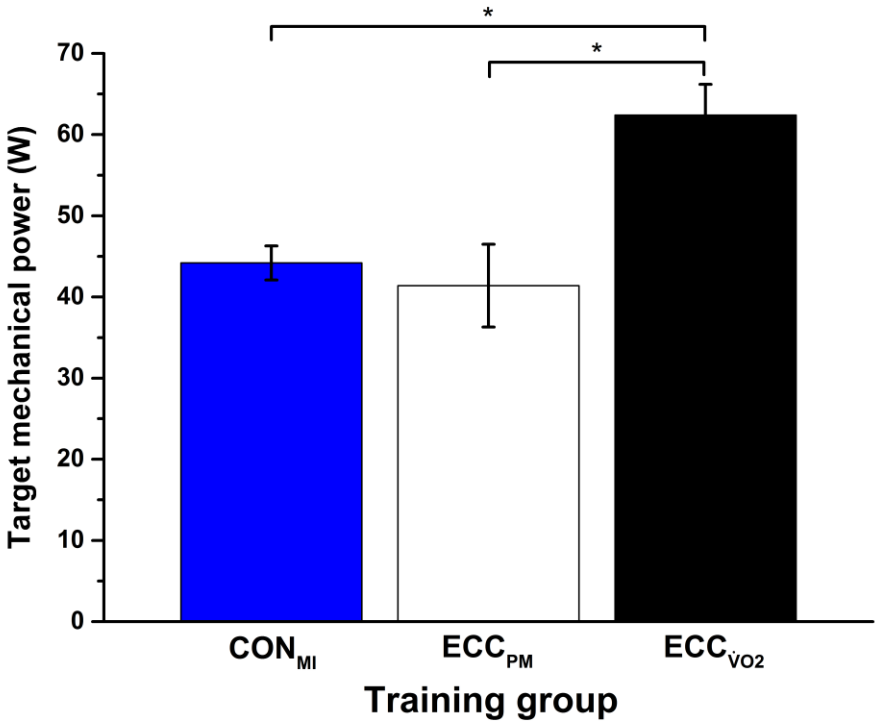

Figure 4.6. Target relative powers for the three training groups (CON_{MI}, ECC_{PM}, and $ECC_{\dot{V}O2}$). Mean ± SE , * = Significantly different (P < 0.05).

4.3.3.2 Rating of perceived exertion

There was no significant effect of training group or time on RPE scores (*n.s.* Figure 4.7). Within the $ECC_{\dot{V}O2}$ and CON_{MI} groups, there was a significantly higher RPE in the 5th training session (first session at the target relative power) compared to the 1st ($P < 0.05$), but no difference in RPE within the ECC_{PM} group throughout training (*n.s.*). The $ECC_{\dot{V}O2}$ training group presented with a non-

significant 20 % and 50 % higher mean RPE within the final 12 sessions (sessions at target relative power) compared to the CON_{MI} or ECC_{PM} groups respectively (49 ± 8 (CON_{MI}), 39 ± 8 (ECC_{PM}) and 59 ± 7 ($ECC_{\dot{V}O2}$)).

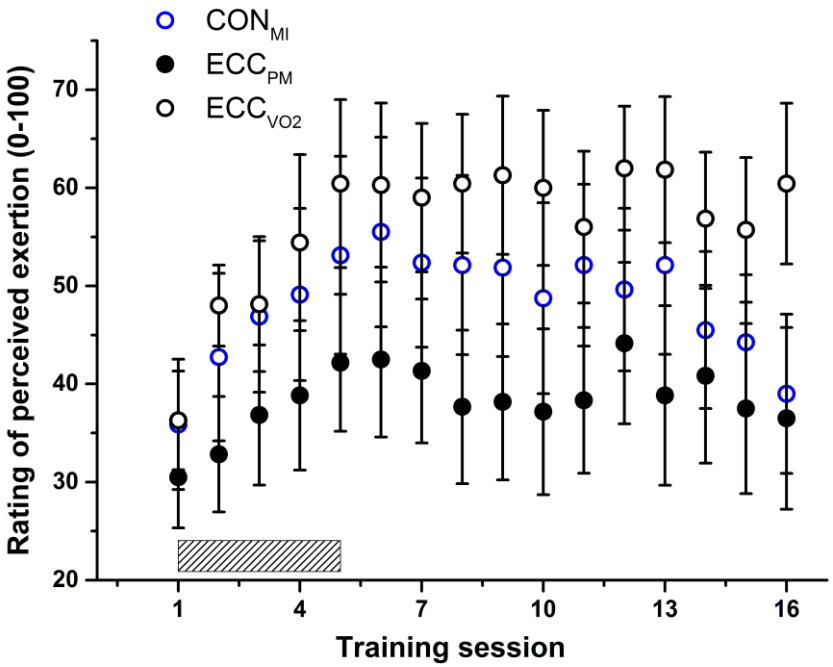

Figure 4.7. Mean ± SE ratings of perceived exertion (RPE) within the three training groups for each training session (n.s). Hashed box represents the first five training sessions where target relative power increased by 10% per session.

4.3.3.3 Muscle soreness rating

Peak muscle soreness experienced by any participant during training was 41/100 for CON_{MI}, 69/100 for ECC_{PM} and 30/100 for the $ECC_{\dot{V}O2}$ training group. Mean muscle soreness values within the final 12 sessions (sessions at the target relative power) were 3.0 ± 1.6 for CON_{MI}, 11.6 ± 10.6 for ECC_{PM}, and 2.7 ± 0.9 for the $ECC_{\dot{V}O2}$ group (*n.s*).

4.3.3.4 Training HR

There was no effect of time (training session) or training group (CON_{MI}, $ECC_{PM,}$ or $ECC_{\dot{V}O2}$) on the HR response during the 16 training sessions (*n.s.*).

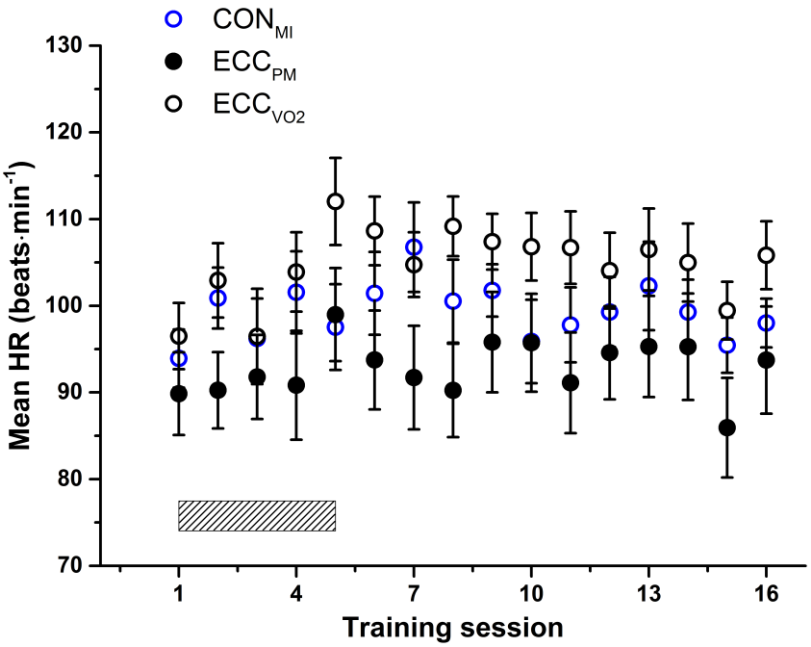

Figure 4.8. Mean ± SE heart rate (HR) response within the three groups for each training session. Hashed box represents the first five training sessions were target relative power increased by 10% per session.

4.3.3.5 Final training session

Within the final training session, the CON_{MI} and ECC_{PM} training groups reached a steady state $\dot{V}O_2$ by 4 minutes (subsequent values *n.s.* Figure 4.9). However, a steady state was not reached within the $ECC_{\dot{V}O2}$ test, with a consistently higher $\dot{V}O_2$ measured after 24 minutes into the test ($P < 0.05$). There was no difference between CON_{MI} and $ECC_{\dot{V}O2}$ $\dot{V}O_2$ throughout the 30 minute session (*n.s.*). ECC_{PM} $\dot{V}O_2$ was lower than CON_{MI} and $ECC_{\dot{V}O2}$ from 2 minutes onwards ($P < 0.05$), and presented with a 43 % and 41 % lower $\dot{V}O_2$ compared to CON_{MI} and $ECC_{\dot{V}O2}$ within the final 2 minutes respectively (1.20 (CON_{MI}), 0.68 (ECC_{PM}) and 1.17 ($ECC_{\dot{V}O2}$) L·min-1; $P < 0.05$). There was no difference in the final 2 minute mean $\dot{V}O_2$ response between the CON_{MI} and $ECC_{\dot{V}O2}$ training groups (*n.s.*).

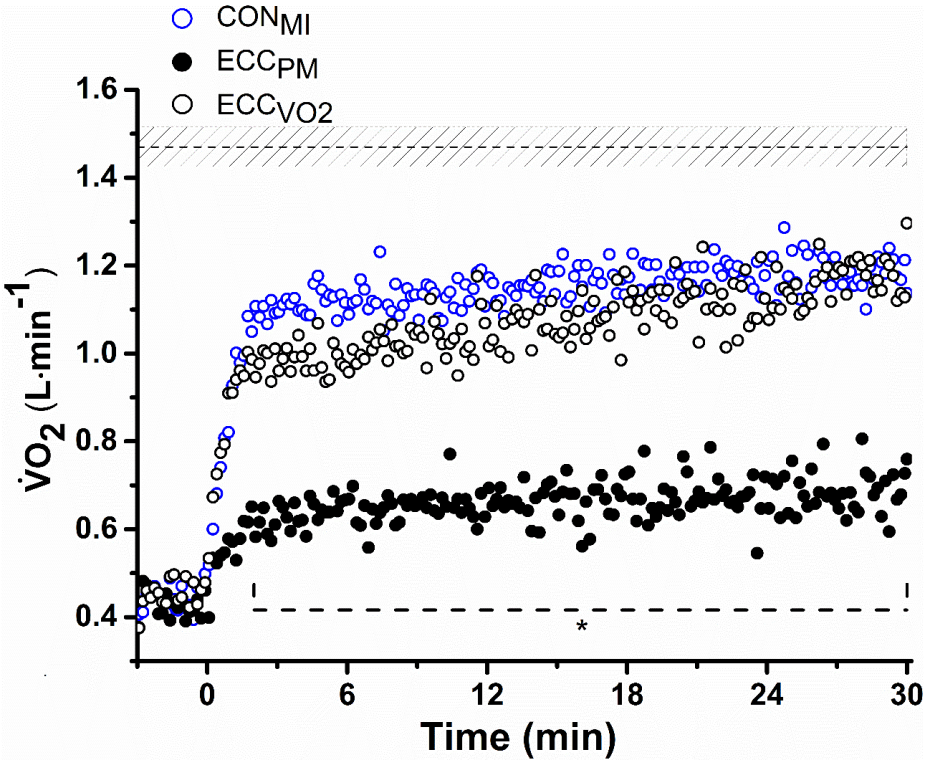

Figure 4.9. Mean interpolated oxygen uptake ($\dot{V}O_2$) during the final 30 minute training session (10s mean applied). Hashed box represents mean concentric LT ± SE. * 2 minute means significantly different (P < 0.05) vs. CON_{MI}.

Within the final session, there was a non-significant 8% and 13% higher $ECC_{\dot{V}O2}$ HR compared to CON_{MI} and ECC_{PM} respectively (98 ± 3 beats·min^{-1} (CON_{MI}), 94 ± 6 beats·min^{-1} (ECC_{PM}), 106 ± 4 beats·min^{-1} ($ECC_{\dot{V}O2}$)).

As the CON_{MI} and $ECC_{\dot{V}O2}$ training groups were matched to 90% of the concentric LT, blood La samples were taken to ensure no significant elevation above resting values occurred. There was no significant change in blood La concentrations relative to resting values at any time point (5, 10, 20 or 30 min), nor between training groups at any time point (both n.s, Figure 4.10).

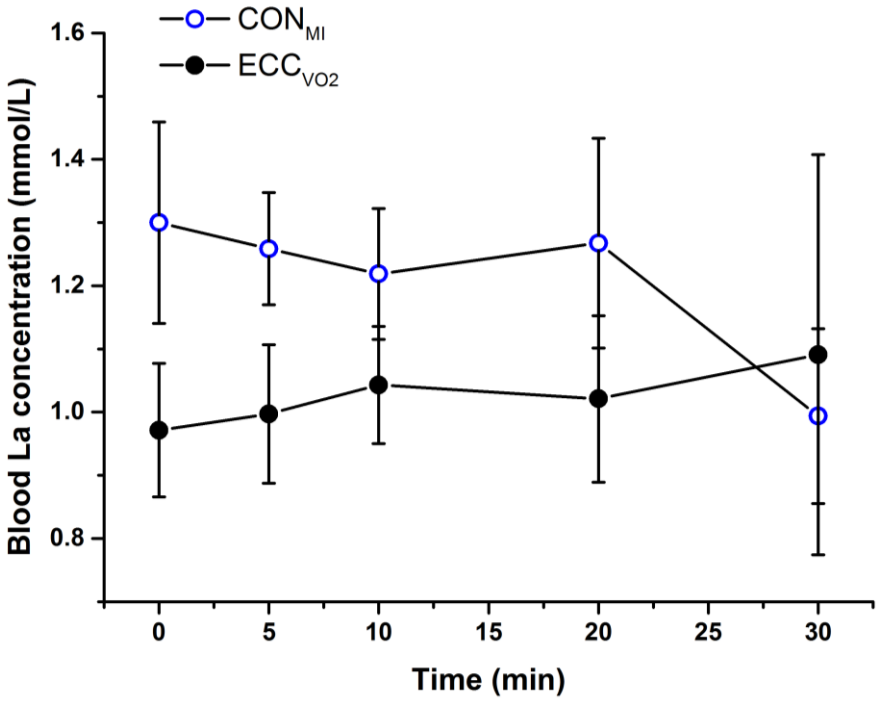

Figure 4.10. Mean ± SE blood Lactate (La) concentrations within the CON_{MI} and $ECC_{\dot{V}O2}$ training groups during the final training session (n.s).

4.3.4 Training responses

4.3.4.1 Voluntary strength

The absolute and relative changes in concentric, eccentric and isometric voluntary strength with training are presented in Table 4.3 and Figure 4.11 respectively. Post-hoc power calculation determined that our study had a power of 0.76 to detect a change in mean voluntary strength between concentric and eccentric training matched for metabolic requirement. The CON_{MI} training group, showed no changes in concentric, eccentric, or isometric voluntary strength (all *n.s.*). Within the ECC_{PM} training group, there were no changes in eccentric or isometric voluntary strength (both *n.s.*), however, there was a 13.0 ± 4.3 % increase in concentric voluntary strength following training (P = 0.023). Within the $ECC_{\dot{V}O2}$ group, there were increases in concentric, eccentric, and isometric voluntary strength (all $P < 0.05$).

Table 4.3. Pre and post concentric, eccentric and isometric voluntary strength (N) for each training group.

	CON_{MI}		ECC_{PM}		$ECC_{\dot{V}O2}$	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Concentric (N)	1629 ± 174	1661 ± 148	1516 ± 127	1687 ± 84 *	1580 ± 104	1795 ± 122 *
Eccentric (N)	2101 ± 252	2326 ± 190	2082 ± 261	2275 ± 244	1909 ± 109	2514 ± 202 *
Isometric (N)	1463 ± 179	1504 ± 172	1409 ± 227	1515 ± 174	1314 ± 101	1572 ± 138 *
Mean (N)	1731 ± 196	1831 ± 166	1669 ± 202	1825 ± 163	1601 ± 94	1960 ± 136 *

* Significant difference ($P < 0.05$) from pre to post training RITs.

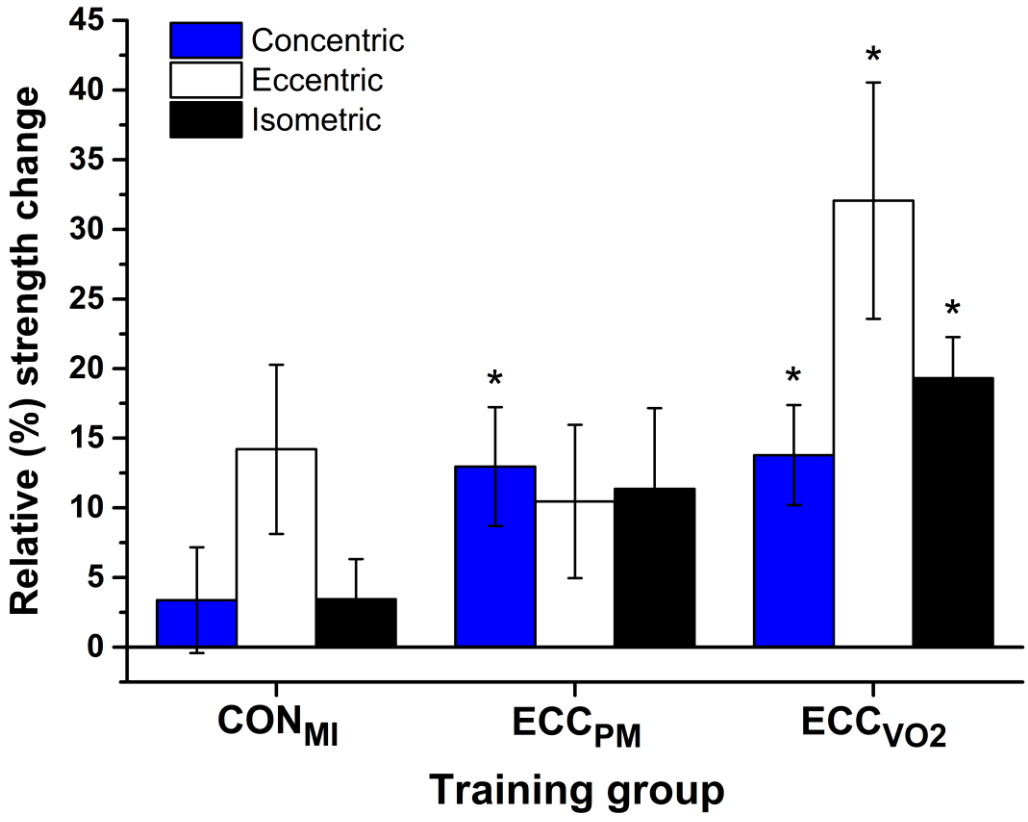

Figure 4.11. Relative (%) concentric, eccentric and isometric voluntary strength changes following training. Mean ± SE, * = significant change relative to pre assessment (P < 0.05).

4.3.4.2 Leg girth

All training groups showed no changes in leg girth as a result of training. CON_{MI} = 0.1 ± 0.9 % change (*n.s.*), ECC_{PM} = 1.9 ± 1.6 % change (*n.s.*), $ECC_{\dot{V}O2}$ = 0.2 ± 1.1 % change (*n.s.*).

4.3.4.3 Ramp-incremental tests

There were no significant changes to any of the cycle ergometer RIT variables as a result of training within any of the training groups (*n.s.* Table 4.4). There remained no differences between groups for all variables at post assessment (*n.s.*).

Table 4.4. Cycle ergometry ramp-incremental test results

	CON_{MI}		**ECC_{PM}**		**$ECC_{\dot{V}O2}$**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
$\dot{V}O_{2gain}$ ($ml \cdot min^{-1} \cdot W^{-1}$)	11.7 ± 0.5	11.7 ± 0.3	11.5 ± 0.4	11.1 ± 0.5	11.3 ± 0.3	11.4 ± 0.4
Peak power (W)	304 ± 16	305 ± 13	289 ± 21	291 ± 18	304 ± 8	304 ± 7
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	3.70 ± 0.19	3.72 ± 0.20	3.37 ± 0.28	3.56 ± 0.26	3.63 ± 0.14	3.67 ± 0.13
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	48.4 ± 2.0	48.5 ± 2.5	45.3 ± 2.8	47.8 ± 2.3	48.0 ± 2.4	48.7 ± 2.4
HR_{peak} ($beats \cdot min^{-1}$)	186 ± 2	184 ± 2	192 ± 3	188 ± 3	187 ± 4	186 ± 4

Training within the CON_{MI} and ECC_{PM} groups did not elicit change to any of the concentric, or eccentric RIT outcomes relative to pre assessment (*n.s.* Table 4.5 and Table 4.6). Within the $ECC_{\dot{V}O2}$ training group, there were no changes in $\dot{V}O_{2gain}$, HR_{gain}, absolute $\dot{V}O_{2peak}$, relative $\dot{V}O_{2peak}$ or HR_{peak} for either RIT (*n.s.*), however, there was a 7 ± 1% (6.1 ± 1.4 W) increase in peak power within the concentric RIT and a 14 ± 2% (16.5 ± 2.5 W) increase in peak relative power within the eccentric RIT relative to baseline (both $P < 0.05$). To correct for the

observed differences in $ECC_{\dot{V}O2}$ and ECC_{PM} absolute and relative $\dot{V}O_{2peak}$ at baseline assessment, an additional one-way ANOVA was conducted on the absolute change from baseline. This again showed no significant difference between all training groups despite the $ECC_{\dot{V}O2}$ group displaying a 0.28L·min^{-1} (11%) increase in absolute $\dot{V}O_2$.

Within the concentric RIT, as with pre assessment, there were no differences between groups (CON_{MI}, ECC_{PM} or $ECC_{\dot{V}O2}$) for any variables at post assessment (*n.s.*). Within the eccentric RIT, $\dot{V}O_{2gain}$, HR_{gain}, peak relative power and HR_{peak} remained similar between groups (*n.s.*), and absolute and relative $\dot{V}O_{2peak}$ remained higher within the $ECC_{\dot{V}O2}$ training group relative to the ECC_{PM} group (both $P < 0.05$).

Table 4.5. Pre and post concentric ramp-incremental test responses

	CON_{MI}		**ECC_{PM}**		**$ECC_{\dot{V}O2}$**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
$\dot{V}O_{2gain}$ (ml·min^{-1}·W^{-1})	33.0 ± 2.6	28.7 ± 1.5	29.7 ± 2.4	30.2 ± 1.4	31.1 ± 1.2	30.5 ± 2.5
HR_{gain} (beats·min^{-1}·W^{-1})	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Peak power (W)	87 ± 5	91 ± 4	86 ± 8	88 ± 6	83 ± 3	90 ± 4 *
$\dot{V}O_{2peak}$ (L·min^{-1})	2.74 ± 0.15	2.74 ± 0.11	2.47 ± 0.21	2.54 ± 0.17	2.60 ± 0.07	2.85 ± 0.12
$\dot{V}O_{2peak}$ (ml·min^{-1}·kg^{-1})	35.8 ± 1.3	35.9 ± 1.9	33.3 ± 2.3	34.2 ± 1.7	34.3 ± 1.0	37.7 ± 1.8
HR_{peak} (beats·min^{-1})	167 ± 4	167 ± 1	175 ± 5	175 ± 5	171 ± 5	173 ± 4

* Significant difference ($P < 0.05$) from pre to post training RIT.

Table 4.6. Pre and post eccentric ramp-incremental test responses

	CON_{MI}		ECC_{PM}		$ECC_{\dot{V}O2}$	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
$\dot{V}O_{2gain}$ ($ml \cdot min^{-1} \cdot W^{-1}$)	16.7 ± 1.3	15.8 ± 1.8	15.4 ± 2.0	14.3 ± 1.5	17.5 ± 1.5	14.8 ± 1.5
HR_{gain} ($beats \cdot min^{-1} \cdot W^{-1}$)	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Peak power (W)	121 ± 8	122 ± 9	120 ± 8	129 ± 12	117 ± 6.5	133 ± 8 *
$\dot{V}O_{2peak}$ ($L \cdot min^{-1}$)	2.31 ± 0.13	2.34 ± 0.18	1.98 ± 0.08	2.13 ± 0.15	2.46 ± 0.08 †	2.74 ± 0.11 †
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	30.1 ± 1.2	30.5 ± 2.3	26.8 ± 1.1	28.7 ± 1.4	33.0 ± 1.9 †	36.5 ± 2.4 †
HR_{peak} ($beats \cdot min^{-1}$)	165 ± 5	165 ± 6	168 ± 2	178 ± 6	177 ± 5	177 ± 4

* Significant difference ($P < 0.05$) from pre to post training RIT, † Significant difference ($P < 0.05$) among eccentric training groups (ECC_{PM} and $ECC_{\dot{V}O2}$).

4.4 Discussion

Comparison of training responses to concentric and eccentric recumbent stepping exercise matched for metabolic or mechanical requirement showed that: 1) a 41% greater target relative power was required during eccentric recumbent stepping to match for concentric metabolic requirement (90% LT); 2) all training was tolerable and resulted in minimal muscle soreness; 3) the higher relative power performed during $ECC_{\dot{V}O2}$ likely gave rise to increases in concentric, eccentric and isometric voluntary strength, as well as increases in peak relative power achieved during CON and ECC RITs.

4.4.1 Acute reduction in cardiovascular and metabolic requirement

As the same acute RIT measures assessed within the previous study were measured at baseline assessment within this training study, validation of the previous results with a greater participant number (n = 21) was possible. Similar results were attained showing a lower $\dot{V}O_{2gain}$, HR_{gain}, absolute and relative $\dot{V}O_{2peak}$, and a higher peak relative power with eccentric *vs.* concentric RIT exercise. These results confirm that eccentric recumbent stepping mirrors previous eccentric modalities showing a reduction in cardiovascular and metabolic requirement compared to concentric activity. The only difference we observed was a lower eccentric unloaded HR, likely due to the greater power to detect the small difference (4 beats·min^{-1} lower) reported.

Baseline comparison of concentric, eccentric and isometric voluntary muscle strength for all participants, showed a 29% and 48 % higher eccentric strength compared to concentric and isometric respectively, consistent with previous eccentric literature (section 1.6.1). These differences however, may have been more pronounced if we used a higher cadence than 23 spm (contraction velocity of ~46 degrees·s^{-1}) as disparity between concentric and eccentric force increases at greater contraction velocities: during knee extensor contractions performed at slow (45 degrees·s^{-1}) or fast (360 degrees·s^{-1}) velocities, a 20%

and 146% higher eccentric torque was found compared to concentric contractions respectively (Westing et al., 1991).

4.4.2 Baseline comparison between training groups

The three training groups within this study were well matched at baseline for age, height, weight, leg girth, voluntary strength (concentric, eccentric and isometric), and RIT responses. However, there was a 24% and 23% higher absolute and relative eccentric RIT $\dot{V}O_{2peak}$ in the $ECC_{\dot{V}O2}$ training group compared to ECC_{PM} group. With there being no differences in $\dot{V}O_{2peak}$ measured within the cycle ergometry RIT, and no differences in baseline demographics or voluntary strength, this result was unexpected and its origins unclear. To correct for these differences, an additional one-way ANOVA was conducted on the change in absolute and relative $\dot{V}O_{2peak.}$

4.4.3 Training period

4.4.3.1 Training intensity

To match for metabolic requirement (90% of concentric LT), a 41% higher eccentric relative power was required during training. This is a similar increase to the previous study (chapter 3) but smaller than previous reverse cycle ergometry studies have shown, with Dufour et al. (2004) showing a fivefold greater WR, and LaStayo et al. (1999) a sevenfold greater eccentric WR at a metabolic demand of ~1$L \cdot min^{-1}$. These differences are likely the result of the lower cadence utilised, but may also be a result of power calculations with the ergometers used. Coupled with the increased force disparity between concentric and eccentric contractions at higher contraction velocities, metabolic disparity also increases, allowing greater eccentric target relative powers to be achieved at matched metabolic requirements (see chapter 1, Abbott *et al*., 1952). Despite the smaller increases, a 41% higher mechanical requirement was still sufficient to induce greater voluntary strength changes, and lower cadences used may allow for faster familiarisation, greater control of power, and be more tolerable for certain clinical populations.

Despite the increased mechanical requirement of ECC$_{\dot{V}O2}$ group training, exercise was still perceived as tolerable, with a mean RPE of ~60/100. This rating does, however, suggest that metabolic targets higher than 90% of concentric LT may encroach on the upper limits of tolerable eccentric endurance exercise. The large variability seen in the RPE measures taken are likely due to the subjective nature of the assessment, and more descriptive assessments such as the Borg RPE scale (Borg, 1970; Scherr et al., 2013) may, in future, allow a more accurate assessment of exercise effort. Based on previous literature, it would have been expected that, when matched for relative power, eccentric recumbent stepping would be perceived as easier to perform, and when matched for metabolic demand would be perceived as slightly harder (Thomson, 1971; Henriksson et al., 1972; Hollander et al., 2003; Meyer et al., 2003). Our results display a similar trend, but a greater statistical power is required to confirm this.

Previous studies have shown that gradual progression of WR reduces or completely eradicates the development of eccentric exercise induced muscle soreness (see section 1.6.5). Within the ECC$_{\dot{V}O2}$ training group that resisted the highest relative powers, a mean muscle soreness rating of just 2.9/100 (VAS) and a peak for any participant of 30/100 (generally considered mild pain (Jensen et al., 2003) provides further evidence for these adaptations. The low muscle soreness scores may also be due to the low contraction velocity utilised, as increased muscle damage and subsequent soreness has been shown with faster velocities of eccentric contraction (Chapman et al., 2006). With muscle soreness being inherently subjective in nature, our results presented with a large variability. Therefore, in future, other measures such as the McGill Pain Questionnaire may improve the accuracy and interpretability of muscle soreness scores (Cleather and Guthrie, 2007).

4.4.3.2 Cardiovascular and metabolic responses during training

Our data showed that there were no differences in HR responses between training groups. Previous research, as well as the acute results identified within this and the previous study (chapter 3), have shown that at the same metabolic demand, eccentric exercise requires a greater cardiovascular requirement (O_2 pulse) (see section 1.6.3). Therefore, it was expected that the $ECC_{\dot{V}O2}$ group would have presented with a higher HR response compared to CON_{MI}. The 8 beat·min^{-1} higher $ECC_{\dot{V}O2}$ HR may have become significant with a greater sample size and power, however our data presented with a large effect size (Cohen's d = 0.84), providing sufficient evidence to conclude that HR demand is not greater during eccentric *vs.* concentric training at low metabolic (90% concentric LT) requirements. To our knowledge, the mean $ECC_{\dot{V}O2}$ HR response of ~106 beats·min^{-1}, is the lowest reported during previous eccentric endurance training studies displaying increases in voluntary strength (~127 beats·min^{-1}, LaStayo *et al*., 2000). This, therefore, further supports adoption of eccentric endurance training as a therapeutic intervention, e.g. for CHF patients who require a lower cardiovascular demand during exercise.

Within this study, CON_{MI} and $ECC_{\dot{V}O2}$ metabolic requirements were accurately matched within the desired moderate intensity exercise domain, confirmed by $\dot{V}O_2$ being below the concentric LT and no rise in La levels. Using the same methods for setting target power, the previous study (chapter 3) showed a gradually increasing $\dot{V}O_2$ within the $ECC_{\dot{V}O2}$ group, resulting in a final minute $\dot{V}O_2$ greater than the concentric LT (1.34 *vs.* 1.28 L·min^{-1}). This suggested that eccentric exercise may have been performed at a higher exercise intensity than concentric. Results within this study also showed a gradually increasing $ECC_{\dot{V}O2}$ $\dot{V}O_2$ within the final training session, however, the rate of increase was not as pronounced (significantly higher than predicted after 24 minutes *vs.* 10 minutes in the previous study) resulting in $\dot{V}O_2$ remaining below LT even after 30 minutes. This may imply that training adaptations may have reduced the magnitude of the slow component, although to confirm this, measurements of $\dot{V}O_2$ and blood La during the 5th session (first session at target relative power) are required. Both endurance and work matched interval training protocols have previously been

shown to reduce the magnitude of the $\dot{V}O_2$ slow component (Berger et al., 2006; Bailey et al., 2009a; Krustrup et al., 2010), but this exercise was performed at far greater metabolic requirements than our study, meaning it remains unclear if lower metabolic demands would result in similar adaptations. Although we lack $\dot{V}O_2$ and blood La measurements throughout training, HR data showed no change from the 5th to last training session, suggesting a reduction in $\dot{V}O_2$ as a result of training is unlikely.

4.4.4 Training responses to eccentric *vs.* concentric recumbent stepping.

4.4.4.1 Increases in voluntary strength at low metabolic requirements

This study demonstrated that 8 weeks of eccentric recumbent stepping performed at a moderate exercise intensity increased concentric, eccentric and isometric voluntary strength, not seen with concentric exercise at the same metabolic requirement. As highlighted within section 1.6.2, the ability to produce higher forces and relative power at the same metabolic requirement with eccentric *vs*. concentric stepping is likely the stimulus for the differential voluntary strength increases, with neural adaptations likely the predominant mechanisms. The majority of eccentric research has focussed on voluntary strength changes to resistance training (Roig et al., 2009), with minimal research investigating eccentric endurance training (LaStayo et al., 2014). Studies that have assessed eccentric endurance training within a young healthy population have utilised reverse cycle ergometry and assessed only isometric voluntary strength on a separate ergometer, making comparisons with our study difficult. Despite this, LaStayo *et al*. (1999, 2000) showed isometric voluntary strength increases of 33% and 36% respectively with eccentric training. By comparison, the current study showed a lower 19.3% increase in isometric voluntary strength, and a 13.8% and 32.1% increase in concentric and eccentric voluntary strength within the $ECC_{\dot{V}O2}$ training group. The previous studies utilised a faster cadence of ~60 rpm, likely increasing the eccentric/concentric force disparity previously mentioned, and increasing the eccentric mechanical stimulus for adaptations. For example, LaStayo et al. (1999) required a seven-times greater eccentric

target relative power to match for a ~1L·min^{-1} $\dot{V}O_2$ response at 60 rpm, and Paddon-Jones et al. (2001) has shown greater increases in voluntary strength with fast (~180 deg·s^{-1}) *vs.* slow (~30 deg·s^{-1}) contraction velocities. This disparity likely explains the greater isometric voluntary strength increases they report, and future utilisation of higher eccentric recumbent stepping cadences may provide a greater training stimulus to induce greater voluntary strength increases.

Our results suggest that the relative power produced within the CON_{MI} and ECC_{PM} training groups is likely too low a stimulus to induce significant voluntary strength changes. However, the ECC_{PM} training group did show significant increases in concentric voluntary strength, and the CON_{MI} training group showed a trend towards increased eccentric voluntary strength (14.2%). Calculation of effect sizes showed a moderate (Cohen's d of 0.65) and low (Cohen's d of 0.38) effect size for these comparisons respectively, suggesting a greater sample size would be required to confidently report a change. It may be possible that eccentric exercise performed at these low metabolic demands provides a sufficient mechanical stimulus to induce small increases, or prevent a decline in voluntary strength, potentially beneficial within exercise intolerant and older adult populations.

Additional strength assessment familiarisation was conducted prior to training, which aimed to reduce the effect of training modality specific increases in voluntary strength (i.e. greater increases in eccentric *vs.* concentric voluntary strength with eccentric training) previously reported (Roig et al., 2009). Greater relative increases in eccentric voluntary strength were evident within the $ECC_{\dot{V}O2}$ group, however the ECC_{PM} group presented with significant increases in concentric voluntary strength, suggesting that the mode of training did not influence the subsequent voluntary strength changes, potentially due to adequate familiarisation.

(Rice et al., 1990; Whitney et al., 1995)No changes were seen in mid-thigh leg girth measurements within any of the training groups. Firstly, although leg girth measurements have been shown to present with a high intra-rater reliability (Rice et al., 1990; Whitney et al., 1995), their accuracy reduces when the lean mass % is low and muscle mass can be overestimated in comparison to CT scans (Rice et al., 1990). Secondly, although there are conflicting results (LaStayo et al., 2000), muscle hypertrophy is considered to only significantly increase after roughly 8 weeks of training (Narici et al., 1989; Akima et al., 1999), and a poor correlation exists between limb girth and voluntary strength changes (Moritani and Devries, 1980; Jones and Rutherford, 1987). Therefore, (Jones and Rutherford, 1987)it is likely that this study duration was too small to elicit muscle mass changes measurable *via* leg girth measurements. (Cooper et al., 1981)(Jones and Rutherford, 1987)More accurate muscle mass assessment methods such as computerised tomography or dual energy X-ray absorptiometry (Levine et al., 2000) may have allowed a more accurate assessment of muscle mass changes with this training.

4.4.4.2 Effect of training on RIT responses

There were no changes to any of the cycle ergometry RIT outcome measures. The metabolic training intensity was below LT, meaning that it is unlikely this exercise placed enough of a cardiovascular or metabolic demand to induce preferential increases in parameters such as $\dot{V}O_{2peak}$. This is in agreement with LaStayo et al. (2000) who showed no difference in $\dot{V}O_{2peak}$ or HR_{peak} with concentric or eccentric training at ~ 60% of HR_{peak}. The cycle ergometer RIT placed a higher peak metabolic demand than the stepping ergometer RITs (~3.7 *vs.* ~2.8 $L \cdot min^{-1}$), supporting our theory that the cycle ergometer LoT was largely due to an inability to deliver and/or utilise required O_2, whereas the stepping ergometers LoT was due to an inability to produce the required relative power (i.e. oxidative capacity limited *vs.* strength limited).

There were also no changes to any of the concentric or eccentric RIT responses within the CON_{MI} or ECC_{PM} training groups, suggesting that the mechanical and/or metabolic demand was not sufficient to induce preferential adaptations.

However, the ECC$_{\dot{V}O2}$ group showed an increase in peak relative power achieved within both the concentric and eccentric RITs, likely due to the higher mechanical requirements of the training stimulating greater neural adaptions that allowed for higher peak forces to be attained (see section 1.7.1). Interestingly, increases in peak relative power seen with ECC$_{\dot{V}O2}$ training did not translate into a significant elevation in $\dot{V}O_{2peak}$ (9.5% increase in concentric RIT absolute $\dot{V}O_{2peak}$ (large effect size of 0.89) and an 11.4% increase in eccentric RIT absolute $\dot{V}O_{2peak}$ (large effect size of 1.13). This may be explained within the eccentric RIT by the 15.5% lower $\dot{V}O_{2gain}$ post training, however, a greater sample size is required to confirm this. $\dot{V}O_2$ gain has been shown to be relatively consistent during cycle ergometry (~10ml·min^{-1}·W^{-1}) and does not change with training (Rossiter, 2011), however, it may be possible that efficiency during eccentric exercise can be increased with training, potentially *via* improved coordination and recruitment of more motor units at greater powers to distribute metabolic requirement.

4.4.5 Conclusions

By matching for metabolic and mechanical requirement between concentric and eccentric recumbent stepping training, we have shown that eccentric recumbent stepping increases the mechanical requirement, and likely results in increases in voluntary muscle strength not seen with traditional (concentric) training performed at a similar low cardiovascular and metabolic demands. This exercise was shown to be tolerable for long durations, and gradual progression of target power resulted in minimal muscle perceived soreness. Although limited comparisons are available, voluntary strength increases during eccentric recumbent stepping are smaller than that seen during eccentric cycling, likely due to the lower cadence used within this study. Utilisation of a lower cadence and a modality that may more closely match normal activity may have allowed for faster familiarisation, less muscle soreness and greater functional improvements. These results further support the potential of eccentric recumbent stepping exercise as a rehabilitative tool within exercise intolerant populations such as COPD or CHF patients and older adults. However, further research within these populations is required that includes an appropriate control group, and tighter control of exercise intensities to fully understand it's potential.

Chapter 5 Feasibility of eccentric recumbent stepping training within an older adult population

5.1 Introduction

As age progresses, natural detriments occur within the neuromuscular (Vandervoort, 2002; Rolland et al., 2008; Verschueren et al., 2013), metabolic (Sharma and Goodwin, 2006), cardiovascular (Lakatta, 1990; Ferrari et al., 2003), respiratory (Sharma and Goodwin, 2006) and neurological systems (Levy, 1994), resulting in reduced mobility, independence and increased social and economic strain (Vina et al., 2016). Of primary concern is the increased prevalence of falls, the leading cause of death in an older adult population (Stevens et al., 2006). Combined with the age-related decline in bone mineral density, traumatic injuries from falls are common, and result in increased morbidity, frailty and mortality (Rubenstein, 2006). Whilst other risk factors contribute to an increased risk of falling (age, gender, medication, inactivity, body mass index (BMI) and co-morbidities (Landi et al., 2012), sarcopaenia, the age-related decline in muscle mass and quality, remains the leading predictor of falls (Goodpaster et al., 2006), (see section 1.7.3).

Incorporation of exercise, specifically resistance training, consistently produces increases in voluntary strength and reduces fall risk within an older adult population (Charette et al., 1991; Schlicht et al., 2001; Vina et al., 2016; Silva et al., 2017). However, research suggests that eccentric exercise is likely a more effective training modality due to: 1) low cardiovascular and metabolic requirement of the exercise; 2) capacity to generate higher forces eccentrically ; 3) low perceived effort; and 4) greater retention of eccentric voluntary strength with age (Hortobágyi et al., 1995; Roig et al., 2010; Gluchowski et al., 2017). These findings have led to a rise in eccentric exercise research, predominantly resistance training, within older adults showing superior increases in voluntary strength, functional capacity and reduced falls risk in comparison to traditional

(concentric) resistance training (Gluchowski et al., 2015). Studies utilising eccentric endurance training report similar improvements (LaStayo et al., 2003; Lastayo et al., 2010; LaStayo et al., 2011; LaStayo et al., 2017). However, training for ~12 weeks, with three sessions a week, is a large commitment for participants. Exercise intensity within these studies progressed slowly (to reduce severity of muscle soreness), and increased only up to a perceived rating of "somewhat hard". Hence, it remains unknown whether shorter eccentric endurance training studies (e.g. 4 weeks) with faster WR progression and higher perceived training intensity can be tolerated and increase voluntary muscle strength, mobility and reduce falls risk.

Therefore, the primary aims of this feasibility study were: 1) to assess if a short (4 week) eccentric recumbent stepping programme at a perceived intensity of "hard" is tolerable; 2) to assess if progressing WR over just three sessions is sufficient to reduce muscle soreness; 3) to report changes in voluntary strength and mobility that occur in response to this training; 4) to report on any changes in voluntary strength and mobility 30-days post-training.

We hypothesise that: 1) this training will be tolerable and result in minimal muscle soreness with low cardiovascular demand; 2) there will be significant improvement in voluntary strength and mobility; 3) Voluntary strength and mobility at 30-day follow up will be greater than baseline, but reduced compared to post-assessment.

5.2 Methods

This study was approved by the Faculty of Biological Sciences Ethical Committee for non-clinical research (University of Leeds, BIOSCI 15-031, Appendix C), and complied with the latest version of the Declaration of Helsinki. Common protocols, measurements and equipment are detailed in Chapter 2 and will therefore only briefly be described here.

5.2.1 Participants

Seven, older adults over the age of 65 years took part in this study (5 female, 2 male; mean ± SE; 70 ± 2 yr, 72 ± 3 kg, 163 ± 2 cm) Participants' were screened using a health and activity status questionnaire, as well as inclusion and exclusion criteria to determine any contraindications for exercise. Additionally, participants' competed the American College of Sports Medicines (ACSM) cardiovascular risk stratification screening questionnaire, with those identified as being at high risk (> 2 risk factors) excluded, and moderate risk participants eligible under supervised exercise (ACSM guidelines, Appendix D). Consent forms were signed by the participant, lead researcher, and assessor prior to commencement of testing. The inclusion and exclusion criteria for the study were as follows:

Inclusion criteria: Male or female individual over the age of 65 years.

Exclusion criteria: 1) any active medical disorder which may alter physiological responses; 2) use of prescription medication or over the counter preparations that may influence their performance; 3) a recent illness or viral infection (within the last 2 weeks); 4) use of recreational or performance enhancing drugs; 5) ingestion of excessive alcohol in the previous 24 hours (pre and post-assessments); 6) history of anaemia, recent asthmatic episodes, diabetes, epilepsy, family history of sudden death, fainting, heart disease, uncontrolled high BP, exercise limiting respiratory disease, recent related muscle or joint injury; 7) unable to provide informed consent or understand English; 8) recent intense exercise less than 48 hours before testing; 9) unable to mobilise independently; 10) identified as high risk on the ACSM cardiovascular risk

stratification screening questionnaire; 11) significant rheumatological or osteoarthritic joint pain during exercise.

5.2.2 Stepping ergometer

All eccentric recumbent stepping exercise was performed on the modified stepping ergometer previously described (Eccentron; BTE, Hanover, MD, USA; see section 2.3). Load cells positioned behind the foot plates were calibrated under no load before every session. Participant set up, and ergometer settings were identical to the previous studies and were maintained for every session (cadence of 23 spm, stride length of 2 (12° displacement), and a knee range from 90 to 30° of flexion). Participants' viewed a custom LabChart screen (LabChart 8; ADInstruments Ltd, Oxford, UK; Figure 5.1) throughout testing that displayed their applied left and right force (N) as a % of the target force.

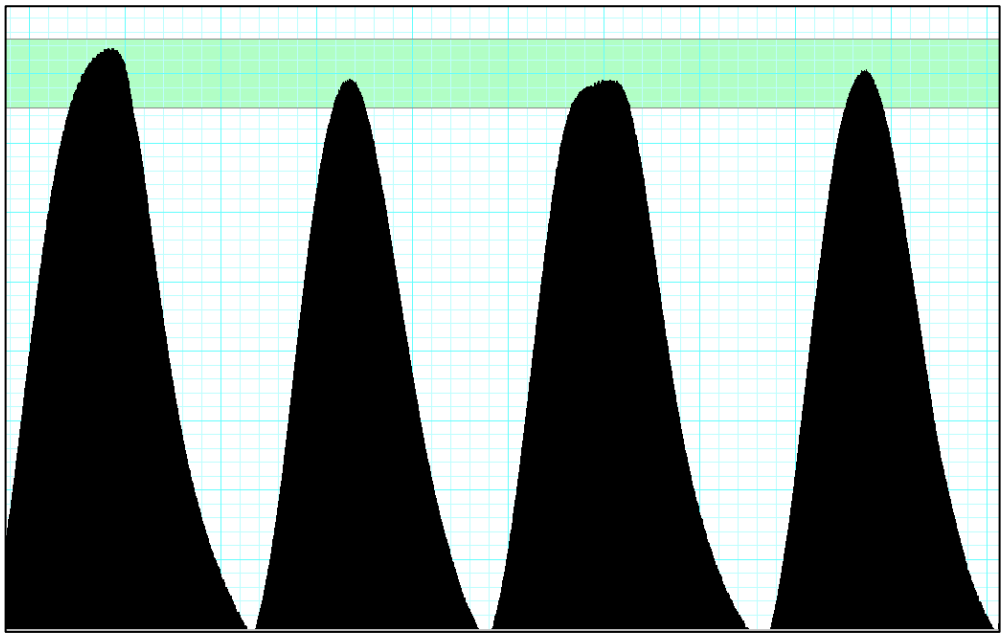

Figure 5.1: Custom LabChart screen viewed by participants' during training. Green band identifies the target force ± 5%. Black peaks represent the left and right real-time force as a % of target force.

5.2.3 Familiarisation

Prior to the commencement of testing, participants' attended a minimum of two familiarisation sessions separated by more than 48 hours. These were conducted to ensure accurate control of force during testing, to reduce the severity of muscle soreness experienced during training, to reduce the anxiety response that often occurs with exercise testing (Pescatello, 2013), and lastly to minimise any observed increases in voluntary strength due to further ergometer familiarisation. Participants' were initially familiarised to the ergometer using the supplied software introduction (see section 2.4.1). The display was then switched to the bespoke LabChart participant view (Figure 5.1) and participants' were instructed on how to correctly perform both concentric and eccentric contractions, matching as close as possible to the target force bar. The target force started low (~200N) and was gradually progressed until the participant felt they were at an intensity rating of 11 (fairly light) on the Borg RPE scale (target for first session).

Familiarisation was also conducted for assessment of voluntary concentric, eccentric and isometric strength, with the protocol being identical to that of the full assessment. Raw force traces were monitored during assessment to ensure participants were contracting during the correct phase of movement. One strength familiarisation was completed per familiarisation session .

5.2.4 Experimental design

An overview of the experimental design is shown in Figure 5.2. Following familiarisation, baseline assessment was completed comprising of: measurement of height, mass, and resting BP; assessment of voluntary concentric eccentric and isometric strength; and assessment of mobility using the elderly mobility scale (EMS), and modified incremental shuttle walk test (MISWT; n=5). Participants' then completed eight, 15 minute training sessions over 4 weeks, with intensity progressing up to "15" ("hard") on the Borg RPE scale by the 3rd session. HR, as well as ratings of muscle soreness and breathlessness (dyspnoea) were taken every session, and voluntary concentric,

eccentric and isometric strength was measured every other session. All baseline testing (excluding the MISWT) was repeated ~2 days following training and at 30 days post-training

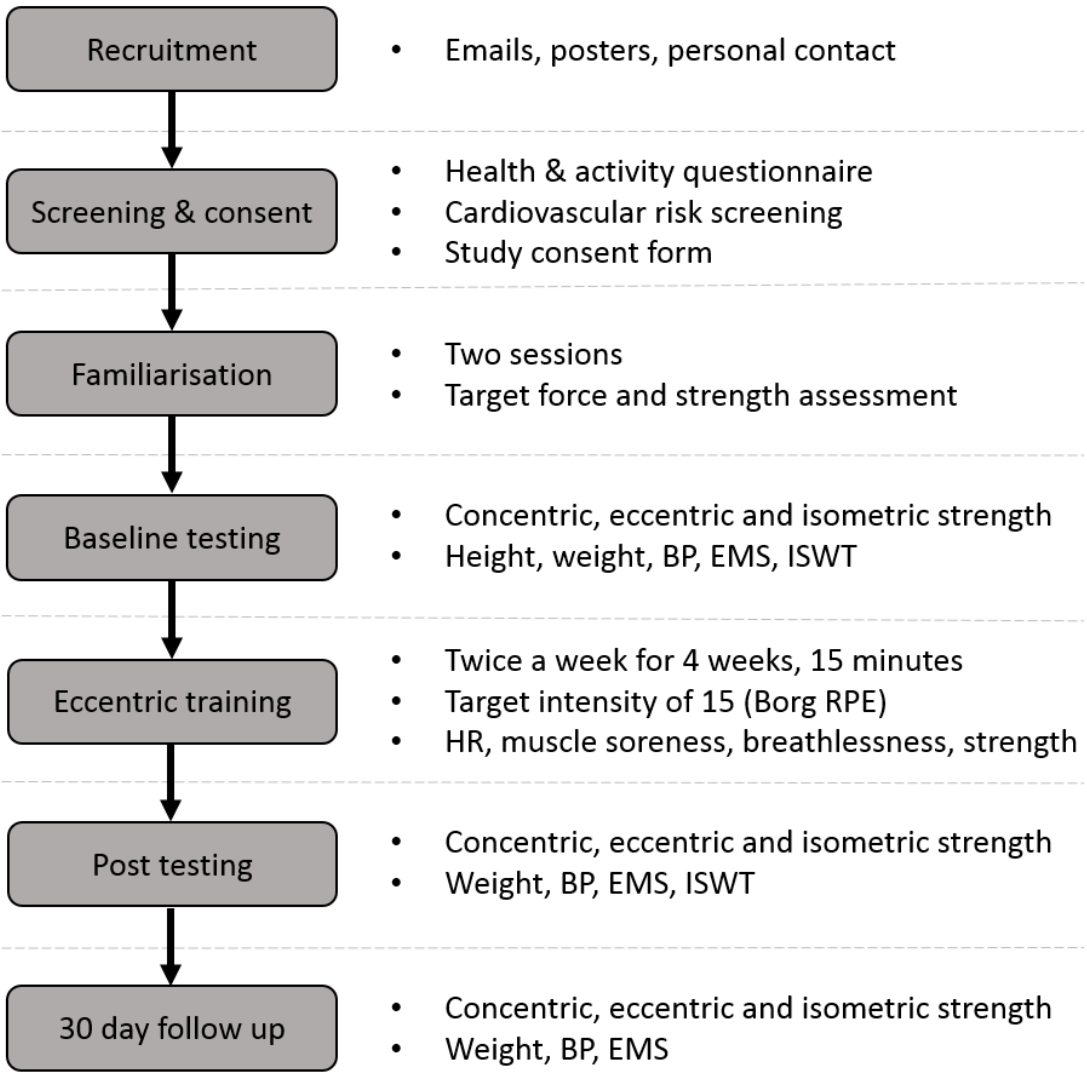

Figure 5.2. Study protocol flow chart.

5.2.4.1 Pre and post-training assessment

During the first visit, and first post-training visit, measurements, of height, weight and resting BP were taken (see section 2.5.1 for details). Assessment of voluntary concentric, eccentric and isometric strength were performed on the modified stepping ergometer as detailed in section 2.4.4. Assessment was repeated twice for each contraction type (total of six contractions for each contraction type) and the peak force value determined using the peak analysis

module within LabChart. The order of contraction type was randomised and the order kept constant for within-training and post-training assessment. Mobility was assessed using the EMS and MISWT >48 hours apart from the voluntary strength assessment.

5.2.4.2 Training period

Following baseline assessment, all participants' completed eight, 15 minute training sessions (twice a week) separated by at least one day. Seat position, stride length and cadence were identical to baseline assessment. Each session comprised of a 1 minute warmup, 15 minutes at the target RPE, and a 1 minute recovery period (Figure 4.3). HR was measured throughout, a rating of dyspnoea was taken at the end of the session, and a rating of muscle soreness experienced during the 48 hours following the exercise was taken the following session. Training intensity was set *via* the use of the Borg RPE (Appendix E) with exercise intensity increased from 11 (fairly light) in session one, to 13 (somewhat hard) in session two, and 15 (hard) for the remainder of the sessions. This progression in exercise intensity was chosen in order to prevent occurrence of severe muscle soreness following eccentric training. Session one target force was identified *via* gradual force increment during familiarisation. Subsequent starting target forces reflected that achieved within the previous session.

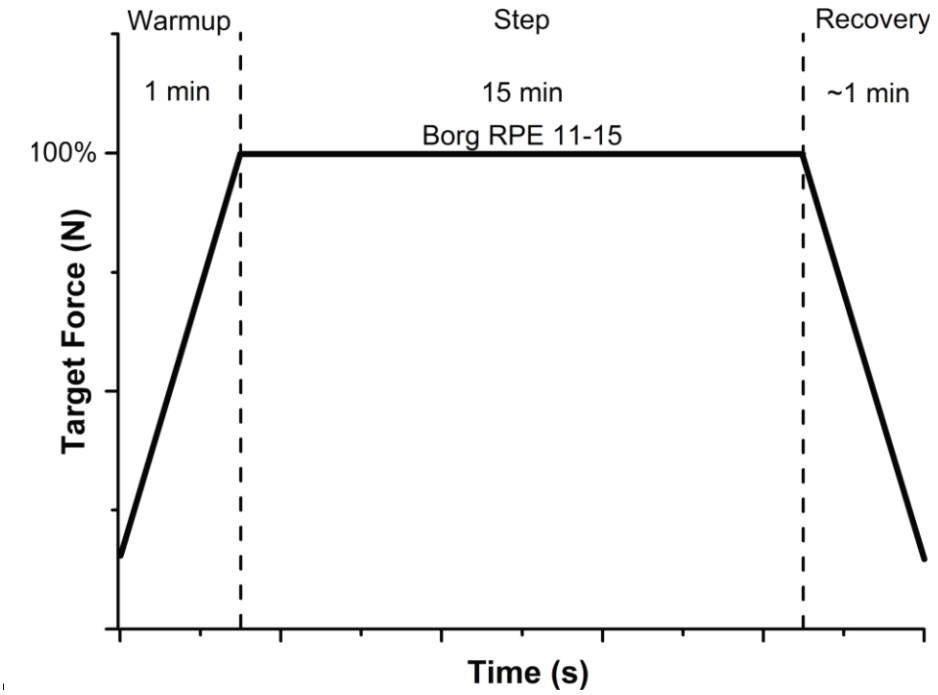

Figure 5.3: Schematic representation of the training session protocol. A 1 min warmup period of increasing force preceded a 15 min target force matching the target perceived intensity (Borg RPE scale). A recovery period followed, in which the participant gradually reduced their applied force.

During the warmup, participants' were instructed to gradually increase their applied force so that they were able to reach the target by the end of the minute. The screen was then refreshed, and participants' asked to keep the peaks of their force as close to the middle of the green target band as possible throughout. Participants' were instructed to vocalise their RPE throughout the training session (scale visible below the ergometer screen throughout), and target force was adjusted to ensure they were exercising at the appropriate RPE. During recovery, participants' were instructed to gradually reduce their applied force and were free to cool down for longer than 1 minute if required. Voluntary strength assessment was completed every other session (1, 3, 5, 7) in an un-fatigued state prior to commencing training.

5.2.5 Outcome measures

5.2.5.1 Mechanical force and power

Force acquisition and unit conversion is detailed in section 2.3. In brief, raw force data were obtained at a rate of 1kHz from calibrated load cells positioned behind the left and right foot plates of the stepping ergometer and were fed into a data acquisition system (ML870 PowerLab 8/30; ADInstruments Ltd, Oxford, UK) connected to a PC for processing (LabChart 8; ADInstruments Ltd, Oxford, UK). Mechanical force (N) was post-hoc converted into power (W) *via* the equations previously detailed.

5.2.5.2 Heart rate

During training, HR data were obtained at a frequency of 0.2Hz using a HR belt (RS800CX; Polar, Broxell Cl, Warwick, UK). The HR sensor was placed at the level of the xiphoid process and the belt moistened prior to application to increase conductance, and therefore accuracy of detection.

5.2.5.3 Mobility

Mobility was assessed using the EMS and MISWT. The MISWT was included within the final 5 participants due to a ceiling effect presenting with the EMS. The EMS is a 20 point ordinal scale commonly used within a hospital or community setting for the assessment of frail older adults (Appendix C). The EMS encompasses assessment of mobility, balance, and position changes *via* seven short assessments, and has been shown to be predictive of fall risk (Duncan et al., 1992; Spilg et al., 2003). A score greater than 14 represents a participant that would be safe mobilising independently at home. A score between 10 and 14 represents a participant with mild mobility impairment that may require assistance at home. And a score less than 10 represents a participant that requires a high degree of assistance with mobility and activities of daily living. The EMS was chosen over other assessment measures due to its greater ability to detect changes in mobility with an intervention (Spilg et al., 2001) as well as its excellent inter and intra-rater reliability, and concurrent validity with other

commonly utilised measures such as the Barthel index, Functional Independence Measure and the Modified Rivermead Mobility Index (Smith, 1994; Nolan et al., 2008).

The MISWT, initially described by Singh et al. (1992), is a maximal exercise test shown to be strongly correlated with peak $\dot{V}O_2$ achieved during a cardiopulmonary exercise test (Singh et al., 1992). Additionally, it has a high test-retest reliability and concurrent validity when compared with the 6 and 12 minute walk test (Campo et al., 2006). Participants' are required to walk around two cones placed 9m apart in the time identified by beeps on an audio track. The initial walking speed was 0.5m/s (20s between cones), and increased by 0.17m/s per minute to a maximum walking speed of 2.87m/s (3.53s between cones). The MISWT is an extension of the ISWT, comprising of an additional 3 minutes of increments, preventing a ceiling effect presenting within our population. To reduce the pre to post learned effect previously reported (Singh et al., 2014), an initial ~3 minute MISWT was conducted with an assessor walking alongside the participant. Following a ~2 minute rest period, the MISWT was repeated and the participant asked to continue until they can no longer keep up with the speed, or feel too breathless or unwell to continue. The same assessor ran every test, and to ensure maximal effort, verbal encouragement was provided throughout. The total distance completed was determined as the last length completed within the beeps (allowance of <1m), and the reason for termination was documented. The MISWT is sensitive to changes with rehabilitation, with a pre-to post change of 47.5m being identified as a clinically relevant increase (Singh, S.J et al., 2008).

5.2.5.4 Muscle soreness and breathlessness

Muscle soreness ratings (VAS, 0-100) were taking at the beginning of each session for the pain experienced in the 48 hours following the previous session (see section 2.5.5 for details). Dyspnoea was assessed at the end of the 15 minute training session using the Modified Borg Dyspnoea Scale (MBDS, Appendix G), which ranges from 0 (nothing at all) to 10 (maximal dyspnoea). The MBDS scale has been shown to correlate better to $\dot{V}E$ than other measures such as the VAS (Wilson and Jones, 1989), and has been shown to be reliable

for assessing dyspnoea over long time periods (Wilson and Jones, 1991). The participant was asked that their rating reflect the peak dyspnoea experienced during the session.

5.2.6 Data analysis

Identification of peak force from the voluntary strength assessments is detailed in section 2.6.3.3. Briefly, the peak force (N) of each contraction during each training session was first detected using the cyclic analysis option within LabChart. A visual check was conducted to ensure the peaks were accurately detected, and detection settings altered if incorrect. All force and power data were exported at 1Hz, and a mean taken for the entire 15 minute training duration. Calculation of peak force achieved during the training session as a % of peak strength was conducted for all sessions in which a voluntary strength assessment preceded training (session 1, 3, 5 and 7). To obtain a mean HR response for each training session, HR data were exported and a mean taken between the 4^{th} minute (sufficient time to exclude initial HR elevation) and 15^{th} minute.

5.2.7 Statistical analysis

All results are expressed as means ± standard error of the mean (SE). GraphPad Prism statistical software (GraphPad v6.0, GraphPad Software Inc, California, USA) was used to conduct all statistical analyses and significance determined when $P < 0.05$. All data was tested for normality prior to choosing the appropriate statistical test. To reduce the risk of type I error, Tukey's or Dunnets corrections were used for all post-hoc multiple comparisons. A one-way repeated measures ANOVA was used to compare pre, post and 30-day follow up measures, changes across the 8 training sessions, and baseline comparison of voluntary concentric, eccentric and isometric strength. In the case of non-normally distributed data Friedmans ANOVA was used. A paired Students t-tests was used to compare pre to post changes for the MISWT.

5.3 Results

5.3.1 Baseline assessment

At baseline, eccentric voluntary strength was 13 ± 9% and 25 ± 9% higher than concentric and isometric voluntary strength respectively ($P < 0.05$; 843 ± 106 N (concentric), 962 ± 168 N (eccentric), 762 ± 103 N (isometric)). There was no difference between concentric and isometric voluntary strength (*n.s.*). All participants were identified as having no mobility impairment (EMS), with six achieving maximum scores (20/20), and one achieving 18/20 due to a low functional reach.

5.3.2 Training period

The training intensities and responses during the 8 training sessions can be seen in Figure 5.4. Mean peak force and relative power increased by 88 ± 13% and 82 ± 11% respectively from the first to last training session (both $P < 0.05$). From the 3rd training session (first session at target RPE), mean peak force and relative power continued to increase showing a 28 ± 4% and 25 ± 4% increase at the final session respectively (both $P < 0.05$).

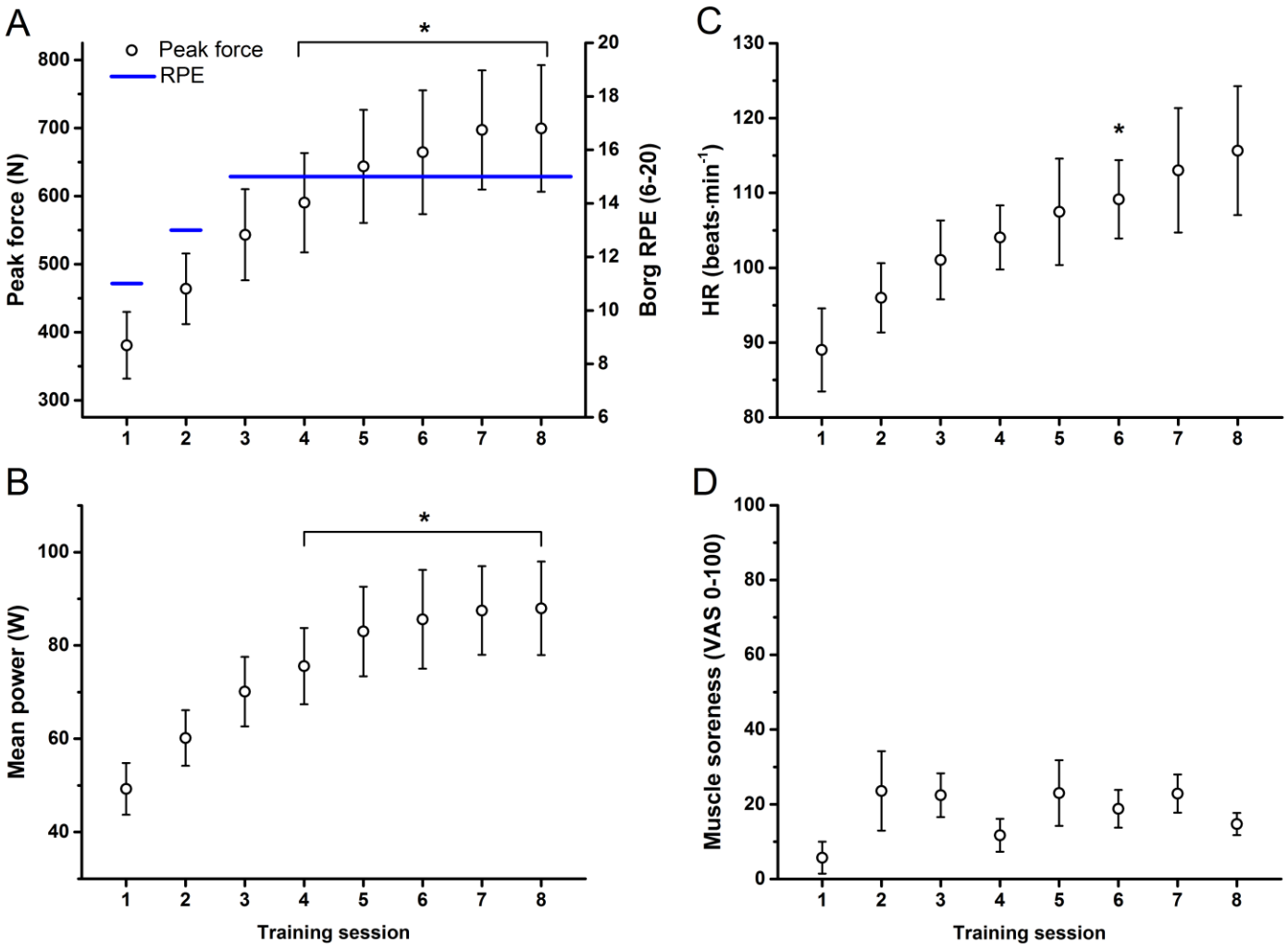

*Figure 5.4. Target training intensity and responses during the 8 training sessions. A) Target Borg rating of perceived exertion (RPE) overlaid onto the mean peak forces attained. B) Mean relative power achieved. C) mean heart rate (HR) response from the 4th to 15th minute. D) Self-reported muscle soreness experienced in the 48 hours following each session. Results presented as means ± SE. * significantly different (P < 0.05) to 3rd training session.*

Excluding the first 3 minutes of data (sufficient time to exclude initial elevation with WR), there was a non-significant increase in HR of 31 ± 10% from the first to last training session, and 14 ± 4% from the 3rd to last training session. Perceived muscle soreness experienced by participants' did not increase with increased peak force and relative power (*n.s.*). The mean muscle soreness experienced during training was 18 ± 2, with a peak muscle soreness experienced by any participant of 60/100. At a RPE of 15 (3rd session onwards), the mean MBDS score was 1.0 ± 0.4 (very slight), with a peak breathlessness rating by any participant of 4 (somewhat severe). There was no change in MBDS score from session 3 onwards.

5.3.3 Training responses

5.3.3.1 Voluntary strength

Concentric, eccentric and isometric voluntary strength increased by 26 ± 9%, 38 ± 9%, and 21 ± 8% respectively ($P < 0.05$; Figure 5.5), was maintained at 30-day follow up assessment ($P < 0.05$), and no difference was evident between voluntary strength at post-assessment and 30-day follow up (*n.s.*). There were no significant changes to concentric, eccentric or isometric voluntary strength assessed weekly during the training period (week 1 to week 4; Figure 5.6).

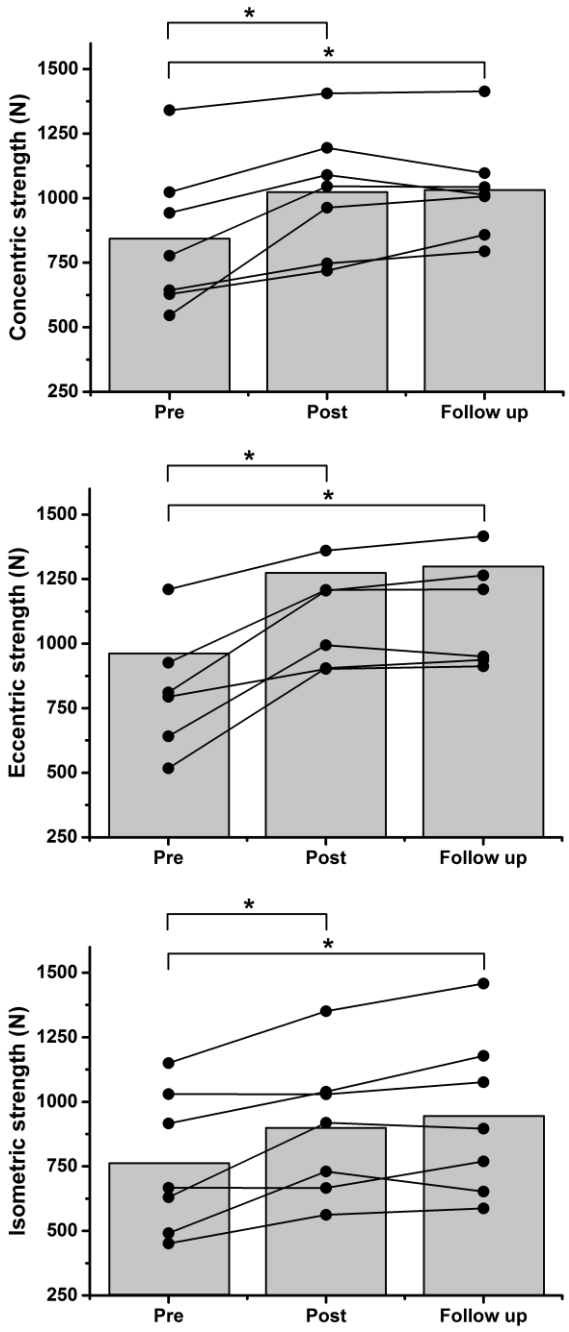

Figure 5.5. Concentric, eccentric and isometric voluntary strength changes. Individual (solid circles) and mean (grey columns) values displayed. Pre-assessment (Pre), post-assessment (Post) and 30-day follow up (follow up). * significant difference between mean responses (P < 0.05).

5.3.3.2 Blood pressure

There were no changes from pre-assessment to post-assessment in systolic (130 ± 4 *vs.* 123 ± 3 mmHg), diastolic (79 ± 2 *vs.* 76 ± 3 mmHg) or mean arterial BP (96 ± 2 *vs.* 92 ± 3 mmHg) (all n.s.). At follow up assessment, there remained no change to diastolic or mean arterial BP, however, systolic BP was 8 ± 2 mmHg lower than at pre-assessment (130 ± 4 *vs.* 121 ± 5 mmHg; $P < 0.05$).

5.3.3.3 Mobility

No change in EMS scores was evident, with a score of 19.3 ± 0.7 at baseline and 20.0 ± 0.0 at post assessment (*n.s.*). There was a non-significant increase in functional reach distance from 30 ± 4 cm at pre-assessment to 34 ± 4 cm at post-assessment (*n.s.*). Functional reach further increased to 38 ± 2 cm at 30 day follow up, resulting in a 34 ± 17 % increase relative to pre-assessment ($P < 0.05$). There were no changes to the 6m walk speed at post-assessment or 30-day follow up (2.9 ± 0.4s (pre), 2.8 ± 0.3s (post), and 2.7 ± 0.3s (follow up) *n.s.*). There was no change in the MISWT distance achieved between pre and post-assessment (730 ± 74m *vs.* 756 ± 40m; n=5; *n.s.*).

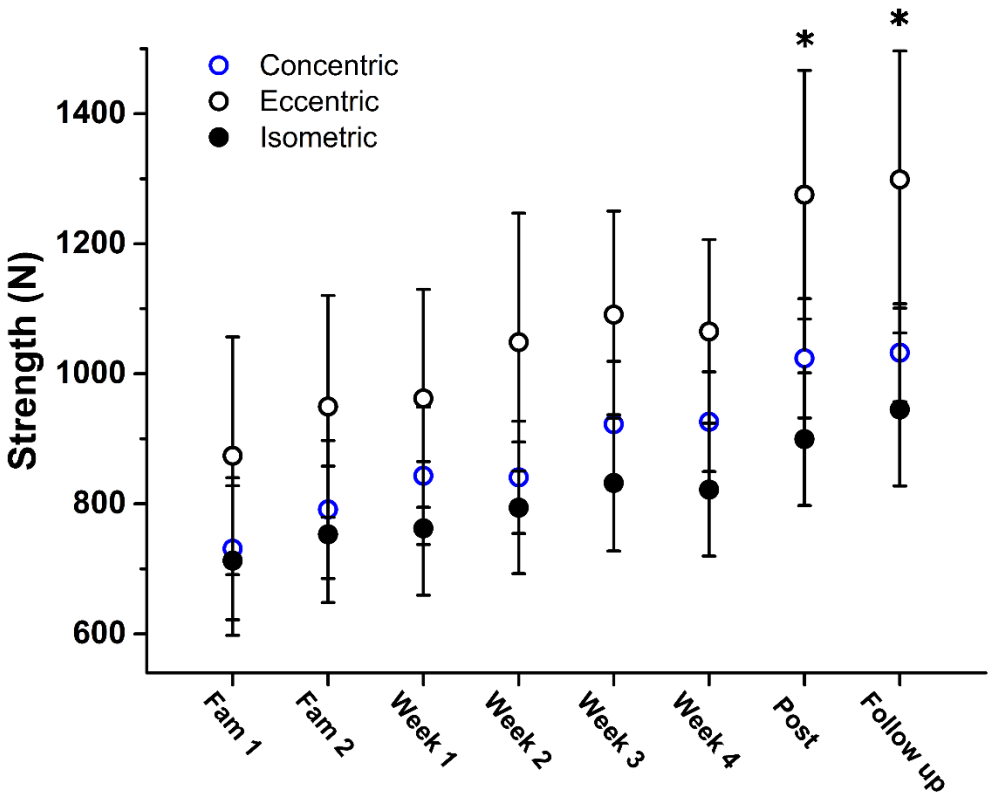

Figure 5.6. Mean concentric, eccentric and isometric voluntary strength during familiarisation, training, post-assessment and follow up. Mean ± SE. * significant difference (P < 0.05) vs. pre-assessment (week 1).

5.4 Discussion

By investigating the effects of eccentric recumbent stepping training within an older adult population, we have shown that: 1) fast progression of WR and high peak intensities are tolerable and result in a low cardiovascular demand and minimal muscle soreness; 2) a short (4 week) program results in significant increases in concentric, eccentric and isometric voluntary strength; 3) At 30-day follow up voluntary strength was maintained and SBP and functional reach were improved; 4) there was no effect of eccentric recumbent stepping on the EMS or MISWT scores.

Note that we aimed to recruit both frail and non-frail participants with low baseline activity levels, enabling the comparison of training effects relative to baseline function. However, due to the recruitment avenues taken, recruitment of frail individuals was not possible, and those included displayed high baseline function and activity levels. This was highlighted by the EMS and MISWT conducted at baseline, with 6 out of 7 participants' achieving maximum scores on the EMS and the mean MISWT score being ~35% higher than predicted within this age range (Probst *et al*., 2012). In collaboration with this study, additional assessment of cognition and frailty was conducted with our participants' at baseline, post intervention and 30-day follow up using the frailty phenotype and Montreal Cognitive Assessment (MoCA). No cognitive impairment was evident, and baseline activity levels were high, further demonstrating the high baseline function within our population.

5.4.1 Duration, frequency and intensity of training

Current research suggests that training interventions of increased duration (80 *vs.* 40 week intervention, Sherk *et al*., 2012) , and frequency (2 *vs.* 3 session per week, Mayer *et al*., 2011) result in greater adaptations and retainment of function within an older adult population. However, long durations and frequency require a large commitment from the participant, and if incorporated within a rehabilitation protocol, are likely to result in poor compliance levels. For example,

a systematic review looking at the compliance of the Otago falls prevention program (~ 1 year duration) showed that just ~37% of patients complied with the prescribed three sessions per week and ~56% with two sessions per week (Thomas et al., 2010). Indeed, average adherence rates within chronic disease rehabilitation with long durations are generally about 50% (Alvarez, 2002). There is therefore a need for shorter rehabilitation programs that still produce substantial increases in voluntary strength and mobility. It has previously been shown that eccentric endurance training studies of shorter durations (~12 weeks), with a typical training frequency of three times a week (LaStayo et al., 2003; LaStayo et al., 2011; Chen et al., 2017; LaStayo et al., 2017), can produce similar increases in voluntary strength and mobility to traditional rehabilitation programs of longer length. However, we have shown that an eccentric program of significantly shorter durations and frequency can still result in increases in voluntary strength that are maintained at follow up.

The fact that we have reported similar increases in voluntary strength with shorter durations can predominantly be attributed to the higher target intensity utilised. Previous eccentric endurance studies have progressed intensity up to a Borg RPE rating of ‘somewhat hard’, a commonly used target within traditional training to restrict dyspnoea within clinical populations (Robertson, 2004). Eccentric exercise has been shown to elicit minimal dyspnoea even at high WRs suggesting higher target intensities could be utilised. For example MacMillan et al. (2017) showed minimal (~2, slight) dyspnoea during eccentric cycling despite the WR being three times greater than concentric. Our low ratings of ~1 (very slight) mirror these findings and suggests that higher RPEs could likely be tolerated within dyspnoea limiting conditions such as COPD, and allow a greater mechanical stimulus, and greater neuromuscular adaptations.

To reduce the severity of eccentric exercise-induced muscle soreness, previous eccentric training studies have typically increased WR over 12 sessions (4 weeks), (LaStayo et al., 2003; LaStayo et al., 2011). However, we have shown that a more rapid increase in WR over just 3 sessions can still prevent severe muscle soreness, with a peak mean of ~24/100 in the first three sessions, and a

mean of ~20/100 throughout the entire study. As discussed in section 4.4.3, the utilisation of a slow cadence throughout training (23 spm) may have reduced the amount of muscle damage and therefore subsequent soreness experienced. Despite the fast progression, these results suggest that the adaptations from previous bouts have resulted in a protective effect against subsequent damage (see section 1.6.5). The development of eccentric induced muscle soreness is currently a large barrier to the implementation of eccentric exercise within clinical populations (Harris-Love et al., 2017). Yet these results suggest that, with appropriate knowledge of WR progression, muscle soreness can be minimised and, therefore, should not limit the incorporation of eccentric exercise into rehabilitation protocols.

Despite working at a perceived intensity of 'hard' (Borg RPE 15), mean HR during the final training session only reached a peak of 116 beats·min^{-1}. This low HR requirement was shown in study 1 and 2, and is lower than previously reported during reverse cycle ergometry at lower perceived exertions (~127 beats·min^{-1}, Borg RPE ~11.7, LaStayo *et al*., 2000). This suggests that the disparity between HR demand and perceived effort during eccentric recumbent stepping may be larger than reverse cycle ergometry, and further highlights the potential usefulness of eccentric recumbent stepping as a rehabilitative tool within patients at risk of cardiac events. Cardiac rehabilitation guidelines (A.C.P.I.C.R., 2015) currently suggest a target HR of 40-70% of HR reserve, a predicted 105-132 beats·min^{-1} within our study population. The HRs achieved within this study are within these targets, suggesting cardiac patients may be able to perform eccentric recumbent stepping exercise at safe HRs, and achieve greater increases in voluntary strength than currently reported (Nishitani et al., 2013). Important to note is that, due to the progressive increase in HR that we have shown occurs at a constant WR (see chapter 3), careful monitoring of HR during training may be necessary to avoid exceeding the target range.

5.4.2 Training adaptations

As the leading predictor of falls within older adults, reversing the muscle mass, quality and strength losses of sarcopaenia is of primary importance (Rubenstein, 2006). Within this study, we have shown increases in concentric, eccentric and isometric strength of 26%, 38%, and 21% respectively, which are considerable and comparable to that of resistance training programs of much longer durations (typical minimum duration of ~10 weeks, Hunter *et al*., 2004). The most comparable eccentric endurance training study within older adults has reported lower isometric voluntary strength gains of ~11% (Lastayo et al., 2010). This study was conducted on the same stepping ergometer as utilised in the present study, but differed in that they required 3 sessions a week for 12 weeks, used a slower WR progression, a lower target intensity ('fairly light' to 'somewhat hard'), and performed the exercise for up to 20 minutes. We have, therefore, shown that with a considerably shorter exercise duration and frequency, greater increases in voluntary strength can be attained. These greater strength changes are likely a result of the increased intensity at which our participants were working, as at the perceived training intensity of 'hard', the peak forces achieved in this study were a considerable ~65%, ~75% and ~85% of eccentric, concentric and isometric voluntary strength respectively. These intensities compare to the ranges used within traditional (Hunter et al., 2004), and eccentric resistance training studies (Roig et al., 2009), and are considerably higher than used during traditional endurance training (Hoppeler, 2015). The ability to resist high loads throughout a large number of repetitions (~350 per session in this study) allows the accumulation of a large total training load within each session, further increasing the total mechanical demand and subsequent adaptations.

Within the current study, due to the relatively short training duration (4 weeks), the mechanisms responsible for increases in voluntary strength are likely neural in origin, rather than via muscle hypertrophy changes (see section 1.7.1). It is generally considered that muscle hypertrophy changes do not become evident until at least 4 weeks into resistance training programs (generally 8 weeks), and may present even later within an older adult population (Moritani and Devries, 1980; Lixandrao et al., 2016). For example, Moritani and Devries, (1980) demonstrated no significant change in muscle CSA, estimated *via* skin fold

measurements, within five older adults following an 8 week resistance training study, whereas CSA was significantly elevated after just 4 weeks within the young healthy population. Lixandrao et al., (2016) later improved upon the poor accuracy of skin fold measurements and low participant numbers utilised by Moritani and Devries, (1980) to demonstrate similar results. They used ultrasonography and MRI to assess changes in vastus lateralis CSA during a 10 week resistance training (leg press) protocol within 14 older adults. They showed that despite their being significant increases in voluntary muscle strength, muscle CSA was only significantly elevated after 9 weeks. A number of neural adaptations have been hypothesised to stimulate these early increases in voluntary strength (see section 1.7.1 for detailed discussion), including; increases in the amplitude and firing frequency of electrical muscle activity (Moritani, 1979; Häkkinen and Komi, 1983; Narici et al., 1989; Patten et al., 2001; Aagaard et al., 2002; Kamen and Knight, 2004), a reduction in antagonist co-activation (Kamen, 1983; Carolan and Cafarelli, 1992), an altered motor unit discharge pattern (Binder-Macleod and Barker, 1991; Griffin et al., 1998; Van Cutsem et al., 1998), and an increase in synchronisation of motor units (Semmler and Nordstrom, 1998).

Maintenance of strength is vital within an ageing population, highlighted by the correlation of declines in strength with increased fall risk, reduced independence, and increased mortality, and maintenance of strength with increased independence and QoL (Rantanen et al., 2000; Marcell, 2003; Rantanen, 2003; Ruiz et al., 2008; Roberts et al., 2017). Our results show promising signs for retainment of voluntary strength following eccentric exercise of short duration, however, it remains unknown whether voluntary strength would be retained over longer durations. It could be hypothesised, that due to the probable lack of muscle hypertrophy changes with the short training duration utilised, retainment of voluntary strength may be lower when compared to studies lasting longer than 4 weeks. Previous studies have demonstrated a reduction in EMG amplitude with limb immobilisation (Wolf et al., 1971; Duchateau and Hainaut, 1987), but maintenance of amplitude with only a limited number of contractions performed has also been demonstrated (Gabriel et al., 1997). Therefore, it may be possible that far lower activity levels than achieved during training may be sufficient to

enable retainment of neural drive and voluntary strength increases. Eccentric exercise has also been shown to display a slower rate of voluntary strength decline with age and de-training compared with traditional exercise (Hortobágyi et al., 1995; Andersen et al., 2005; Roig et al., 2010). Therefore, it may also be the case that maintenance of voluntary strength over longer periods may be greater with eccentric training. The maintenance in voluntary strength we have observed may have been accentuated by relative high activity levels within our population, as volume of activity measured *via* the frailty phenotype assessment (with collaboration group) showed an increase of ~145% (*n.s.*). This assessment was only conducted on the previous week's activity and presented with a large variability, hence making it difficult to accurately correlate retainment of voluntary strength with activity levels. Of importance to note is a study by LaStayo et al. (2000) that showed a continued increase in isometric knee extension voluntary strength following eccentric endurance training, reaching a peak at 10 days post training intervention. This suggests an initial reduction in voluntary strength due to EEIMD agreeing with acute studies showing reduced voluntary strength for up to 7 days post exercise (Byrne et al., 2001). Therefore, it is possible that our participants' voluntary strength could have increased further after post-assessment and then reduced back to similar levels. This would suggest a lower maintenance of voluntary strength than initially apparent, although, more frequent strength assessments and longer follow up periods are needed to fully characterise voluntary strength changes with time.

The reductions in SBP at follow up assessment (8mmHg reduction) are similar to those seen with traditional concentric training in older adults (Barone et al., 2009). These studies, however, required a far greater cardiovascular demand, perceived effort, and likely metabolic demand than we present, suggesting that eccentric recumbent stepping may be a more tolerable exercise intervention to reduce BP. A recent study by Chen et al. (2017) reported greater reductions in SBP with descending *vs*. ascending stair walking in older adults (-9% *vs*. -4%), providing further support for preferential cardiovascular adaptations with eccentric exercise. The mechanisms responsible for these adaptations remain unclear. However, due to the slow velocity of eccentric contractions similar mechanisms that occur with isometric training, that is known to stimulate greater

reductions in SBP compared to aerobic endurance or dynamic resistance training (Cornelissen and Smart, 2013), may be at play. Possible adaptations include: improvements in endothelium-dependent dilatation, reduced oxidative stress, and autonomic neural adaptations (Millar et al., 2014). Despite our population presenting with normative, healthy systolic BP at baseline (Lloyd-Jones et al., 2005), the reductions we have observed, if maintained, are still likely to improve vascular health and reduce mortality, with benefits evident down to systolic BPs of ~115 mmHg (Grossman, 2011).

Due to our population presenting with a high functional capacity at baseline, the EMS presented with a celling effect and was not sensitive enough to detect any change with our intervention. To address this issue, the MISWT was incorporated within the final 5 participants. There was a small (26m) increase in the mean distance completed, with previous research concluding that a minimum increase of 47.5m is needed to observe a clinically relevant change in functional performance (Singh et al., 2008). Although we did not demonstrate any significant increase in the distance achieved, we did note a pre to post training difference in the reason for terminating at the LoT. At baseline assessment, four out of five participants' stated that they stopped due to not being able to maintain the required speed, whereas at post-assessment four out of five reported that they became too breathless to carry on. This suggests that the voluntary strength changes observed may have resulted in an increased ability to maintain higher speeds, allowing greater WRs to be maintained, increasing the metabolic demand and subsequent dyspnoea. These results are speculative, and further assessment with a higher participant number is required to confidently assess mobility changes with a short eccentric recumbent stepping program.

Although the full EMS score presented with a ceiling effect, we were able to assess changes to the functional reach and walk speed components. At baseline assessment, functional reach was similar to the normative ranges within a 70-87 year old population (Williams et al., 2017), whereas at 30-day follow up, the 8cm increase matched more closely to the normative ranges of 41-69 year olds. This suggests that this exercise intervention may result in improved balance and

potentially reduce fall risk (Spilg et al., 2003). The high correlation of functional reach with falls was highlighted by Duncan et al. (1992) who measured functional reach within 270 community dwelling older adults and monitored falls for 6 months. They found that, compared to participants that could reach further than 25cm, the chance of recurrent falls was twice as likely in those that could reach between 15 and 25cm and four times more likely in those that could reach less than 15cm. The increased balance at follow up is likely due to the increases in quadriceps voluntary strength we have observed, with lower limb strength shown to be strongly correlated with balance (Wang et al., 2016). We can also speculate that, due to the bracing nature of eccentric activity on this ergometer, core activation may increase during eccentric activity, although measurement of core muscle activity during eccentric and concentric stepping are required to confirm this. We did also see small improvements in the 6 metre walk speed, but the reduction were too small and the error too large to draw any valid conclusions.

5.4.3 Conclusions

We have shown that a short eccentric recumbent stepping program, with fast WR progression and a high target intensity results in minimal muscle soreness, low cardiovascular demand, and also achieves significant improvement in voluntary strength that are maintained for 30-days. Mobility and balance changes within this study show trends towards improvement, but further research with greater participant numbers are required to provide more robust outcome measures. These results add to the growing literature demonstrating the superior training effects of eccentric *vs.* traditional training, and suggest that shorter interventions may be possible that increase rehabilitation compliance, and reduce age-related loss of strength and falls risk.

Chapter 6 General discussion

The aims of this thesis were to adapt a novel eccentric recumbent stepping ergometer for use within a research setting, and to characterise the acute and chronic responses of eccentric recumbent stepping within a young and older adult population. The adaptations applied to the ergometer enabled tight control of exercise parameters, and crucially allowed the comparison of concentric and eccentric recumbent stepping on the same ergometer. We have shown that, compared to concentric, eccentric recumbent stepping requires a significantly lower cardiovascular and metabolic demand to produce the same relative power. Therefore, when incorporated within training programs, a far greater eccentric relative power can be performed for the same metabolic requirement. This translates into substantial voluntary strength increases at low cardiovascular and metabolic requirements within both a young and older adult population, and crucially, the exercise is tolerable and results in minimal muscle soreness. These findings help further our understanding of eccentric exercise and hopefully provide evidence for its implementation within clinical populations that may benefit.

6.1 Summary of key findings

Within the first study we assessed the acute responses of concentric and eccentric recumbent stepping matched for mechanical or metabolic requirement. By initially conducting concentric and eccentric RITs, not previously performed, we were able to accurately determine the mechanical WRs required to achieve a target metabolic requirement. This allowed us to accurately place participants into the moderate intensity exercise domain, allowing greater consistency of metabolic responses. The reductions in cardiovascular and metabolic requirement at the same WR, and increases in WR at the same metabolic requirement, are consistent with previous studies; however, demonstrated smaller disparities, likely due to the lower cadences utilised. Interestingly, we

showed a progressive increase in $\dot{V}O_2$ and HR at a constant relative power, suggesting a higher eccentric exercise intensity, and highlighting the need for careful exercise prescription within certain clinical populations.

By utilising the WR determination methods from study one, we were able to tightly control mechanical and metabolic requirement during concentric and eccentric training in young healthy participants. We demonstrated that, even at low metabolic requirements, the increases in required eccentric relative power were sufficient enough and likely responsible for the substantial voluntary strength increases not evident with concentric training. Importantly, participants familiarised quickly to the exercise, it remained tolerable throughout, and the gradual increase in exercise intensity resulted in minimal muscle soreness.

After gaining a greater understanding of the acute and chronic responses of eccentric recumbent stepping in a tightly controlled manner, we were able to assess eccentric recumbent stepping as a feasible rehabilitation protocol within an older adult population. We demonstrated that fast WR progression to a higher target intensity than previously used is tolerable, and results in minimal muscle soreness. The high WR intensity again likely stimulated increases in voluntary strength that were maintained for 30-days and required a significantly shorter training duration than typically used. Further assessment of mobility, falls risk and BP changes, as well as longer follow up times are required, but preliminary results suggest that eccentric recumbent stepping may provide a more effective training stimulus than current rehabilitation programs, and may increase rehabilitation compliance, improve maintenance of function, reduce falls risk and ultimately improve QoL.

6.2 Applications of eccentric endurance training

The results within this thesis, as well as previous eccentric literature, highlight the potential benefit of eccentric exercise as a rehabilitative tool within exercise intolerant clinical populations. The low cardiovascular and metabolic requirement, high force generating capacity, low perceived effort, and greater retention of eccentric voluntary strength (Hortobágyi et al., 1995; Roig et al., 2010; Gluchowski et al., 2017), suggest that eccentric exercise will likely be a more tolerable and effective form of exercise to perform. Despite the abundance of literature supporting these claims, eccentric exercise is not yet routinely used within any clinical rehabilitation program. This is likely due to the cost and availability of equipment, as well as safety concerns and potential for generating DOMS. There is clearly a need for further development of eccentric equipment; however, alternative cheap and readily available forms of eccentric exercise are currently available and still not utilised. For example, it was demonstrated that descending stair walking is a safe and effective modality to improve strength and mobility, and furthermore, that muscle soreness can be prevented with progression of repetitions (Chen et al., 2017).

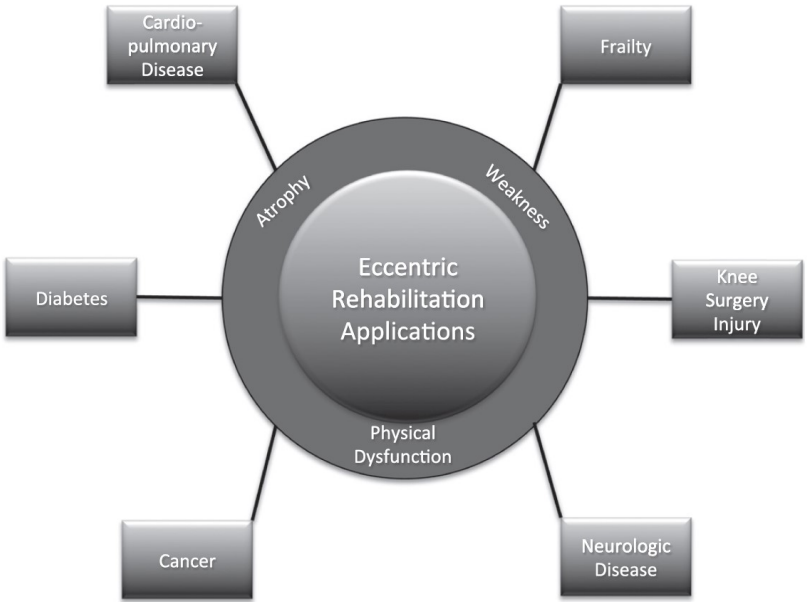

Figure 6.1. Possible clinical applications of eccentric endurance exercise (LaStayo et al., 2014).

The investigation of eccentric endurance rehabilitation has grown over the recent decades with its applications spreading across exercise intolerant populations to post-operative musculoskeletal rehabilitation (Figure 6.1). Improvements in voluntary strength and physical function have been demonstrated in patients with cancer (Hansen et al., 2009; Lastayo et al., 2010), diabetes (Marcus et al., 2008; Marcus et al., 2009), and Parkinson's disease, as well as within anterior cruciate ligament rupture (Gerber et al., 2006; Gerber et al., 2007b; Gerber et al., 2007a; Gerber et al., 2009) and total knee arthroplasty patients (LaStayo et al., 2009). Two populations which stand to benefit greatly from eccentric endurance rehabilitation are patients with COPD and CHF.

COPD and CHF are extremely prevalent conditions, with COPD estimated to be present in approximately 25% of over 40 year olds (Mannino and Buist, 2007; Decramer et al., 2012), and CHF resulting in over one million annual inpatient bed days and 5% of all A&E admissions (Stewart et al., 2002; Al-Mohammad and Mant, 2011). Despite distinct pathological origins, barriers to exercise within CHF and COPD are similar, with dyspnoea and exercise intolerance at low WRs being the primary reasons for exercise avoidance. This avoidance often results in a vicious decline in symptoms, with reduced activity resulting in reduced strength and mobility, leading to further reductions in activity levels and a worsening of disease symptoms. Therefore, breaking this cycle, either by improving disease state, or increasing strength and mobility to increase activity levels is required. Traditional (concentric) exercise rehabilitation has been shown to be effective at improving $\dot{V}O_{2peak}$ (Giannuzzi et al., 2003; Erbs et al., 2010), a marker strongly correlated with mortality and QoL, and has led to the implementation of traditional exercise protocols within cardiac and pulmonary rehabilitation (O'Connor et al., 2009). Unfortunately, the cardiovascular, metabolic and mechanical demands within these sessions are not sufficient enough to elicit meaningful improvements in mortality, morbidity and QoL (West et al., 2012), suggesting an alternative approach is required.

Eccentric exercise may provide this alternative, allowing a greater mechanical stimulus to improve voluntary strength, whilst reducing restrictive sensations. Two studies have highlighted the benefits of incorporating eccentric exercise within COPD rehabilitation. Rooyackers et al. (2003) and Rocha Vieira et al. (2011) have demonstrated that up to five-times greater eccentric *vs*. concentric loads are possible, resulting in minimal dyspnoea scores (~3 on the Borg RPE, moderate) and not requiring supplementary O_2. Responses are similar within CHF patients, with acute studies showing the ability to produce far greater relative power at the same cardiovascular demand (Agarwal et al., 2017; Haynes et al., 2017), and training studies reporting significant increases in voluntary strength at low perceived efforts and with minimal muscle soreness (Besson et al., 2013; Theodorou et al., 2013). Importantly, eccentric exercise can be performed without any negative impact on vascular or platelet function (Haynes et al., 2017), and has even been shown to elevate interleukin-6 levels that may lessen the development of cardiovascular disease (Agarwal et al., 2017).

These results provide clear evidence that eccentric exercise is likely an effective modality to break the cycle of decline in strength and mobility within COPD and CHF patients. There is, therefore, a need for well-designed large-scale studies that assess the potential therapeutic benefit of incorporating eccentric exercise within standard rehabilitation practice.

6.3 Pilot investigations

6.3.1 Estimation of fatigue mechanisms

The data presented within study 1 and 2 (chapters 3 and 4) suggest that the mechanisms leading to task failure during concentric and eccentric recumbent stepping compared to cycle ergometry are very different. We propose that the LoT within the stepping RITs is likely determined by a peripheral inability to produce the required relative power, rather than an inability to meet the metabolic requirements of the exercise. In addition, due to the higher peak relative powers and lower peak metabolic strain during eccentric *vs.* concentric RITs, different

fatigue mechanisms likely occur between contraction modes as well. Determining these mechanisms will provide essential insight; however this usually involves invasive or costly procedures. We have, therefore, adapted a new non-invasive and relatively cheap method that utilises synchronous EMG and force measurements to estimate relative contributions of peripheral and central fatigue.

The contributions to total fatigue are generally considered to reside either proximal or distal to the neuromuscular junction (NMJ). Activation fatigue (AF) occurs proximal to the NMJ and comprises of a reduced ability to activate efferent pathways, namely corticospinal and motor neurone pathways, whereas muscle fatigue (MF) occurs distal to the NMJ, and is a result of disrupted excitation-contraction coupling. Discerning the relative contributions of AF and MF to total fatigue gives crucial insight into the fatigue mechanisms of an exercise task and allows a tailored approach to rehabilitation. To quantify the relative peripheral and central fatigue of a task, studies have previously either used magnetic or electrical peripheral nerve stimulation, or direct stimulation of the motor cortex *via* transcranial magnetic stimulation (Polkey et al., 1996; Cairns et al., 2005; Jubeau et al., 2014). This methodology removes the effect that a lack of voluntary effort has on reductions in force, thereby, allowing quantification of peripheral muscle fatigue (Lepers et al., 2002). However, these techniques are limited in that they often assess contractions very different to the activity that caused fatigue, are uncomfortable for the participant to perform, present with a time delay before measurement, and produce a low % of maximum force generating capacity (Verges et al., 2009; Gruet et al., 2014).

A novel method that involves measurement of EMG and force data at rest and task failure can provide estimations of the relative contribution of AF and MF to total fatigue (Coelho et al., 2015). Raw EMG activity is composed of the superposition of all underlying motor unit action potentials (Loeb and Gans, 1986), with increased EMG activity a result of increased recruitment of motor units and, therefore, increased muscle force. In fact, in an unfatigued state, a strong linear relationship exists between the force produced by a muscle and its

electrical activity (Coelho et al., 2015; Ferguson et al., 2016). This relationship can be used to allow the comparison of the EMG/power relationship in an unfatigued and fatigued state, and can be used to infer the relative contribution of AF and MF to total fatigue. This method, although useful, only provides a snapshot of fatigue mechanisms at the LoT, and does not allow for the assessment of changes in MF throughout the exercise task. Assessment throughout is impeded due to the requirement of isokinetic or isometric exercise that maintains the force-velocity relationship (Elmer et al., 2013). As our methods involve performing a RIT to the LoT at the same contraction velocity (cadence) throughout, this in theory should allow us to quantify changes in MF for every contraction.

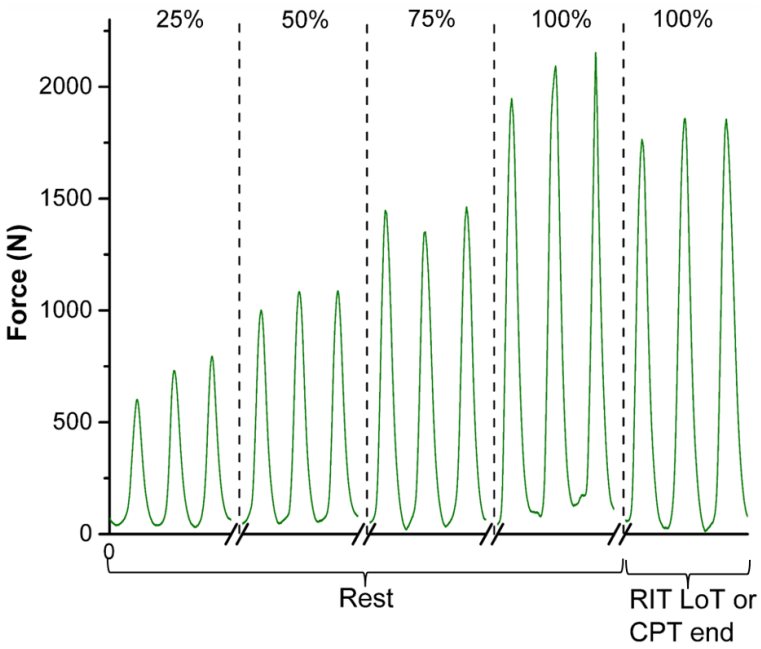

Figure 6.2: Representative participants raw force data during assessment of fatigue. Unfatigued (rest) contractions performed at 25, 50 , 75 and 100% of maximal. At task failure (LoT), maximal contractions were repeated.

Methods for this pilot work have been adapted from Coelho et al. (2015) and are detailed in Appendix H. Briefly, throughout concentric and eccentric RIT exercise (separated by > 48 hours), EMG data was recorded from nine muscles using a wireless EMG system (Telemyo DTS; Noraxon U.S.A. Inc, Scottsdale, AZ, USA), and mechanical force was obtained from the eccentric steppers load cells. At baseline, the participant performed three concentric or eccentric contractions at

25%, 50%, 75% and 100% of maximal effort, and at the LoT, three more maximal contractions were immediately performed (Figure 6.2). EMG and force data were normalised to the highest values attained in the unfatigued state and a linear regression fit to determine the unfatigued EMG/power relationship (Figure 6.3). The relationship of the LoT point to the unfatigued relationship allows for estimation of relative contributions of AF and MF to total performance fatigue (PF).

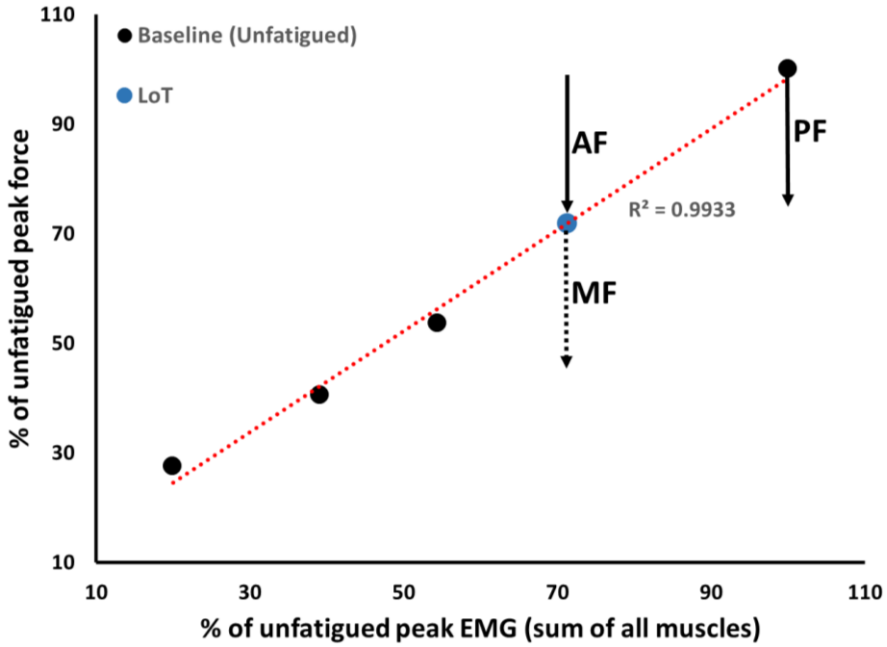

Figure 6.3. A representative example of the force/EMG relationship at baseline and task failure. Black circles represent the force/EMG relationship during the 25%, 50%, 75% and 100% (of peak) unfatigued contractions of which a linear regression line is fit (red dotted line). Blue circle represents the force/EMG relationship at the LoT. Performance fatigue (PF) represents the total reduction in force at the LoT. Muscle fatigue (MF, dotted line) represents the reduction in force from the unfatigued linear relationship. Activation fatigue (AF) represents the remaining proportion of PF due to reduced peak EMG amplitude. In this example, as the EMG/power relationship at the LoT lies on the unfatigued linear relationship, no MF is present and, therefore, all PF can be solely attributed to AF.

By normalising the EMG and force data for every RIT contraction, we can plot the ratio of force/EMG against time (Figure 6.4), or overlay each contraction onto the original relationship (Figure 6.5). These methods may allow us to determine the onset time of muscle fatigue, the rate of its development, and visualise the time course of changes relative to fatigue at task failure. Important to note, is that

we cannot determine the relative contributions of AF throughout the RIT, as this can only be assessed when voluntary maximal effort is provided at the LoT. By measuring EMG from multiple muscle groups, we may also be able to identify if one muscle group is fatiguing faster than others and causing task failure. Together, these results may provide additional insight into the fatigue mechanisms throughout exercise and may allow a more focussed rehabilitation approach.

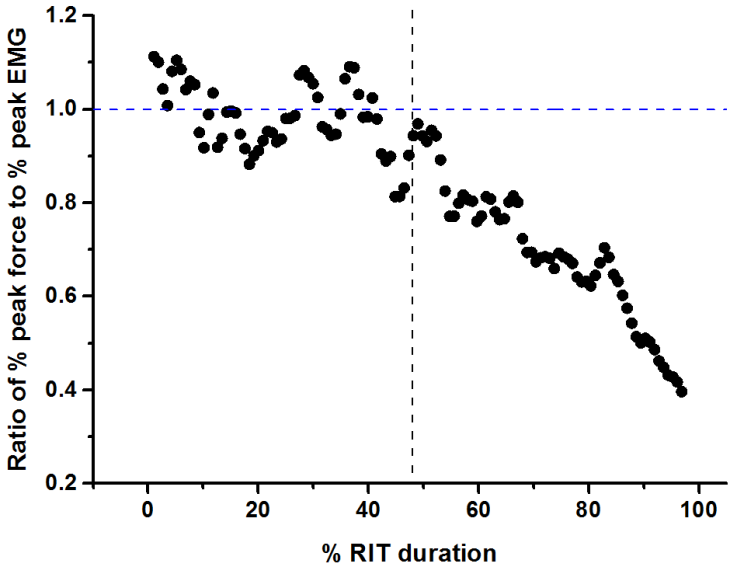

Figure 6.4. A representative example ratio of normalised force/EMG for every contraction throughout a concentric RIT. Horizontal blue dashed line represents the 1:1 ratio present in an unfatigued state. Vertical dashed line represents the breakpoint identified by fitting a piecewise linear regression, potentially useful in identifying the onset of muscle fatigue.

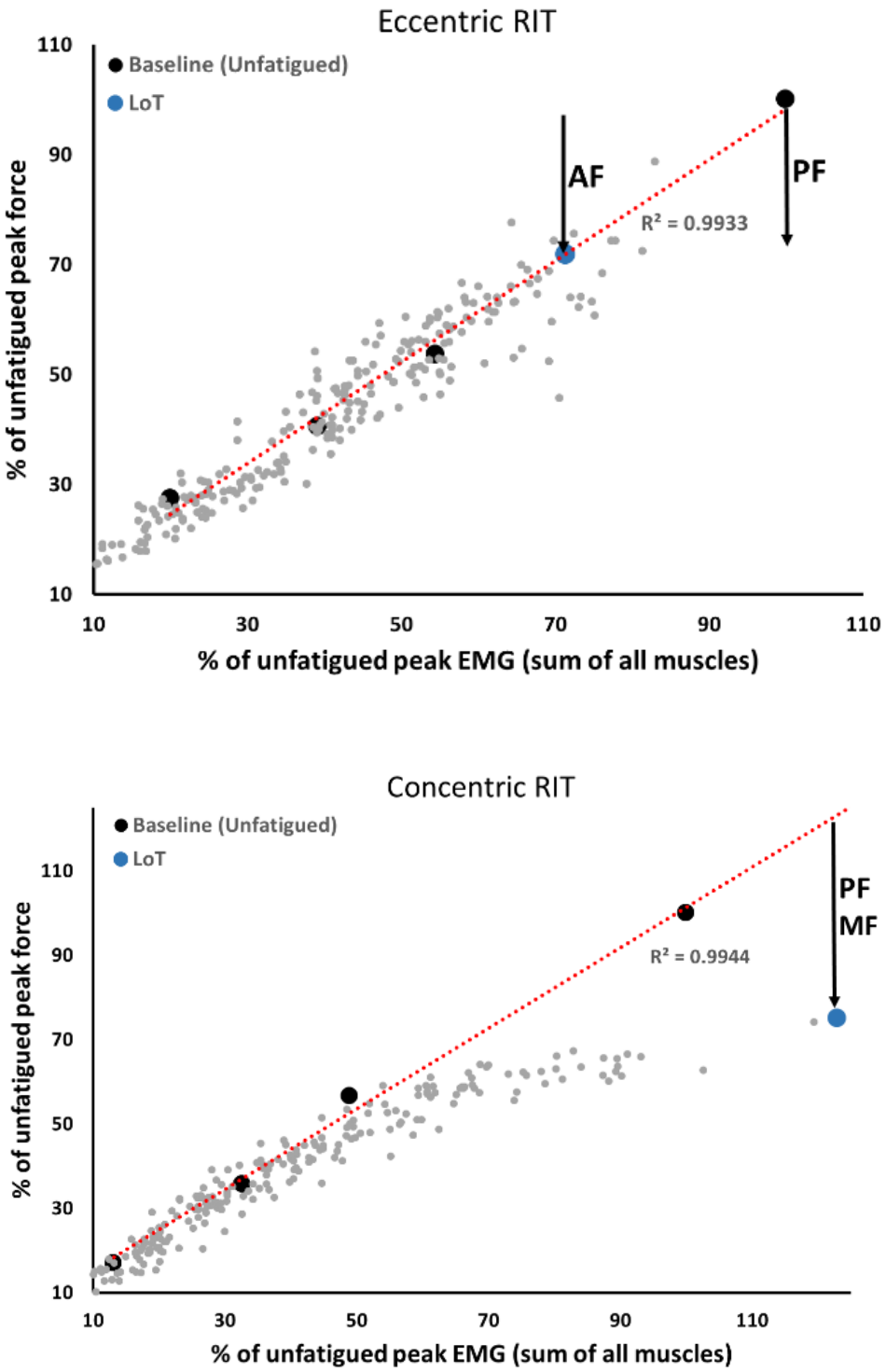

Figure 6.5. Representative force/EMG relationships displaying all RIT contractions. Black circles represent the force/EMG relationship during the 25%, 50%, 75% and 100% (of peak) unfatigued contractions of which a linear regression line is fit (red dotted line). Blue circle represents the force/EMG relationship at the LoT. Light grey circles represent the force/EMG relationship for each RIT contraction. In these examples, within the eccentric RIT, there is no MF, with solely AF at the LoT. The RIT data confirms no MF development via the close relationship to the unfatigued force/EMG relationship. Within the concentric RIT, there is no AF, with solely MF at the LoT. The RIT contractions show a progressive increase in MF tracking towards the force/EMG relationship at the LoT.

6.3.2 Assessment of muscle oxidative capacity

We conducted additional pilot work utilising a novel method of non-invasively estimating muscle oxidative capacity ($m\dot{V}O_2$). $m\dot{V}O_2$ is a measure of the muscles capacity to utilise the available O_2 which often becomes impaired with inactivity, ageing, and within chronic conditions such as COPD, CHF and diabetes (Drexler et al., 1992; Mancini et al., 1992; Esposito et al., 2010; Hood et al., 2011; Adami et al., 2017). Measuring $m\dot{V}O_2$ can provide information regarding exercise limitation, and can serve as a useful marker to assess the effectiveness of exercise interventions. At present, measurement of $m\dot{V}O_2$ involves invasive techniques such as muscle biopsies to assess enzyme activity and concentrations (Holloszy, 1967; Gollnick et al., 1973), as well as isolated mitochondria (Chance and Williams, 1955) and permeabilised muscle fibre preparations (Gnaiger, 2009). Non-invasive measurement can be achieved by utilising magnetic resonance spectroscopy to measure phosphorus metabolites (Chance et al., 2006); however, this technique is very costly. A new non-invasive and economical method of $m\dot{V}O_2$ assessment has recently been developed, demonstrating good repeatability and reliability (Hamaoka et al., 1996; Ryan et al., 2012; Adami et al., 2017), and was, therefore, assessed for its feasibility within our research.

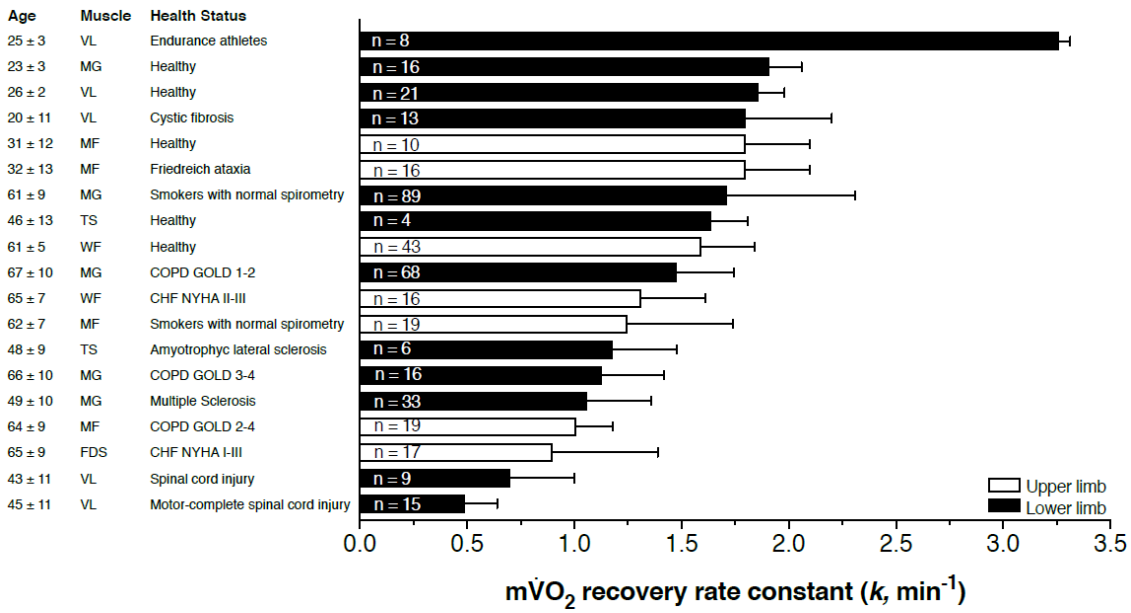

Figure 6.6. $m\dot{V}O_2$ within varied populations and muscle groups. Measured via NIRS. See article for abbreviations, (Adami and Rossiter, 2017).

Methods have been adapted from those of Ryan *et al*., (Ryan et al., 2012; Ryan et al., 2013; Ryan et al., 2014). Details of the procedure used and raw NIRS signals obtained can be seen in Figure 6.7 and Figure 6.8. This novel method utilises NIRS technology which measures the changes in relative concentrations of O_2Hb and HHb. The $m\dot{V}O_2$ measures obtained using this method have been cross-validated against MRS measurements of phosphate (Ryan et al., 2013) and permeabilised muscle fibres (Ryan et al., 2014), leading to its utilisation within a variety of populations and muscle groups (Figure 6.6). Based on the reduced metabolic requirement of eccentric exercise, it has previously been suggested that when matching for metabolic demand, increases in $m\dot{V}O_2$ may be enhanced. Although a recent study suggests that these adaptations may actually be reduced during eccentric *vs.* concentric exercise (MacMillan et al., 2017), they failed to accurately match for metabolic requirement, instead matching for HR response. This would likely result in a lower eccentric metabolic demand, reducing the stimulus for adaptations. Therefore, future investigation is necessary and non-invasive assessment *via* NIRS may provide a simple and effective method to assess the adaptations that occur as a result of eccentric training.

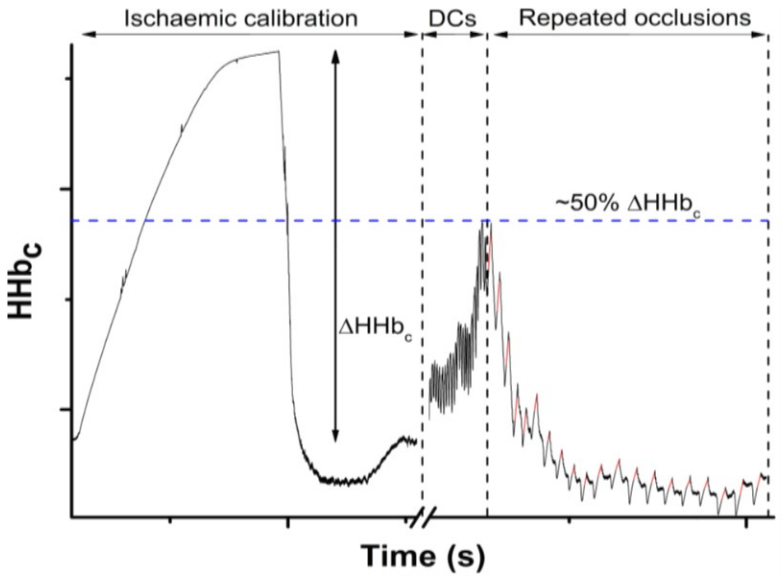

Figure 6.7. Representative example of blood volume corrected HHb signal (HHb_c) during assessment of $m\dot{V}O_2$. Following priming contractions, an ischaemic calibration is performed involving determination of maximum and minimum steady state HHb_c values via sustained supra-systolic occlusion. Light dynamic contractions (DCs) of the associated muscle are then performed to activate mitochondrial oxidative enzymes, elevate $\dot{V}O_{2m}$, and increase HHb_c to ~ 50% of the range (ΔHHb_c) previously determined. Repeated rapid supra-systolic occlusions are then applied at rest, restricting oxygen delivery and allowing assessment of the rate of $\dot{V}O_{2m}$ via linear regressions (red lines). Blue dashed line represents the desaturation induced by the dynamic contractions, with a target of ~50% ΔHHb_c.

The pilot NIRS $m\dot{V}O_2$ data collected to date presents with a large test-retest variability, contradicting previous studies (Adami and Rossiter, 2017), and therefore not meeting the requirements to be included within our training studies. Adaptations such as performing the ischaemic calibration prior to the assessment, and limiting desaturation during dynamic contractions improved consistency; however, further method refinements such as repeating the assessment within the same session, and increasing the consistency of probe placement, may improve repeatability and allow its future implementation.

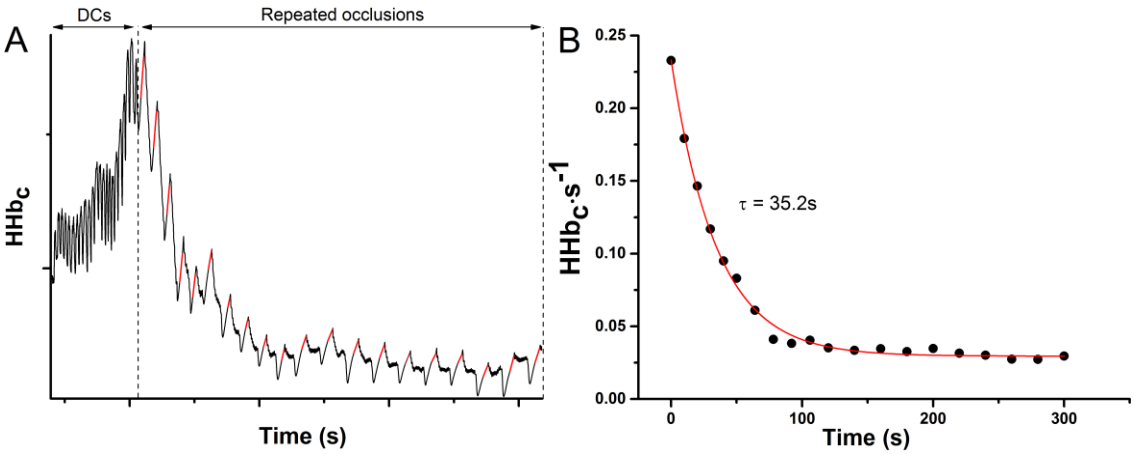

Figure 6.8. Process of determining muscle oxidative capacity ($m\dot{V}O_2$) from the raw NIRS data. Panel A) raw HHb_c NIRS signal during dynamic contractions (DCs) of the medial gastrocnemius muscle and repeated supra-systolic occlusions (red lines identify the linear regressions fit during each occlusion). Panel B) exponential fit (red line) to slope values of panel A. Time constant (τ) presented, which is the reciprocal of the rate constant (k).

6.4 Future directions and conclusions

As previously highlighted, eccentric exercise has great potential within a variety of clinical conditions. In addition, the adaptations implemented with the stepping ergometer may allow for tighter control of methodology, and further enhance our understanding of the acute and chronic physiological adaptations to eccentric exercise.

One area of eccentric research that has received little attention, is the potential of eccentric training to increase bone mineral density within frail populations at risk of falls. The combination of falls with osteoporosis often results in traumatic injuries that ultimately reduce mobility and independence, and result in an increased frailty status, morbidity, and mortality (Rubenstein, 2006). Bone tissue is in a constant state of remodelling, continuously adapting to the mechanical demands placed upon it (Moreira et al., 2014), especially *via* the contractile pull of muscles (Vieira et al., 2013). Current rehabilitation programs such as the Otago falls prevention program fail to provide increases in BMD (Brooke-Wavell et al., 2015), highlighting the need for alternative rehabilitation strategies that present a greater mechanical stimulus. With the positive correlation of increased

bone strain to increases in BMD (Rubin and Lanyon, 1985; Rhodes et al., 2000; Vieira et al., 2013), it can be postulated that the increased forces possible during eccentric training should result in greater increases in, or a slower decline of BMD. Although there are minimal results comparing concentric and eccentric training on BMD, results within young adults suggest that even when similar increases in voluntary strength are observed, only the greater forces applied with eccentric exercise result in BMD increases (Hawkins et al., 1999). It has also recently been shown that 12 weeks of descending stair walking within elderly obese women results in a 6% increase in BMD not seen with the ascending group. We have demonstrated that forces equivalent to ~85% of peak isometric torque are tolerable within older adults; therefore, it is likely that we would see similar, if not greater increases in BMD with our training. Pre and post-training measurements such as dual-energy X-ray absorptiometry or blood biomarkers of bone formation and resorption may in future allow us to quantify the potential therapeutic benefit (Karabulut et al., 2011). Blood biomarkers of bone formation (Procollagen I N-Terminal Propeptide, PINP) and resorption (C-terminal cross-linking telopeptide of Type-I collagen, CTX) are awaiting analysis from study 2 (chapter 4), *via* enzyme-linked immunosorbent assay (ELISA). Changes in these markers occur far more rapidly than BMD changes and may, therefore, be more applicable for use within our shorter training studies.

Within all three of the studies included in this thesis we have utilised a low contraction velocity (23 spm, ~46 deg·s^{-1}), which in hindsight, may be limiting the acute differences between concentric and eccentric responses, as well as the potential training responses observed. Due to the different methods of force generation, large differences in the torque-velocity relationship exist (see section 1.6.1). Concentric contractions exhibit a reduced ability to generate force with increasing velocity, whereas eccentric contractions show an initial sharp rise in the force with increased velocity, followed by a plateau at higher velocities (Duchateau and Baudry, 2014; Herzog et al., 2016). This results in up to ~153% higher eccentric forces at fast velocity contractions (Westing and Seger, 1989). Coupled with this increased force generating capacity is a greater disparity between concentric and eccentric metabolic requirement at increased velocities. The ratio of concentric to eccentric $\dot{V}O_2$ increases from 5:1 at 30 rpm, to 10:1 at

100 rpm (Bigland-Ritchie and Woods, 1976). These results suggest that if higher cadences are utilised within future studies, we should be able to match for metabolic requirement as before, but further increase the eccentric mechanical stimulus. This would likely enhance strength and mobility changes, although it should be noted that the low contraction velocities we utilised likely contributed to the high tolerability, lower muscle soreness, and faster familiarisation of the exercise. Therefore, thorough assessment of these outcomes within young and older adults is required to assess the feasibility of increasing velocity.

Finally, although we have shown that eccentric recumbent stepping is a safe, tolerable and effective form of exercise within young and older adults, a direct comparison against other eccentric modalities, namely the most commonly utilised reverse cycle ergometry, is required. Methods vary widely across acute and training studies utilising different eccentric modalities, making it hard to assess their relative benefits. Tightly controlling exercise parameters across devices may allow for a greater understanding of these benefits and give health care professionals greater knowledge of what modality would be best suited to their clinical populations. Future studies should aim to control for all aspects that effect physiological outcomes, allowing the sole assessment of the modality's potential. This would likely be challenging and involve matching for parameters such as: joint angles, cadence, stride length, relative power and metabolic requirement. However, with appropriate matching, training adaptations, as well as personal factors such as perceived difficulty of the task, muscle soreness experienced, and personal preference may be better assessed. The similarity of eccentric recumbent stepping and other eccentric modalities to that of common activities of daily living should also be assessed, with techniques such as kinematic and EMG activity analysis as well as subjective opinions likely useful. We hypothesise that eccentric stepping will more closely replicate natural activities and, therefore, result in greater translation to functional mobility improvements within these activities as previously shown (Millet et al., 2002; Misic et al., 2009; Garber et al., 2011), but, this correlation between ecological validity and functional improvements will need to be further assessed by comparing modalities of varying ecological validity, e.g. stair descent *vs.* recumbent stepping ergometer *vs.* reverse cycle ergometer training.

6.5 Concluding remarks

The studies we present have quantified the acute physiological responses of concentric and eccentric recumbent stepping exercise and demonstrate how the large disparity in cardiovascular and metabolic demand allows eccentric exercise to be utilised as an effective training stimulus within young and older adults. The potential applications of eccentric exercise are far reaching; however, it remains underutilised within standard rehabilitation. Eccentric recumbent stepping may provide a safe, tolerable and effective modality within exercise intolerant populations, and it is hoped that by contributing to this growing literature field, we may play a part in promoting and implementing eccentric exercise within populations that stand to benefit.

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Appendices

Appendix A ACSMs cardiovascular risk screening questionnaire

Participants completed the ACSMs cardiovascular risk screening questionnaire prior to undertaking study 3 (chapter 5). Those identified as being at high risk (> 2 risk factors) were excluded, and moderate risk participants were eligible to take part under supervision.

ACSM risk stratification screening questionnaire completed by all participants.

You have had:

___ a heart attack
___ heart failure
___ cardiac arrhythmia
___ known heart murmur
___ congenital heart disease
___ any heart surgery
___ coronary angioplasty
___ heart palpitations

You have:

___ experienced chest pain with mild exertion
___ experienced dizziness, fainting, or blackouts with mild exertion
___ experienced unusual fatigue or shortness of breath during usual activities
___ been prescribed heart medications (please indicate):

Check all that apply:

___ you are a man older than 45 years
___ you smoke
___ your blood pressure is greater than 140/90
___ you take blood pressure medication
___ you are completely physically inactive
___ you currently have bone/joint problems
___ you have had a recent injury/surgery
___ you are a diabetic or take medicine to control your blood sugar
___ you have been diagnosed with high cholesterol >200 (or HDL is less than 35 mg/dL or LDL is greater than 169 mg/dL)
___ you have a close blood relative who had a heart attack before age 55 (father/brother) or age 65 (mother/sister)

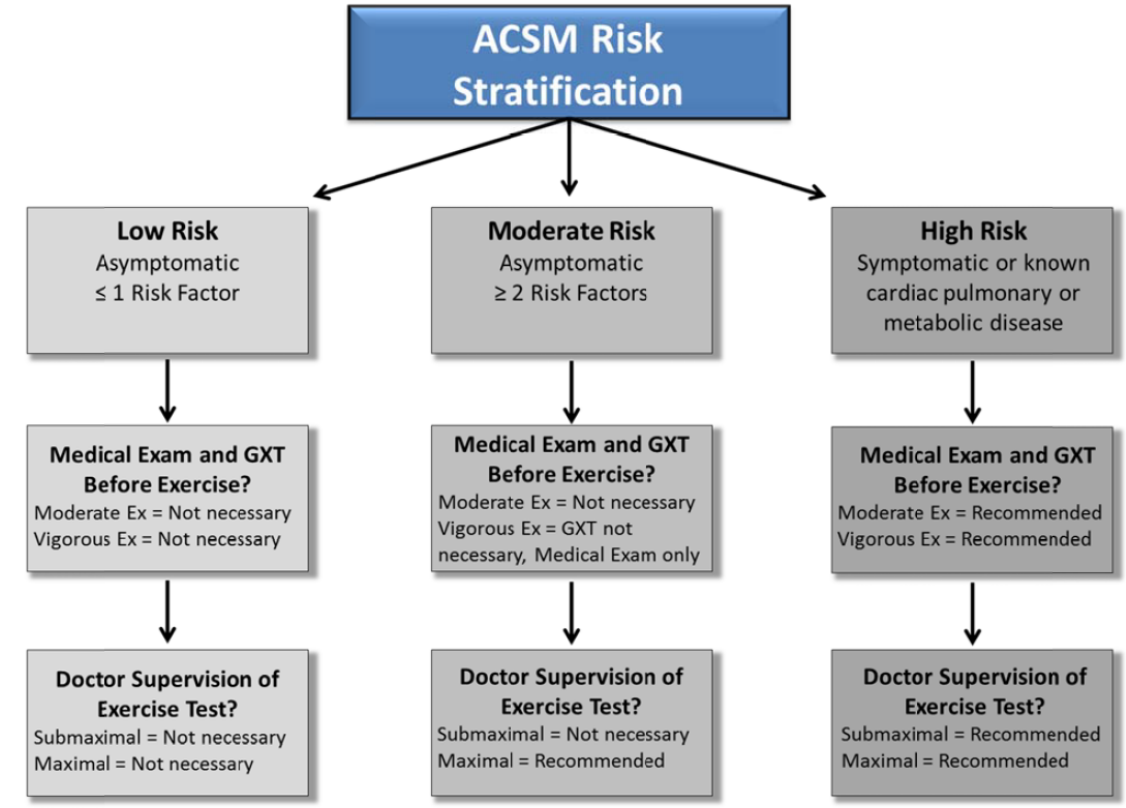
___ Other (specify) _____

ACSM risk Stratification Scoring completed by assessor.

Positive Risk Factors	Defining Criteria	Points
Age	Men ≥ 45 years, Women ≥ 55 years	+1
Family History	Myocardial infarction, coronary revascularization, or sudden death before 55 years of age in father of other 1st degree male relative or before 65 years of age in mother or other 1st degree female relative	+1
Cigarette Smoking	Current cigarette smoker or those who quit within the previous six months, or exposure to environmental tobacco smoke (i.e., secondhand smoke)	+1
Sedentary Lifestyle	Not participating in at least 30 minutes of moderate-intensity physical activity on at least three days/week for at least three months	+1
Obesity	Body mass index ≥30 kg/m^2 or waist girth >102 cm (40 inches) for men >88 cm (35 inches) for men	+1
Dyslipidemia	Low-density lipoprotein (LDL) cholesterol ≥ 130mg/dL (3.37 mmol/L) or high-density lipoprotein (HDL) cholesterol <40mg/dL (1.04mmol/L) or currently on lipid-lowering medication; If total serum cholesterol is all that is available, use serum cholesterol >200 mg/dL (5.18mmol/L)	+1
Prediabetes	Fasting plasma glucose ≥100 mg/dL (5.50 mmmol/L) but <126 mg/dL (6.93 mmol/L) or impaired glucoe tolerance (IGT) where a two-hour oral glucose tolerance test (OGTT) value is ≥140 mg/dL (7.70 mmol/L), but <200 mg/dL (11.00mmol/L)	+1
Negative Risk Factors	**Defining Criteria**	**Points**
High HDL Cholesterol	≥60 mg/dL (1.55 mmol/L)	-1

Total CVD Risk Score: _____

ACSM risk stratification screening classification completed by assessor.



GXT = Graded Exercise Test

Appendix B Borg rating of perceived exertion (RPE) scale

Borg RPE scale used to assess the difficulty of exercise throughout training in study 3 (chapter 5). First training session was performed at a rating of 11, second session at 13, and the remainder of sessions at 15. (Borg, 1982).

Table 1. The 15-grade scale for ratings of perceived exertion, the RPE Scale. (3)

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Appendix C Elderly mobility scale (EMS) assessment form

Elderly mobility scale (EMS) assessment form used to assess mobility within study 3 (chapter 5). (Smith, 1994)

TASK	Date	
Lying to Sitting	2 Independent 1 Needs help of 1 person 0 Needs help of 2+ people	
Sitting to Lying	2 Independent 1 Needs help of 1 person 0 Needs help of 2+ people	
Sitting to Standing	3 Independent in under 3 seconds 2 Independent in over 3 seconds 1 Needs help of 1 person 0 Needs help of 2+ people	
Standing	3 Stands without support and able to reach 2 Stands without support but needs support to reach 1 Stands but needs support 0 Stands only with physical support of another person	
Gait	3 Independent (+ / - stick) 2 Independent with frame 1 Mobile with walking aid but erratic / unsafe 0 Needs physical help to walk or constant supervision	
Timed Walk (6 metres)	3 Under 15 seconds 2 16 - 30 seconds 1 Over 30 seconds 0 Unable to cover 6 metres *Recorded time in seconds.*	
Functional Reach	4 Over 20 cm. 2 10 - 20 cm. 0 Under 10 cm. *Actual reach*	
	SCORES	/ 20

Appendix D Modified Borg dyspnoea scale

Instructions provided to participant.

Use this scale to rate the difficulty of your breathing.

It starts at number 0 where your breathing is causing you no difficulty at all and progresses through to number 10 where your breathing difficulty is maximal.

How much difficulty is your breathing causing you right now?

Modified Borg dyspnoea scale used during study 3 (chapter 5). Modified from (Borg, 1982).

0	**Nothing at all**
0.5	**Very, very slight (just notiveable)**
1	**Very slight**
2	**Slight**
3	**Moderate**
4	**Somewhat severe**
5	**Severe**
6	
7	**Very severe**
8	
9	**Very, very severe (almost maximal)**
10	**Maximal**

Appendix E Methods for assessing fatigue mechanisms via force/EMG relationship

Methods have been adapted from (Coelho et al., 2015).Force and EMG activity of the left leg were recorded at rest and at the LoT of the RIT. The RIT protocol followed is identical to that detailed within section 2.4.2. Following 5 minutes of rest, the participants were instructed to either resist (eccentric) or push the pedals away (concentric) at 25, 50, 75 and 100% of their self-perceived maximal effort. Only the contraction type corresponding to the RIT being performed was assessed. Participants were asked to perform 3 contractions for each relative % effort, with a 1 minute rest period interspersed between increments. Raw force and EMG data were time aligned *via* a trigger switch and recorded throughout the RIT duration. As soon as the participant reached the LoT, they were immediately asked to produce three more consecutive contractions at 100% effort, whilst verbal encouragement was given.

A wireless EMG system (Telemyo DTS; Noraxon U.S.A. Inc, Scottsdale, AZ, USA) was used to assess the electrical activity of 9 muscles on the left leg during testing. This system consisted of 9 wireless EMG sensors and a wireless 3D accelerometer which transmitted their data to a differential amplifier. The wireless EMG sensors were composed of the wireless transmitter with a reference electrode on the base, and a 2-pin lead wire connector that connected to the 2 electrode pads. The differential amplifier acts to only amplify the signal that is different for each electrode (common mode rejection), therefore acting to eliminate external noise that would reach both electrodes at the same time, and amplify the electrical muscle activity. The 3D accelerometer was positioned at the top of the ergometer foot plate corresponding to the leg EMG was measured on, and the Z axis was orientated in the plane of the pedal movement. This allowed us to standardise the muscle activity analysed.

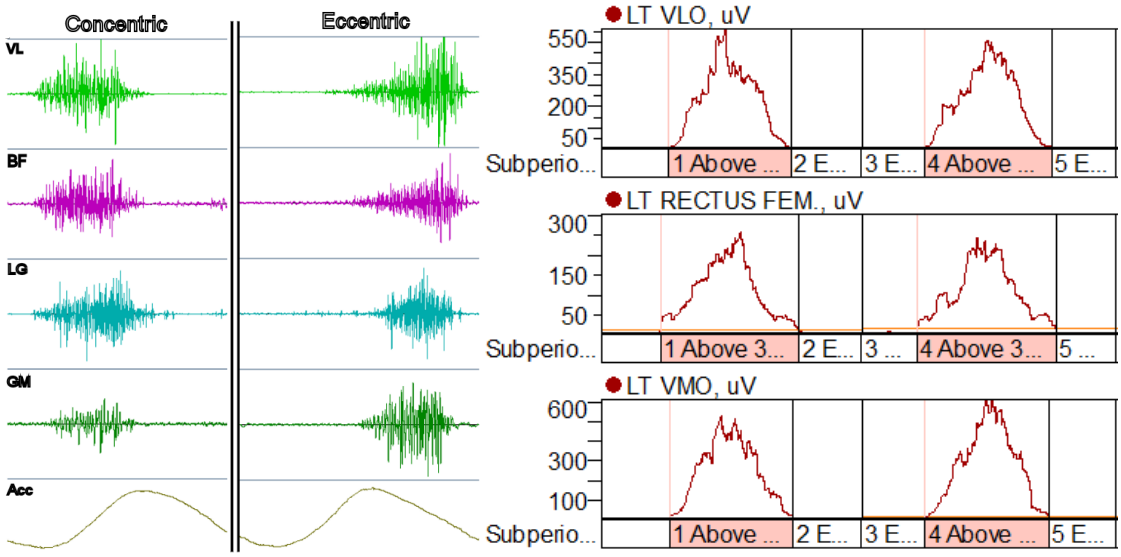
The electrode locations, shape, size, skin preparation, inter electrode distance and signal assessment were all based on the European recommendations for surface electromyography (Hermens et al., 1999). Two Kendall electrodes

(H93SG, Covidien, Minneapolis, MN, USA) were placed directly next to each other (inter-electrode distance of 24mm) at the recommend electrode placement site parallel to the muscle fibres. The wireless transmitter (reference electrode) was placed as far as possible from the two electrodes without placing tension on the wires, and was stuck directly to the skin with strong double sided adhesive tape. To improve electrode conductance, the electrodes skin location was prepped by shaving excess hair, abrading the skin (Nuprep gel; Nuprep, Weaver and company, Aurora, CO, USA) and was cleaned with alcohol wipes. The 9 muscles assessed were vastus lateralis, rectus femoris, vastus medialis, semitendinosus, biceps femoris, lateral and medial heads of gastrocnemius, tibialis anterior and gluteus maximus. Selection of these muscles was based on their high contribution to human locomotion and high activity during stepping exercise (Winter and Yack, 1987; Ivanenko, Y. P. et al., 2004), a movement that the stepping ergometer replicates.

All data were collected, processed and analysed using Noraxon Myoresearch XP software (Noraxon U.S.A. Inc, Scottsdale, AZ, USA). Once all electrodes were connected, the signal quality was checked by assessing the baseline level of noise, the offset before and after contractions, and the signal during voluntary contraction of the associated muscle. The participant was positioned on the stepping ergometer and asked to completely relax whilst the sensors were started. If the baseline value was greater than 15 microvolts, was not close to 0 at rest and after contractions, or if there was a noisy or low signal during contraction, the electrodes were removed, the skin re-prepped and the new electrodes positioned. In order to time align the collection of EMG and force data, an external trigger was connected to both the LabChart software and Noraxon software and set up to trigger the start of the recording. All EMG and accelerometer data was recorded at 1500Hz throughout the whole test.

The EMG data was initially assessed for signal quality, with any channels presenting with excess noise, or minimal muscle activity signal eliminated. Raw data were rectified and smoothed *via* a root mean squared (RMS, 100ms windows, Coelho *et al*., 2015). The accelerometer data was used to determine

each step cycle and the peak EMG for each step exported. The EMG activity from all muscles was summed for each contraction. Details of determination of peak force can be seen in section 2.6.3.3. For both EMG and force data, at baseline unfatigued assessment, a mean was taken of the three contractions peak EMG/force for each percentage of maximal effort contraction (25, 50, 75, 100%). All data is reported relative to the 100% maximal effort, eliminating the effect of high day-to-day variability in the EMG signal.



Representative raw (left) and processed (right) EMG data collected from 4 muscles during concentric and eccentric RIT stepping. Vastus lateralis (VL), biceps femoris (BF), lateral gastrocnemius (LG), gluteus maximus (GM) and accelerometer (Acc). The accelerometer was used to determine each step cycle. The raw EMG data has been rectified and smoothed via a 100ms RMS.