Exploring the mechanistic basis, and plasticity of “exercise resistance” to improve human health

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Abbreviations

1-RM	one-repetition maximum
ACSM	American College of Sports Medicine
AT	anaerobic threshold
BMI	body mass index
BP	blood pressure
CRP	C-reactive protein
CSA	cross-sectional area
DBP	diastolic blood pressure
DXA	Dual-energy X-ray absorptiometry
EET	endurance exercise training
HDL	high-density lipoprotein
HOMA-IR	homeostasis model assessment of insulin resistance
HR	heart rate
IL-6	interleukin-6
LBF	leg blood flow
LDL	low-density lipoprotein
LM	lean muscle mass
MAP	mean arterial pressure
MPB	muscle protein breakdown
MPS	muscle protein synthesis
MVC	maximal voluntary contraction
NCD	non-communicable disease
RET	resistance exercise training
SBP	systolic blood pressure
T2DM	type 2 diabetes mellitus
TC	total cholesterol
VO_2	volume of oxygen
VO_2max	maximal oxygen uptake
WHO	World Health Organization

Chapter 1: Introduction & Experimental Methods 4
1.1 Background 4
1.2 Physiological adaptations to exercise 5
1.2.1 Cardiorespiratory function 6
1.2.1.1 Cardiac contribution to cardiorespiratory function 7
1.2.1.1 Vascular contribution to cardiorespiratory function 8
1.2.1.3 Mitochondrial contribution to cardiorespiratory function 8
1.2.1.4 Assessment parameters of cardiorespiratory function 9
1.2.1.5 Heterogeneity in cardiorespiratory responses to exercise training 11
1.2.2 Muscle mass, structure and function 11
1.2.2.1 Muscle strength 12
1.2.2.2 Muscle mass 13
1.2.2.2.1 Assessment methods of muscle hypertrophy 15
1.2.2.2.2 Heterogeneity in hypertrophic responses to exercise training 15
1.2.3 Muscle architecture 16
1.2.3.1 Assessment methods of muscle architecture 16
1.3 Exercise for health 17
1.3.1 Metabolic health 17
1.3.1.1 Assessment methods of metabolic health 18
1.3.1.2 Heterogeneity in glycaemic and insulinaemic responses to exercise training 18
1.3.2 Vascular health 19
1.3.2.1 Haemodynamic parameters 19
1.3.2.2 Lipid parameters 20
1.3.2.3 Inflammatory parameters 21
1.3.3 Body composition 22
1.3.3.1 Assessment methods of body composition 22
1.4 “Exercise resistance” 23
1.4.1 Heterogeneity in exercise training responses 23
1.4.2 “Exercise resistance” and health 23
1.5 Study design & aims 24
Aim 1: To assess the heterogeneity of physiological adaptations to exercise training 24
Aim 2: To assess the mode-specificity of responder status to exercise training 24
Aim 3: To determine the relationship between “fitness” and health adaptations to exercise training 25
1.6 Experimental methods 25
1.6.1 Subject recruitment 25
1.6.2 Exercise training 26
1.6.2.1 Resistance exercise training (RET) 26
1.6.2.2. Endurance exercise training (EET) 29
1.6.2.3 “Wash-out” phase 29
1.6.3 Acute studies 29
1.6.3.1 Blood tests 30
1.6.3.2 Oral glucose tolerance test (OGTT) 30
1.6.3.3 Dual energy X-ray absorptiometry (DXA) 30
1.6.3.4 Blood pressure (BP) and heart rate (HR) 30
1.6.3.5 Vascular reactivity 31

1.6.3.6 Nutrient provision 31
1.6.3.7 Cardiopulmonary exercise testing (CPET) 31
1.6.3.8 Muscle architecture and quadriceps cross-sectional area (CSA) 32
1.6.3.9 Dynamometry 33
1.6.3.10 One repetition maximum 34
1.6.4 Statistical analysis 34
Chapter 2: Exploring the heterogeneity in exercise responses 35
2.1 Does heterogeneity exist in response to EET? 35
RESULTS 35
2.2 Does heterogeneity exist in response to RET? 36
RESULTS 37
2.2.1 Hypertrophic responses 37
2.2.2 Strength responses 38
2.2.3 “Wash-out” period 40
2.3 Summary 40
Chapter 3: Is “responder” status dependent on exercise modality? 42
RESULTS 42
3.1 Are fitness responses mode dependent? 42
3.2 Are health responses mode-dependent? 44
RESULTS 44
3.2.1 Metabolic health 44
3.2.2 Vascular health 45
3.2.2.1 Haemodynamic factors 45
3.2.2.2 Biochemical factors 46
3.2.3 Body composition 47
..... 48
3.3 Summary 48
Chapter 4: Are fitness- and health-related responses linked? 49
4.1 Cardiorespiratory fitness and health 49
4.1.1 Metabolic health 49
4.1.2 Vascular health 50
4.1.3 Body composition 51
4.2 Hypertrophic responses and health 52
4.2.1 Metabolic health 52
4.2.2 Vascular health 53
4.2.3 Body composition 53
4.3 Relationships between health parameters 54
4.3.1 EET 54
4.3.2 RET 55
4.4 Summary 55
Chapter 5: Discussion & Conclusions 57
5.1 Study design 57
5.2 Heterogeneous responses to exercise 57
5.2.1 Variation in cardiorespiratory responses to EET 57
5.2.2 Variation in hypertrophic responses to RET 59
5.3 Mode-specificity of exercise responses 61
5.3.1 Performance 61

5.3.2 Health 63
Metabolic health 63
Vascular health 63
Body composition 64
5.4 Effect of improving fitness on improving health 65
5.4.1 Metabolic health 65
5.4.2 Vascular health 66
5.4.3 Body composition 68
5.4.4 Relationships between health parameters 68
5.6 Study limitations & Future directions 69
5.6.1 Study design 69
Participants 69
Exercise training 69
Washout phase 70
Habitual physical activity and diet 71
5.6.2 Scope for future work 71
Molecular analyses 71
5.7 Overall conclusions 72
References 73
Appendix 86
Appendix I: Recruitment letter 86
Appendix II. Volunteer information sheet. 87
Appendix II. Volunteer screening sheet 92

Chapter 1: Introduction & Experimental Methods

1.1 Background

Non-communicable diseases (NCDs) are non-infectious chronic diseases that cause more deaths than all other causes combined (WHO 2014). Even more alarmingly, the incidence of deaths attributable to NCDs is projected to increase from 38 million in 2012 to 52 million in 2030 (WHO 2014). The four main NCDs – cardiovascular disease, cancer, chronic respiratory disease, and diabetes – give rise to 82% of NCD deaths, approximately 42% of which will occur before the age of 70 years.

In recent years, evidence has accumulated to suggest a causal relationship between NCDs and preventable risk factors, with physical activity playing a predominant role (Blair and Morris 2009). In fact, a direct epidemiological link has been identified between low physical activity and NCDs, suggesting low physical activity to be causal for ~16% of all deaths, greater than that of obesity, diabetes, and tobacco use combined (Blair *et al.* 2012). Therefore, the prevailing view is that a causal association exists between physical inactivity and obesity, insulin resistance, and hypertension, hence inactive lifestyles are a major global health threat. For these reasons, the solution to prevent and reduce NCDs would appear simple; to lead more active lifestyles. However, recent suggestions of "non-responders" to exercise may complicate this "one-size-fits-all" assumption, a premise which will be discussed in detail throughout this thesis.

Structured physical activity can be divided into two classes: resistance exercise training (RET), involving overcoming resistive forces of external loads (i.e. weight training), and endurance exercise training (EET), which involves prolonged aerobic activities (i.e. running and cycling). Both modes of exercise training lead to physiological adaptations that may be beneficial for health; for example, adequate muscle mass (they key physiological adaptation to RET is muscle hypertrophy) is epidemiologically linked to aerobic fitness and morbidity/mortality (Evans 1995; Newman *et al.* 2006; Kokkinos *et al.* 2008). In this respect, the American College of Sports Medicine (ACSM) guidelines encourage participation in exercise for optimal health and well-being, recommending ~150 min per week of moderate intensity EET and RET of the main muscle groups ~3 times per week (Garber *et al.* 2011). Despite both of these training modes being clearly related to improved whole-body health, such as improved insulin sensitivity (Henriksen 2002), and lowered LDL-cholesterol (Kelley *et al.* 2005b) and blood pressure (Green *et al.* 2014), RET and EET also result in separate and distinct physiological adaptations. Fundamentally, RET is associated with muscle hypertrophy (ACSM 2009; Erskine *et al.* 2014), and consequently improved strength and power (Phillips *et al.* 2012b; Franchi *et al.* 2014); while in contrast, EET is more likely to lead to improvements in aerobic/cardiorespiratory function (Garber *et al.* 2011), resulting from microvascular angiogenesis (Keller *et al.* 2011) and mitochondrial biogenesis (Hoier *et al.* 2013).

Although exercise training (of both or either mode) improves health (Blair and Morris 2009), and results in the expected physiological changes (hypertrophy for RET and improved aerobic capacity for EET) in most cases, not all individuals achieve the expected positive responses (Figure 1). For example, following several weeks or even months of supervised EET, which typically improves aerobic fitness, approximately 20% of people show no or

negligible increases in aerobic capacity (Timmons *et al.* 2010). Similarly, ~30% show no improvement in insulin sensitivity, and ~20% of subjects even show reduced insulin sensitivity (Boule *et al.* 2005). Likewise, hypertrophic responses to supervised RET are shown to vary from -1.2–10.4 cm^2 (muscle cross-sectional area, CSA; Raue *et al.* 2012) and +3–28% (Phillips *et al.* 2013; Figure 1). These responses are invariable of inter-individual characteristics, such as gender, age, muscle mass, or habitual physical activity and thus the mechanisms underlying this "exercise resistance" are unclear.

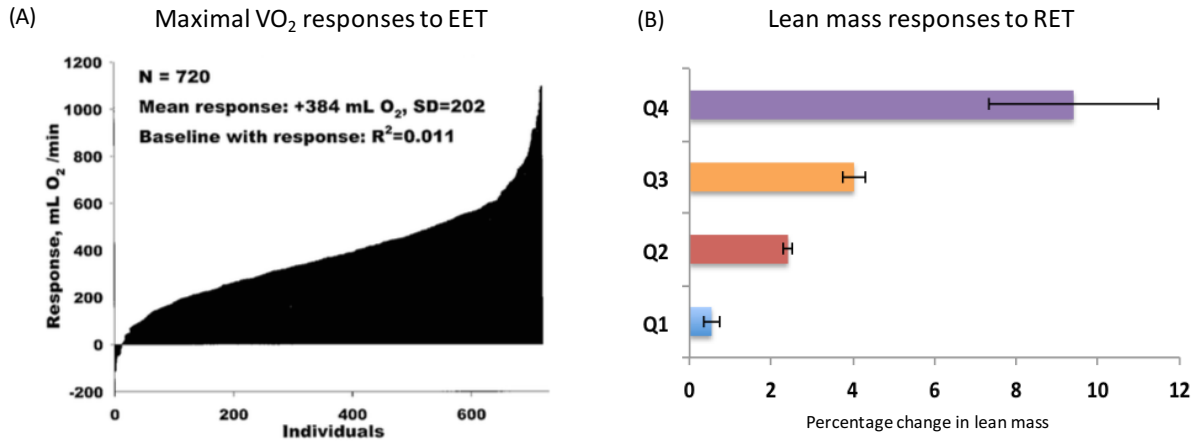


Figure 1. Heterogeneous responses to standardised exercise training. (A) VO_2peak responses to 20 weeks of EET from the HERITAGE Family Study (image from Bouchard and Rankinen 2001). (B) Lean mass responses to RET (image from Phillips et al. 2013).

In summary, major inter-individual differences exist in physiological responses to both RET and EET; with associated "positive" health outcomes, such as the lowering of blood pressure or improvements in insulin sensitivity, also exhibiting similar between-subject variation (Cornelissen and Smart 2013; Dalzill *et al.* 2014). These observed impediments to physical conditioning with exercise provide a strong foundation for the exploration of mechanisms regulating these adaptive processes and the relationship between health and "fitness" (e.g. hypertrophy, aerobic capacity and physical performance; acknowledging that these factors are also key for health across the lifespan) responses. In addition to issues surrounding exercise adherence (Perri *et al.* 2002), explanation and understanding of these heterogeneous responses are crucial for exercise prescription in metabolic pathology.

1.2 Physiological adaptations to exercise

As outlined in section 1.1., endurance exercise training (EET) and resistance exercise training (RET) lead to distinct physiological adaptations in skeletal muscle, as well as other tissues. Primarily, EET is associated with improvements in cardiorespiratory fitness (e.g. VO_2max), whereas RET is associated with physiological endpoints of strength and hypertrophy. The improvement of aerobic capacity, such as that commonly observed with EET, involves enhancement of the processes required for oxygen extraction and utilisation. This may involve, for example, the activation of pro-angiogenic pathways (Gustafsson *et al.* 1999) or the induction of muscle mitochondrial protein synthesis (Wilkinson *et al.* 2008). In contrast, hypertrophic responses in skeletal muscle in response to RET are associated with enhanced myofibrillar muscle protein synthesis (MPS; Wilkinson *et al.* 2008), as well as the activation of established key anabolic signalling pathways (i.e. AKT-mTORc1) (Wilkinson *et al.* 2008),

satellite cell activation (Blaauw and Reggiani 2014), and myocellular incorporation (Petrella *et al.* 2008). Furthermore, molecular adaptation in response to RET and EET can overlap, as well as showing distinct differences (Phillips *et al.* 2013). It is therefore necessary to characterise these adaptations and identify potential limiting factors in physiological responses to training.

1.2.1 Cardiorespiratory function

The oxygen transport and utility chain describes factors influencing and limiting training responses in regards to aerobic fitness and potentially associated health benefits (e.g. heart rate, blood pressure, etc.). Oxygen transport can be viewed as the time taken for O_2 to travel from the blood to the muscle cells, whereas oxygen demand encompasses the mitochondrial oxidative capacity of skeletal muscles. Oxygen transport can be further categorised into "peripheral" (muscle O_2 transport conductance) and "central" factors (blood flow and Hb-O_2 dissociation: O_2 extraction) (Bassett and Howley 2000; Figure 2). Therefore, O_2 uptake is regulated at many points on this pathway, from the cardiorespiratory system to the mitochondria, and a limitation at any of these points may lead to limited maximal aerobic capacity (VO_2max) improvements in response to exercise training.

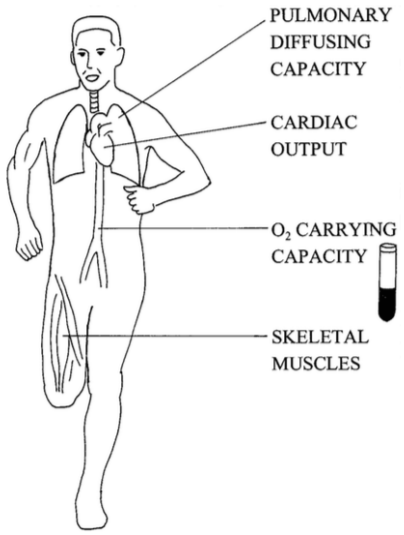


Figure 2. Factors that may limit cardiorespiratory function (maximal oxygen capacity; VO_2max). Image from Bassett and Howley (2000).

Maximal oxygen uptake, or VO_2max, is defined as the oxygen uptake attained during maximal exercise intensity, defining the limits of the cardiorespiratory system (Hill and Lupton 1923). VO_2max marks the highest rate of oxygen uptake and utilisation by the body, and is usually measured by a graded exercise test. VO_2max has implications in both health and athletic performance. A low VO_2max is a risk factor for premature mortality, as well as metabolic and cardiovascular disease (Kokkinos *et al.* 2008). VO_2max and aerobic performance are correlated in athletes of mixed ability, and well-trained athletes can achieve VO_2max values over two-times those of their sedentary counterparts (Saltin and Astrand 1967).

The cardiovascular system and the respiratory system work together to supply O_2 to the working muscles and remove CO_2. Four processes are involved in this function: 1) pulmonary ventilation, the movement of air into and out of the lungs; 2) pulmonary

diffusion, the exchange of O_2 and CO_2 between the lungs and the blood; 3) transport of O_2 and CO_2 in the blood; and 4) capillary gas exchange, the exchange of O_2 and CO_2 between capillary blood and the working muscle (Balady *et al.* 2010). These processes are linked by the circulatory system. The consensus view is that VO_2max is limited by the rate of oxygen delivery, as opposed to the ability of the muscle to take oxygen from the blood (reviewed in Bassett and Howley 2000). Physiological factors that may limit VO_2max include pulmonary diffusion capacity, maximal cardiac output, and oxygen carrying capacity of the blood (central factors), in addition to peripheral factors such as skeletal muscle characteristics (e.g. fibre type; Bergh *et al.* 1978). All of these variables may impede or enhance O_2 flux on the pathway from the air to the mitochondria in skeletal muscle.

Exercise tolerance is another physiological parameter related to VO_2max that commonly improved by EET. Exercise tolerance is determined by three factors: 1) pulmonary gas exchange; 2) cardiovascular performance (including the peripheral vascular tree); and 3) skeletal muscle metabolism (i.e. oxidative phosphorylation) (Albouaini *et al.* 2007). The Fick equation describes VO_2 in relation to cardiac output (CO); at maximal exercise, the Fick equation is expressed as:

$$VO_2max = (SVmax \times HRmax) \times (CaO_2max \times CvO_2max),$$

whereby maximal values in the equation are for stroke volume (SV), heart rate (HR), arterial oxygen content (CaO_2), and mixed venous oxygen content (CvO_2). VO_2max may be compromised by factors affecting any one or more of these components, i.e. reduction in maximal HR, maximal SV, maximal CaO_2, or increased resting CvO_2; this is also relevant to pathological cardiac conditions (e.g. heart failure), whereas exercise training can improve VO_2max (Albouaini *et al.* 2007). As a whole, this concept reflects the ability of the individual to maximally take in, transport, and utilise oxygen, reflecting the functional aerobic capacity of an individual. VO_2max is thus measured as a parameter of cardiorespiratory fitness.

1.2.1.1 Cardiac contribution to cardiorespiratory function

As outlined by the Fick equation above, cardiac output (CO or Q; the amount of blood ejected by the heart during a 1-min period) is an important determinant of individual differences in VO_2max. In normoxia, VO_2max is primarily limited by CO and locomotor muscle blood flow (Mitchell *et al.* 1958), whereby CO = stroke volume (SV) × heart rate (HR). Heart rate is the number of contractions the heart performs per minute, measured in beats per minute (bpm). Changes in CO vary due to changes in SV, with less variation in HR and systemic oxygen extraction. During maximal exercise, individuals exhibit greater exercising CO, which can be up to 6 times that of resting, and CO is also redistributed away from non-active tissues (splanchic and renal blood flow) to skeletal muscle. Endurance-trained athletes have greater exercising COs and SVs, due to the adaptation of left ventricular hypertrophy (Osborne *et al.* 1992), which consequently has greater blood-pumping capability. At rest, CO may be relatively similar in trained and untrained subjects, and adaptation to training is due to an increased SV of the trained subject with a lower resting HR; for trained individuals, at maximal exercise, an increased CO is attributed to an increased exercising SV, with limited effects on maximum HR (Mitchell and Blomqvist 1971). A higher CO is therefore beneficial for performance in endurance athletes, to meet the high demands for O_2 in the tissues.

EET allows the sinus node of the heart to be brought under greater influence of acetylcholine, which is a parasympathetic hormone that slows resting HR, and also decreases resting sympathetic activity. There are 3 physiological mechanisms that work to increase SV during exercise. Firstly, the myocardium incurs enhanced cardiac filling in diastole, which leads to a greater force of systolic contraction. The second mechanism is neurohormonal and involves forceful ejection and emptying during systole. Thirdly, training adaptions that expand blood volume and reduce resistance to blood flow in peripheral tissues constitute another mechanism of increased SV from exercise training. Maximal CO is therefore viewed as the main limiting factor of VO_2max in cycling and running tests (Saltin and Calbet 2006), and it has been estimated that 70–85% VO_2max is limited by maximum CO.

1.2.1.1 Vascular contribution to cardiorespiratory function

In addition to the central cardiac parameters outlined above (1.2.1.2) VO_2max is also limited by skeletal muscle blood flow (Saltin and Calbet 2006). During maximal exercise, the majority of oxygen available is extracted from the blood that perfuses the active muscles, and blood flow is primarily distributed to the respiratory muscles (Harms *et al.* 1997). This presents a perfusion limitation and therefore limitation for O_2 delivery. As an additional mechanism to enhance O_2 delivery to the working muscles, blood is redistributed during exercise. Indeed, Saltin (1985) observed a 2–3-fold higher O_2 uptake in the quadriceps compared with whole body maximum effort, implying a diversion of greater blood flow to the quadriceps upon local muscle stimulation. During whole-body exercise, the maximum vascular conductance of the limbs outweighs the pumping capacity of the human heart (Calbet *et al.* 2004). Thus, the body must have an exceptional capacity to increase blood flow and O_2 delivery to skeletal muscles during exercise, and VO_2max is limited by oxygen delivery to some extent.

An improvement in muscle blood flow, such as with exercise training (Laughlin and Roseguini 2008), leads to improvements in VO_2max, such as by vascular remodelling (i.e. angiogenesis) and/or altered vascular resistance properties of skeletal muscle. As the primary site of O_2 resistance occurs between the surface of the RBC and the sarcolemma (Honig *et al.* 1992), inadequate blood flow to skeletal muscles may thus play a role in limiting VO_2max. There are therefore multiple cardiovascular levels of regulation of VO_2max, from the organ to the cellular level.

1.2.1.3 Mitochondrial contribution to cardiorespiratory function

Already described as the culminating location for O_2 in the O_2 transport and utility chain, skeletal muscle mitochondria are likely to play a role in the VO_2max training responses. For example, six-weeks endurance training has been shown to increase mitochondrial enzyme activity, indicated by citrate synthase activity (Vollaard *et al.* 2009). However, although EET has been shown to increase skeletal muscle mitochondrial capacity, other work has contended that mitochondrial capacity is not limiting for aerobic adaptation (Lessard *et al.* 2013). Hoppeler *et al.* (1990) reviewed the relationship between VO_2max and muscle oxidative capacity (muscle mitochondrial volume), emphasising that although there is a linear relationship between VO_2max and muscle mitochondria, humans seem to have "excess" mitochondrial volume, and thus the cardiovascular system has a surplus ability to

undergo muscle oxidative capacity at VO_2max. Indeed, individuals with a similar VO_2max can have a striking differences in their mitochondrial enzyme activity (Holloszy and Coyle 1984). Thus, although these data indicate that mitochondrial enzymes may not be limiting for aerobic adaptation, mitochondrial do appear to need to be in sufficiency to allow VO_{2max} to increase with EET via other adaptive mechanisms. It therefore appears that skeletal muscle mitochondria have more of a role in enhancing exercise performance than increasing VO_{2max}.

Mitochondrial capacity may contribute to exercise tolerance (Taivassalo *et al.* 2003). EET training results in fatigue resistance, which may be due to increased oxidative capacity and mitochondrial biogenesis (Menshikova *et al.* 2006; Cho *et al.* 2016). Increasing skeletal muscle mitochondria with EET may also be involved in other metabolic mechanisms such as oxidising fat at a higher rate (Wolfe 1998), and reduce lactate production during exercise (Lindholm *et al.* 2004), both of which contribute to improved exercise tolerance. Overall, VO_{2max} is considered to be dependent on the interaction of O_2 transport and mitochondrial O_2 uptake, both of which can be enhanced, *on average*, by EET (Honig *et al.* 1992).

1.2.1.4 Assessment parameters of cardiorespiratory function

During cardiopulmonary exercise testing (CPET), VO_{2max} (the maximal amount of O_2 that can be utilised in a given time-period (Hill and Lupton 1923)), the gold-standard measure of cardiorespiratory function (Mezzani *et al.* 2009) is theoretically achieved when intensity of exercise increases without a concomitant increase in oxygen utilisation (VO_2). This premise first postulated in the 1920s by Hill and Lupton (1923), with VO_2max indicated by a plateau in oxygen uptake during CPET despite a continued increase in exercise intensity (Figure 3). The plateau represents the maximal achievable level of oxidative metabolism involving large muscle groups (Albouaini *et al.* 2007). However, in practice, subjects cannot always reach a VO_2max plateau at maximal effort, and this is estimated to occur in approximately 50% of cases (Howley *et al*. 1995). Thus, in more recent years, secondary criteria have been developed to characterise oxygen uptake at its maximal rate (Edvardsen *et al.* 2014), such as respiratory exchange ratio (RER), HRmax, and blood lactate concentration (described later), which aim to verify VO_2max at an apparent plateau.

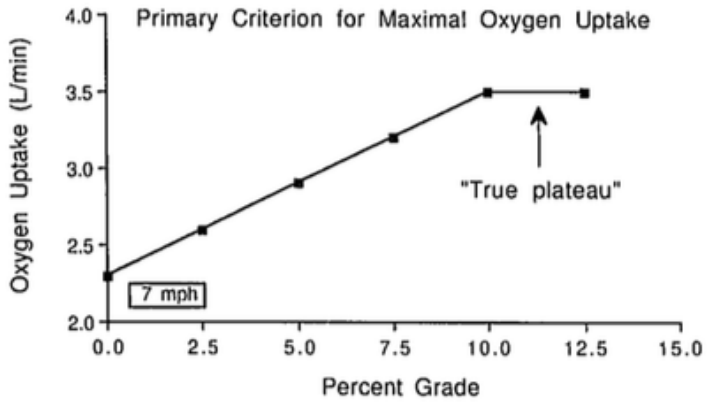


Figure 3. Theoretical representative of achievement of "true" VO_2max, showing a plateau in VO_2 (oxygen uptake) as percentage grade continues to increase. Image from Howley et al. (1995).

During CPET, it is important to monitor the interactions between pulmonary, cardiovascular, and metabolic dynamics, with the expectation that ventilation (VE), VCO_2, VO_2, and HR will increase in a linear fashion in respect to the rise in workload until a set-point. As most activities of daily living do not require maximal effort, and based on the inability of many individuals to achieve true VO_2max, the anaerobic or ventilatory threshold (AT or VT, respectively) is a widely used submaximal index of exercise capacity (Balady *et al.* 2010). VT occurs when the exercise level reaches a point where VE increases exponentially relative to increasing VO_2. VT is thought to reflect AT, as AT describes when the oxygen supply to the muscle is insufficient to meet the oxygen requirements. Consequently, metabolism becomes increasingly reliant on anaerobic glycolysis for energy to produce lactate; this point is known as the lactate threshold (LT). A subsequent increase in VE is required to eliminate excess CO_2 produced as a result of lactic acid conversion to lactate, as illustrated in Figure 4. The respiratory compensation point (RCP) marks the onset of hyperventilation during a CPET and is therefore known as "respiratory compensation," which is likely the cause of the subsequent metabolic (lactic) acidosis (Meyer *et al.* 2004). This can be seen by a loss of linearity between VE and VCO_2, and is clearly distinguishable from the AT. The onset of hyperventilation may therefore result from the subjects' inability to compensate for this metabolic acidosis (inability to maintain blood pH). AT occurs at ~45–60% peak or maximal VO_2 in healthy untrained individuals (Balady *et al.* 2010), which may be dependent on aerobic fitness levels, such as endurance-trained compared with untrained individuals. In the former case, the AT will be higher, whereas in the latter case AT may be at the lower end of the scale. AT may be lower in patients with heart failure (Sullivan *et al*. 1989), highlighting the role for this parameter in cardiorespiratory health.

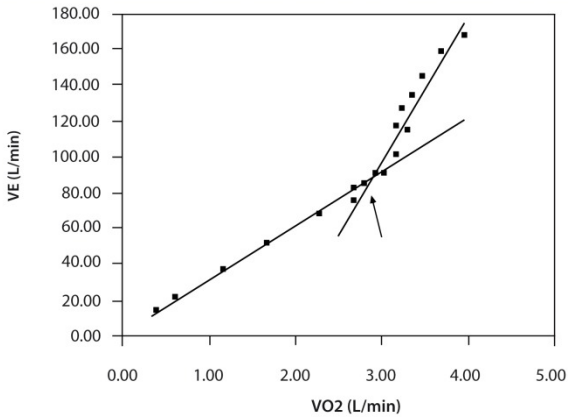


Figure 4. Determination of anaerobic threshold (AT) from a CPET. AT is indicated by the arrow. Image from Ghosh (2004).

The respiratory exchange ratio (RER) indicates CO_2 expiration and O_2 inspiration during a breath. RER is defined as the ratio between VCO_2 and VO_2, which is obtained from from ventilatory expired gas analysis, and can be used to determine sufficient subject effort during a CPET. At high exercise intensities, lactic acid buffering leads to increased VCO_2 output, increasing at a higher rate than VO_2, the denominator. RER therefore is a useful indicator of cardiovascular function, detecting the threshold for anaerobic metabolism without the need for blood sampling procedures to measure blood lactate or arterial blood bicarbonate or pH. A peak RER of >1.10 is considered as excellent subject effort during CPET (Balady *et al.* 2010), indicating a significant physiological demand and sufficient effort exerted by the subject.

1.2.1.5 Heterogeneity in cardiorespiratory responses to exercise training

VO_2max sets a theoretical upper limit to aerobic performance and is often used to assess adaptation to exercise training, however responses to a structured EET programme vary considerably. For example, Timmons *et al.* (2005) showed that "high responders" to EET improved peak VO_2 consumption (+0.71 ± 0.1 L·min^{-1}), whereas "low responders" showed no significant changes (+0.17 ± 0.1 L min^{-1}), determined by cycle ergometry. This VO_2max response phenotype has been shown to be independent of baseline VO_2max values, and may result from complex interactions between genetics, environment, and perhaps epigenetics; this complexity likely contributes to the heterogeneous exercise adaptation observed in VO_2max. The HERITAGE Family Study showed significant familial aggregation of VO_2max responses to EET, estimating the heritability for gains in VO_2max to be ~47% (Bouchard *et al.* 1999). Similarly, Timmons *et al.* (2010) used RNA expression profiling to produce a molecular classifier to predict the hereditary response in VO_2max from a structured EET programme. They discovered a 29-RNA expression signature to predict the heterogeneous VO_2max training response. From these results, it was shown that 11 single nucleotide polymorphisms (SNPs; i.e. variations in DNA) explained 23% of the variance in VO_2max changes, corresponding to ~50% of the variation in VO_2max responses to training. Overall, it was concluded that ~30 genes could be used to predict VO_2max responses to endurance training, from transcriptome analysis of the muscle.

Variance in VO_2max responses is complex, and it has been shown that heterogeneous VO_2max changes with exercise are not necessarily correlated with other physiological and metabolic responses, particularly in "low responders" (Vollaard *et al*. 2009). For example, Vollaard *et al*. showed that variance in VO_2max responses were related to maximal parameters (VE/VO_2max), accounting for 64% of the variance, whereas variance in aerobic performance responses to training were more related with submaximal parameters. Training-induced changes in VO_2max may therefore have a complex relationship with aerobic performance, with each physiological parameter associated with distinct physiological and biochemical endpoints. As highlighted throughout this section (1.2.1), VO_2max responses to exercise training will be dependent on the particular limitations imposed on an individual. This will contribute to the inter-individual variation in response to a standardised training programme.

1.2.2 Muscle mass, structure and function

Muscular strength is important for many aspects of life such as physical function and mobility for every day living as well as for athletic performance; loss of muscular strength (dynapenia) is associated with the pathology of age-related muscle wasting (sarcopenia), both of which have deleterious consequences for an ageing population (reviewed in Mitchell *et al.* 2012).

Skeletal muscle has immense plasticity and as such responds and adapts to changes (acute and chronic) in locomotory and metabolic demands, such as those imposed by exercise. The nature of the activities performed (i.e. stimulus intensity and duration) (Mangine *et al.* 2015), and the genetic foundation of the individual (Raue *et al.* 2012) will shape adaptive responses in skeletal muscle. For example, EET and RET result in similar (e.g. vascular remodelling) as well as unique skeletal muscle adaptations (e.g. muscle fibre hypertrophy).

However, it is well known that RET is the primary stimulus (with adjuvant adequate nutrition) involved in skeletal muscle hypertrophy and strength improvements, with EET having a minor effect on these outcomes (Atherton *et al.* 2005).

Trainable characteristics of musculoskeletal fitness include muscular strength, power, hypertrophy, and local muscular endurance (ACSM 2009), all of which can be enhanced, *on average*, by RET. Additionally, other measures of motor performance may also be enhanced by RET, such as speed, agility, balance, and coordination (ACSM 2009). RET increases motor unit recruitment capacity, and motor unit firing rate, along with increased muscle hypertrophy (Cadore *et al.* 2014). These neuromuscular adaptations lead to improved muscle strength and power development. RET has is also known to reduce the time to peak force and to increase the rate of force development (Hakkinen *et al.* 1994).

Many factors determine muscle strength in an individual. Muscle fibre cross sectional area (CSA) is positively associated with maximal force production, and the arrangement of fibres according to the angle of pennation, muscle length, joint angle, and contraction velocity all contribute to the muscle strength phenotype (described in ACSM 2009). Additionally, force generation is dependent on motor unit activation. Muscle strength has been shown to significantly increase after just one week of RET (Coburn *et al.* 2006), with long-term improvements possibly due to enhanced neural function, increased muscle CSA, altered muscle architecture, and perhaps tolerance to increased metabolites such as H^+. Globally, the magnitude of strength improvements with RET will be dependent on the type of training programme used (e.g. the muscle actions, intensity, volume, exercise order, rest time, frequency, etc.); however, regardless of these variables and as will be explored in this work, 'responder status' of the individual likely also has a significant role in determining strength and hypertrophic adaptation in response to RET.

1.2.2.1 Muscle strength

One repetition maximum (1-RM) tests are widely used predictors of maximal muscle strength, specific to the motion performed. Determined as the maximal external load that can taken through one complete (most commonly concentric and eccentric phase) repetition, true 1-RM assessments are challenging to perform (limited attempts to ascertain 1-RM before fatigue becomes a limiting factor) and may not be suitable for all (e.g. frail elderly). 1-RM can be predicted from submaximal repetitions (e.g. 5-RM, 10-RM; Taylor and Fletcher 2012), however, the degree of accuracy will vary dependent on the subject's RET experience and muscular composition. RET is often prescribed (as in this study) as a percentage of 1-RM (measured or predicted). Moderate to high training intensities (65–85% 1-RM) result in greater improvements in maximal strength compared with lower intensities (Steib *et al.* 2010).

A second commonly utilised measure of muscle strength (in a research setting) is that of maximal voluntary contraction (MVC) performed on an isokinetic dynamometer. MVC is a measure of strength, defined as the maximal exertion of force that can be applied around a joint. MVC may be reported as an absolute force (e.g. Newtons) or a moment (e.g. Newton-metres). MVC is a static measure of isometric muscle contraction, usually by a limb, whereby the dynamometer is used to assess (for example, knee extension) contractions at different muscle tendon unit (MTU) lengths. As reduced MTU capacity (e.g. with ageing) has

been shown to be indicated by lower muscle strength and lower tendon stiffness (Karamanidis and Arampatzis 2005), and isometric MVC can be used to assess the properties of MTUs.

High-intensity weight training leads to improvements in muscle strength, indicated by both 1-RM and MVC, after only 4 weeks of training, in a load- (MVC only) and time course-dependent manner (Laidlaw *et al.* 1999). Furthermore, there is a linear relationship between improvements in 1-RM and MVC. Contrary to RET, EET does not appear to improve muscle strength, or have a beneficial effect on MTUs, as runners and non-active individuals show similar MTU properties (Karamanidis and Arampatzis 2005).

1.2.2.2 Muscle mass

Increases in muscle mass (hypertrophy), the primary physiological adaptation to RET, are a result of increased muscle anabolism. Anabolism is defined as the synthesis of complex molecules from the storage of energy, such as muscle protein from constituent amino acids (AAs) (Reidy and Rasmussen 2016). Currently, the most effective and safe way to maintain or increase muscle mass is through RET with sufficient dietary protein (Parise and Yarasheski 2000), specifically EAA provision (with vast evidence for the role of Leucine) (Wilkinson *et al.* 2013). Anabolism can vary in its AA requirement, and healthy adults need AAs to replace those lost during protein turnover and in tissue growth. Major sources of body protein exist in the blood plasma, visceral tissues and skeletal muscle; however, this nutrient cannot be stored in the body as reserves. The protein content of skeletal muscle, which is ~65% of the body's total protein, can increase with resistance training, due to a prolonged and amplified window of postprandial MPS (normally ~90 minutes). However, despite the enhancement of MPS with RET, a "muscle-full" set point still exists, in which MPS rates return to baseline despite continued availability (plasma) of AA and continuation of amplified anabolic signalling responses (Atherton *et al.* 2010). There is however suggestion that after a single bout of exercise, the "muscle-full" response is delayed for ~24 h (Atherton and Smith 2012), therefore this mechanism may underlie favourable adaptations to chronic exercise, leading to muscle hypertrophy over time.

Rates of MPS depend on numerous factors, such as activity levels, nutrient availability, and health status. Skeletal muscle proteins are turned over at a rate of ~1.2% day^{-1} and exist in dynamic equilibrium: in the fasted state, muscle protein breakdown (MPB) predominates over MPS, whereas MPS predominates over MPB in the fed state (Phillips *et al.* 2012a). Furthermore, MPS signalling may be altered in an anabolic or catabolic state, which may respectively increase or reduce the MPS response in amplification or duration (Figure 5). After RET, MPS transiently increases and chronic exercise-induced increases in MPS likely drive (hypertrophic) adaptation to RET. Muscle hypertrophy can be observed within weeks of exercise beginning, with recent work from Brook *et al.* (2015) demonstrating the most demonstrable gains in the first 3 weeks of RET, attributable to hypertrophic remodelling. Conversely, the same premise can be applied when assessing losses in lean muscle mass, where malnutrition (inadequate protein intake) and/or muscle disuse result in a situation of negative net protein balance (MPB>MPS) and subsequent losses of muscle mass (Phillips *et al.* 2012a).

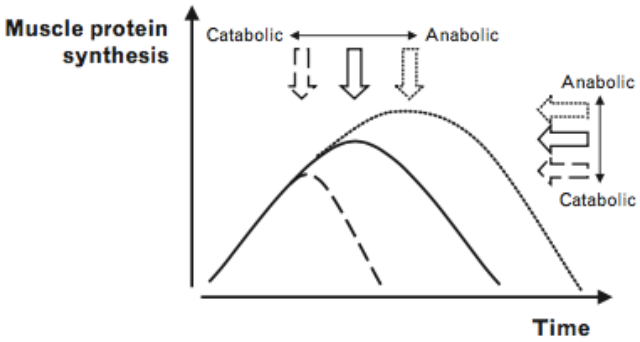


Figure 5. Schematic of muscle protein synthesis (MPS). Arrows indicate the "muscle-full" set point. When this set-point shifts in an anabolic (muscle-building) or catabolic (muscle-wasting) state, this results in an increased or decreased MPS, respectively, as indicated by the dotted and dashed curves, which are altered with respect to peak and duration of MPS. Image from Phillips et al. (2012a).

As outlined above, muscle hypertrophy is considered to be the product of nutrition and exercise-induced MPS (Moore *et al.* 2011). Muscle hypertrophy is the most recognised adaptation to RET, and is associated with corresponding activation of muscle satellite cells (muscle stem cells) for growth (Blaauw and Reggiani 2014) and the stimulation of angiogenesis (Phillips *et al.* 2013) along with this fibre growth, as well as sufficient remodelling of the extracellular matrix. Therefore, the muscle hypertrophic response to RET integrates these physiological mechanisms of tissue remodelling to produce the resultant muscle phenotype.

RET induces muscle hypertrophy through mechanical, metabolic, and hormonal mechanisms. Hypertrophy leads to proportionate increases in contractile proteins (actin and myosin). RET has also been shown to increase the myosin heavy chain and mixed-fraction (myofibrillar and mitochondrial) MPS rates (Hasten *et al.* 2000). Mechanical loading transduces a series of intracellular events that leads to the regulation of gene expression and protein synthesis (Martineau and Gardiner 2001). Indeed, RET is implied to affect the activity of ~70 genes, upregulate myogenic factors (e.g. myogenin, MyoD), and downregulate inhibitory growth factors (e.g. myostatin). Other factors, such as fibre type shifts, metabolome changes and endocrine signalling (testosterone, growth hormone, cortisol, insulin, and insulin-like growth factor I) also play a role in the magnitude of hypertrophic responses to RET (Velloso 2008).

The hypertrophic response to RET will combine mechanical and metabolic stimuli to produce relevant adaptations. Hypertrophic and neural adaptations are required in synergy for the expression of strength gains from RET. In untrained individuals, neural adaptations occur in the early stages of training. Similarly, muscle hypertrophy is observable within the first 6 weeks, with changes in protein quality and MPS also occurring at early stages (Brook *et al.* 2015). Once this initial adaptation has taken place, less muscle mass is recruited during training to overcome the forces of resistance. This leads to reduced adaptive improvements in strength; thus progressive overloading is required for continuity in strength improvements from RET.

Muscle fibre hypertrophy is considered as the main cause of increased muscle mass from RET, and it is typically greater for fast-twitch than slow-twitch fibres (Tesch and Karlsson 1985). It has been proposed that there is an optimum or maximum degree of hypertrophy for individual fibres, from which further increases in size can only be obtained by

hyperplasia (Antonio and Gonyea 1993); however, this notion has not been verified in human studies.

1.2.2.2.1 Assessment methods of muscle hypertrophy

Changes in whole-body muscle volume (i.e. muscle hypertrophy), reflecting anabolism (tissue building) or catabolism (tissue breakdown), can be measured using numerous techniques including: dual-energy x-ray absorptiometry (DXA), ultrasound, magnetic resonance imaging (MRI), or computerised tomography (CT) scans (Wilkinson *et al.* 2014). All of these techniques have their own strengths and limitations, both methodological (e.g. inter-operator variability for ultrasound) and practical (e.g. cost and access issues for MRI). Commonly regarded as the 'gold-standard' for localised hypertrophy in response to RET, anatomical CSA can be assessed by MRI; however, regional differences have been reported in RET-induced changes when comparing the mid and distal portions of the m. vastus lateralis (Franchi *et al.* 2014).

1.2.2.2.2 Heterogeneity in hypertrophic responses to exercise training

The degree of hypertrophy from an exercise training programme may be attributed to factors such as the intensity and volume of the mechanical stimulus, the type of contraction performed, and nutritional availability, which will interact with the genotype to give the phenotypic response to RET (Phillips *et al.* 2013). However, significant heterogeneity has been observed in muscle CSA responses to RET, whereby responses varied from -1.2 to +10.4 cm^2 (Raue *et al.* 2012). Furthermore, these results were irrespective of gender, age, pre-existing muscle mass, physical activity levels, or dietary habits.

Enhanced MPS and ensuing hypertrophy in response to exercise training is dependent on workload and intensity. For example, resistance exercise at intensities above 60% of the 1-RM leads to 2–3-fold increases in MPS (Kumar *et al.* 2009), with suggestion of further enhancement in MPS at intensities higher than this. Indeed, Kumar *et al.* showed that when the MPS responses after a single bout of exercise at 90% 1-RM to failure, 30% 1-RM to failure and 30% 1-RM work-matched to 90% 1-RM to failure were compared, 30% 1-RM to failure appeared to be most effective at inducing anabolic responses.

Mixed MPS is stimulated by both EET and RET, however, the differential training adaptations of individual proteins within this collective protein pool in response to exercise is incompletely elucidated. Exercise leads to mechanotransduction and associated complex physical and chemical sensory responses in muscle. These signalling cascades predominantly lead to acute post-translational protein turnover via phosphorylation, and changes in gene expression. Mixed MPS responses occurs with both EET and RET; however, EET does not lead to the same hypertrophic responses as seen with RET. This is due to the subfractional protein responses in muscle to the different training modes. For example, RET leads to increased myofibrillar not mitochondrial protein synthesis, whereas EET leads to increased mitochondrial not myofibrillar protein synthesis (Wilkinson *et al.* 2008). These differences are likely to be responsible for the specific phenotypic responses of the two exercise modes, i.e. hypertrophy with RET and mitochondrial biogenesis with EET.

1.2.3 Muscle architecture

The structural arrangement of the muscle is determined by its anatomical architecture. In turn, these qualities contribute to the function and biomechanics of the MTU that determine force production and velocity. Mechanical stimuli, such as exercise training, are able to modify these structural and consequently functional adaptations, in both healthy individuals as well as in physiopathology or aging (Narici *et al.* 2016).

It is known that muscle hypertrophy induces a greater fibre pennation angle as shown in the human triceps (Kawakami *et al.* 1993). Heightened muscle fibre pennation allows for the physiological muscle CSA to greatly exceed the anatomical muscle CSA, through the addition of muscle fibres in parallel (Narici *et al.* 2016). Force production capacity of the muscle, which is proportional to the total physiological CSA, thus increases with increasing pennation angle (Narici *et al.* 1992). In contrast, the contractile force exerted onto the aponeurosis or tendon will decrease with increasing pennation angle. Pennate muscles can therefore exert greater contractile forces than non-pennate muscles. Greater anatomical CSAs and greater pennation angles have been reported in bodybuilders compared with age-matched sedentary subjects, indicating that there is a relationship between muscle fibre pennation and muscle hypertrophy (Kawakami *et al.* 1993). In contrast, muscle fascicle length (Lf), another architectural parameter altered by RET, is more strongly associated with the addition of sarcomeres in series.

RET involves a combination of the lifting (concentric contraction; CC), and lowering (eccentric contraction; EC) phases of an action, which in turn reflect muscle shortening, and lengthening, respectively. When comparing isolated CC and EC RET, Lf is reported to be increased with EC RET, whereas pennation angle (PA) predominantly increased with in CC RET (Franchi *et al.* 2014). Conversely, with CC RET, PA is increased and Lf is unchanged. The training stimulus may also influence the result of muscle lengthening. For example, EC RET produces an increase in muscle length and thus likely an increase in serial sarcomeres (Reeves *et al.* 2009), whereas CC RET results in muscle shortening due to the bundling of more contractile units along the tendon aponeurosis (Kawakami *et al.* 1993). Furthermore, regional differences in muscle hypertrophy with exercise training may be due to the differential addition of sarcomeres in series and in parallel. These different architectural adaptations could be due to differences in muscle 'damage' induced by the type of contraction. Intramuscular signalling proteins use mechanotransduction to relay architectural responses leading to exercise-specific architectural adaption in skeletal muscle and different contraction modes may trigger different signalling pathways (Martineau and Gardiner 2001).

1.2.3.1 Assessment methods of muscle architecture

Muscle architecture can be assessed *in vivo* via ultrasound imaging, both at rest and during passive and active motions. Skeletal muscle ultrasound imaging is a reliable, non-invasive technique that can be used in real-time (Franchi *et al.* 2014). Fibre length, pennation angle and muscle thickness can all be measured via ultrasound (in addition to subsequent image analysis) and are easy to image in the *m. vastus lateralis* (VL), due to the superficiality of the muscle and the structural clarity of the VL. Compared to the other muscle heads of the quadriceps, the VL has the most uniform architecture (Blazevich *et al.* 2006). Architecture is similar between the three Vasti of the quadriceps muscle group, with only the *m. rectus*

femoris differing in muscle CSA, thickness, and pennation angle (Ema *et al.* 2013). Therefore, VL architecture can be considered representative of the majority of the quadriceps.

1.3 Exercise for health

Exercise training is generally a well tolerated low-cost method of improving numerous aspects of health (Green *et al.* 2008), although not all of the mechanisms of how exercise adaptations improve health have been fully elucidated, nor indeed how these adaptations are associated with each other. It is however, well accepted that a number of physiological effects of chronic exercise training, such as alterations in cardiovascular parameters or changes in skeletal muscle metabolism, provide direct health benefit, such as reduced blood pressure and improved insulin sensitivity, for example.

Furthermore, numerous clinical conditions have been associated with physical inactivity (Slentz *et al.* 2005), including the metabolic syndrome, for example. The metabolic syndrome is often referred to as a prelude to type II diabetes mellitus (T2DM), and is characterised by abdominal obesity, atherogenic dyslipidaemia, hypertension, insulin resistance with or without glucose intolerance, as well as a proinflammatory and prothrombotic state (Grundy *et al.* 2004). Metabolic syndrome significantly contributes to an increase in cardiovascular disease risk (Grundy *et al.* 2004), and is associated with increased body fat and sedentary behaviour. In addition to reducing metabolic syndrome and associated cardiovascular disease risk, exercise training often results in favourable body composition adaptations, such as reduced visceral body fat which will also play a role in improved whole-body metabolic status (Miyazaki *et al.* 2002). Indeed, the Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) protocol was developed specifically to target these areas for investigation (Kraus *et al.* 2001). Different exercise doses and intensities were investigated to determine skeletal muscle metabolic changes in relation to health improvements from exercise training. STRRIDE determined that cardiovascular risk parameters were reduced primarily through improvements in skeletal muscle metabolic health from exercise.

As a whole, RET has many benefits on health, and contributes to exercise-induced improvements in cardiovascular function, reduces risk factors of CHD and T2DM, may reduce risk of certain cancers, prevents osteoporosis, promotes weight loss and maintenance, improves dynamic stability and preserves functional capacity, and contributes to psychological well-being (ACSM 2009).

1.3.1 Metabolic health

Metabolism is defined as “the sum of the physical and chemical processes in an organism by which its material substance is produced, maintained and destroyed, and by which energy is made available.” In the context of human health, much emphasis is placed on the metabolism of the three major macronutrients: fats, protein and carbohydrate. The importance of protein metabolism (in skeletal muscle), and its relevance to exercise adaptation (primarily RET) has already been discussed in the context of hypertrophy. Conversely fat metabolism (oxidation) is predominantly enhanced with EET. It is however, carbohydrate metabolism that is most commonly associated or assessed in relation to “health status”.

Carbohydrate metabolism entails the various mechanisms in the human body holds in place to control blood sugar levels and store excess energy as glycogen and fat. Carbohydrates are a short term fuel for the body, and aerobic respiration involves the metabolism of glucose and oxygen in cells to release energy. Blood glucose levels are controlled by the central metabolic hormone insulin. Insulin release (e.g. stimulated by feeding) causes cells to store glucose internally as glycogen, as well as having other effects such as lipid and amino acid uptake, and controls electrolyte balance in cells. Absence of insulin (e.g. during fasting) reduces glucose uptake into cells, causes glycogen breakdown (in the liver), causes release of lipids from adipose cells, and reverses electrolyte adjustments. Blood glucose is the major signal to activate insulin production by pancreatic beta-cells. Therefore, diet controls many aspects of carbohydrate metabolism as the major determinant of blood glucose is from ingested carbohydrate. Notably, the hormone glucagon has opposing actions to insulin, releasing glucose from glycogen (in the liver), as well as other hormones such as growth hormone, cortisol, and catecholamines (Koeslag *et al.* 2003; Sandoval and D'Alessio 2015).

One clinical condition associated with dysregulated glycaemic and insulinaemic responses is diabetes mellitus (DM). DM is a group of metabolic diseases characterised by high blood sugar levels over a prolonged period (WHO 2016). Type 2 diabetes mellitus (T2DM), or "adult-onset diabetes" can be acquired and is caused by insulin resistance, whereby cells fail to respond to insulin properly or may reduce insulin production. This is often associated with excess body weight and physical inactivity (WHO 2016). Pre-diabetes is a clinical term used to describe individuals at risk for developing T2DM, with respect to insulin secretion and resistance patterns causing hyperglycaemia. Pre-diabetes is heterogeneous and three distinct phenotypes exist: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both (IFG + IGT) (Malin *et al.* 2016), indicating that there are multiple pathophysiological mechanisms leading to the T2D phenotype.

Positive associations exist between changes in the acute insulin response to glucose and reductions in body weight, waist circumference, plasma TGs, and total cholesterol (Boule *et al.* 2005). These associations offer some insight as to why structured regular exercise has been shown to improve insulin sensitivity, however, the optimal dose of exercise to improve insulin sensitivity has not been elucidated, nor have all of the mechanisms underlying the development, and exercise-induced improvement in the T2DM phenotype.

1.3.1.1 Assessment methods of metabolic health

Traditionally, an oral glucose tolerance test (OGTT) has been used to diagnose impaired glucose tolerance with the shape of the plasma glucose curve during an OGTT indicative of insulin sensitivity and therefore risk of developing T2DM (Bervoets *et al.* 2015). An OGTT performed over 2 h, can be used to assess a subject's insulin sensitively and β-cell function (Tschritter *et al.* 2002) by obtaining values of plasma glucose and insulin concentrations at set intervals over the OGTT. Plasma glucose area under the curve (AUC) is a continuous measure of glucose tolerance, which can be correlated with the plasma insulin AUC during an OGTT, as well as age, BMI, waist-to-hip ratio (WHR), and HbA_{1c} (Tschritter *et al.* 2002).

1.3.1.2 Heterogeneity in glycaemic and insulinaemic responses to exercise training

As with VO_2max and hypertrophic responses to exercise training, Boule *et al.* (2005) showed that there is great inter-individual variability in insulinaemic and glycaemic responses to

exercise training, and changes in insulin sensitivity are unrelated to the participant's initial body mass, age, or pre-existing physical fitness levels. For example, in response to EET, ~30% of subjects show no improvement in insulin sensitivity (Boule *et al.* 2005; Timmons *et al.* 2005); further highlighting the necessity to understand these 'negative' responses to allow for the provision of exercise prescription in these individuals.

1.3.2 Vascular health

1.3.2.1 Haemodynamic parameters

The vascular endothelium, the lining of the body's circulatory system, has a number of physiological functions outwith its role in the development of new blood vessels (angiogenesis). These roles include: the regulation of smooth muscle tone, control of thrombosis, inhibition of leukocyte and platelet cell adhesion, and promotion of intra-arterial permeability (Harris *et al.* 2010). Vascular endothelial dysfunction (ED) is a condition characterised by an alteration in the physiological balance between endothelial vasodilation and vasoconstriction; ED represents an initial step towards hypertension and cardiovascular disease, and can thus be measured as a vascular health parameter (Harris *et al.* 2010). For example, with regard to atherosclerosis, ED is described as the primary aetiology (Verma *et al.* 2003), and is the earliest identifiable event in the process of atherosclerotic CVD, the leading cause of mortality in the United States (Ross 1999). ED can also be observed in several conditions associated with CVD risk, such as DM, insulin resistance, hypertension, hypercholesterolaemia, and smoking (Franzini *et al.* 2012).

Blood pressure (BP) can be defined as the force that circulating blood exerts on the walls of the blood vessels. Blood pressure is decreased as the blood moves away from the heart, and branches off into smaller vessels to supply blood to the tissues for the delivery of O_2 and nutrients and the extraction of waste products (e.g. CO_2). Systolic blood pressure (SBP) is the peak arterial pressure at the beginning of a cardiac cycle; diastolic blood pressure (DBP) is the blood pressure at the resting phase of the cardiac cycle. Blood pressure is usually expressed in units of mmHg and as values of SBP/DBP. Recommended values for a healthy adult 120/80 mm Hg, with clinical hypertension defined as a resting SBP of >140 mmHg and/or DBP of >90 mmHg. Mean arterial pressure (MAP), a parameter encompassing SBP and DBP, is often used to represent blood pressure and is calculated as:

MAP = 1/3 SBP + 2/3 DBP,

with the equation roughly representative of the relative periods of the cardiac cycle spent in systole (1/3) and diastole (2/3).

Hypertension is a significant modifiable risk factor for cardiovascular disease, such as coronary heart disease, stroke, and heart failure (Heidenreich *et al.* 2013), thus blood pressure is an important parameter of vascular health and subsequent disease risk.

In a systematic review and meta-analysis on the role of chronic exercise on changes in BP, Cornelissen and Smart (2013) showed that adult resting BP (SBP and DBP) was lowered by endurance, dynamic resistance, conventional resistance, and isometric resistance training. Most pronounced reductions in BP resulted from dynamic endurance training and were found in males and hypertensives, although significant reductions were still found in

participants with normal BP and prehypertension. Resting heart rate (HR) is also altered by chronic exercise training. Describing the frequency of the cardiac cycle, in a unit of beats per minute (bpm), resting HR is 60–80 bpm for a healthy adult. Resting HR has been shown to be positively affected (reduced) by EET (D'Souza *et al.* 2014) and RET (Poelkens *et al*. 2007).

The distensibility of the arterial tree, or arterial stiffness, impacts on cardiac performance, perfusion, and homeostasis. Arterial stiffness is associated with adverse cardiovascular events (Boutouyrie *et al.* 2002) and as such is another cardiovascular parameter indicative of health. A decline in vascular function, such as that seen with aging, may be represented as a reduction in peripheral (limb) blood flow, at rest and in response to exercise and feeding (vasodilatory stimuli). Doppler ultrasound can be used to non-invasively measure leg blood flow (LBF) (Phillips *et al.* 2012b). Phillips *et al.* reported age-related declines in LBF in response feeding and exercise that were alleviated by chronic RET. RET also improved 'baseline' LBF in all age groups. Similarly, EET has been shown to be associated with improved LBF with masters athletes demonstrating significantly higher LBF values than age-matched controls (DeVan and Seals 2012).

1.3.2.2 Lipid parameters

Lipids encompass triglycerides ("fats"), fatty acids and their derivatives, and sterol-containing metabolites such as cholesterol. Triglyceride (glycerol + 3 fatty acids) is the main constituent of body fat in humans and animals, and is present in the blood to allow the bi-directional transference of adipose and blood glucose from the liver (Gong *et al.* 2015). During lipolysis, triglycerides are broken down (monoacylglycerol + free fatty acids) and are absorbed in to the enterocytes lining the intestines. Here, they are packaged with cholesterol and proteins to form chylomicrons. Various tissues capture the chylomicrons to use triglycerides as an energy source, and subsequent triglyceride breakdown signalled by glucagon (Stryer and Lubert 1995). For the brain, which cannot use fatty acids for energy, the glycerol component of triglycerides is instead converted to glucose. In humans, high triglycerides levels in the bloodstream are linked to the pathogenesis of atherosclerosis, as well as heart disease and stroke (Talayero and Sacks 2011).

The lipid profile of an individual is related to the risk of coronary artery disease (CAD) (Gordon *et al.* 2014). Cholesterol is the most widely known derived lipid and exists only in animal tissue. Cholesterol can be obtained through the diet (exogenous cholesterol) or be made through cellular synthesis (endogenous cholesterol). More endogenous cholesterol forms with a diet rich in saturated fatty acids, facilitating cholesterol synthesis in the liver (70%), however, cholesterol synthesis also occurs in the arteries and intestines (Jackson *et al.* 1990). Low-density lipoprotein cholesterol (LDL-C) is the primary lipid carrier of cholesterol on the body, thus total cholesterol (TC) and LDL-C are causally linked, and a reduction in LDL causes a consequent reduction in blood TC (The Lipid Research Clinics Coronary Primary Prevention Trial results 1984). Lowering LDL-C is therefore the primary approach to blood lipid and lipoprotein control. In contrast, high density lipoprotein cholesterol (HDL-C), the "good" cholesterol, is a positive lipid marker in the blood. HDL-C content of the blood is also a good indicator of CAD risk, and increased CAD risk is associated with increased fasting LDL-C and non-HDL-C (including intermediate density lipoproteins [IDL], very low density lipoproteins [VLDL], and lipoprotein A) (Duff and McMillan 1951). Furthermore, elevated blood lipid and lipoprotein levels are known to be

involved in the pathogenesis of metabolic syndrome, insulin resistance, T2DM, and sarcopenia.

High HDL-C levels are observed in active individuals as compared to their sedentary counterparts. HDL particles have been shown to have a beneficial role in glucose metabolism, as reviewed in Drew *et al.* (2012). It is known that low plasma HDL is a risk factor for atherosclerosis, however, recent evidence suggests that low HDL may also be involved in the pathophysiology of T2DM. For example, the TG:HDL-C ratio has been considered to be an early indicator of insulin resistance in obese youths (Giannini *et al.* 2011). Conversely, elevated HDL-C is thought to reduce blood glucose levels, increasing glucose uptake in skeletal muscle, through the activation of the AMP-activated protein kinase (AMPK) signalling pathway and stimulation of pancreatic beta-cell insulin secretion (Drew *et al.* 2012).

Both EET and RET are implicated in positively affecting blood lipids and lipoprotein profiles. Recently, Gordon *et al.* (2014) reviewed the roles of EET and RET on lipid profiles, particularly in relation to CAD risk factors, such as postprandial lipidaemia and metabolic syndrome. Regular EET plus abstinence from smoking significantly increases HDL-C, decreases LDL-C, and favourably improves the HDL:LDL ratio. EET produces decreases in TC, LDL-C and TG, and increases HDL-C (Halbert et al. 1999). Furthermore, fatty acids are a major energy source during EET and high HDL-C levels are associated with increased postprandial TG clearance (Patsch *et al.* 1987). The lipid profile in response to RET may be improved (lower LDL, higher HDL; Gordon *et al.* 2014), however, training programmes less than 12 weeks may not elicit a significant change (Goldberg *et al.* 2012).

1.3.2.3 Inflammatory parameters

Inflammation is implicated in metabolic health with high serum levels of inflammatory factors released by adipose tissue, such as IL-6, reported to predict disability and sarcopenia, independently of other known risk factors (Gallucci *et al.* 2007). There appears to exist a strong inverse relationship between physical activity levels and serum inflammatory markers, such as IL-6 and C-reactive protein (CRP). IL-6 is a circulating cytokine, which is released from adipose tissue and skeletal muscle; IL-6 is produced by macrophages and adipocytes in response to acute and chronic inflammatory conditions. The most commonly used marker of global inflammation is CRP, which is produced in the liver mainly in response to IL-6 (Heinrich *et al.* 1990) as well as other response factors released by macrophages and adipocytes. CRP activates the compliment system, which promotes phagocytosis by macrophages. CRP is thus an acute phase protein, and levels in blood plasma rise in response to inflammation.

Although contentious literature does exist, exercise may positively affect inflammatory status. For example, IL-6 has been shown to be significantly reduced after 12 weeks of moderate EET (Thompson *et al.* 2010). Similarly, RET was also shown to reduce markers of inflammation (Garber *et al.* 2011). Upon assessing the relationship between different adaptations to exercise training, waist-to-hip ratio has been shown to positively correlate with IL-6 levels, and IL-6 is positively correlated with total cholesterol. Indeed, 16 weeks of RET has been shown to decrease TC, LDL-C, waist circumference, and waist-to-hip ratio, as well as lower IL-6 compared with a non-exercise control group (Nunes *et al.* 2016).

1.3.3 Body composition

Body composition, such as fat/muscle ratios and quantities, are key to physical performance and health implications. Overweight individuals are at an increased risk of cardiovascular diseases, T2DM, and other health complications such as osteoporosis and hormonal imbalances (Klein *et al.* 2004). Certain phases across the lifecourse, such as ageing and puberty, are associated with body composition changes that are almost inevitable. Outwith these phases significant (negative) alterations in body composition are key in the pathogenesis of obesity and associated comorbidities.

The percentage body fat (%BF) of the population varies greatly. For example, %BF is lower in trained athletes than that of the general population. The average %BF in the US of 20–29-year olds is 15.9% for men and 22.1% for women (ASCM 2000), whereas competitive runners typically have values of 4–10% for men and 7–15% for women (Boileau and Horswill 2000), also exemplifying the gender-related difference in body composition. In general, exercise leads to increases in muscle mass and reductions in %BF, shown with both RET (Nunes *et al.* 2016) and EET (Slentz *et al.* 2005).

Location of excess fat is also an important factor in the pathogenesis of many conditions associated with increased %BF. Visceral adiposity is considered to link central obesity with disease risk (Miyazaki *et al.* 2002). For example, visceral fat, but not subcutaneous fat, increases with age in both men and women (Ferrannini *et al.* 2008), combined with the physical inactivity of aging it is this fat deposition that is therefore likely to increase cardiometabolic risk associated with advancing age. However, the exact mechanisms by which fat is preferentially stored in the viscera is not known. Sedentary overweight adults subject to a short duration (6 months) of continued physical inactivity have been shown to significantly increase visceral abdominal fat (Slentz *et al.* 2005). However, for the same study, in age- and body composition matched subjects, low intensity exercise was adequate to prevent this increase in visceral fat. Furthermore, a high amount of exercise was able to produce significant reductions in both visceral and subcutaneous fat. This highlights the detrimental effect of physical inactivity and the effectiveness of exercise-based lifestyle interventions, as well as a dose-responsiveness, to ameliorating body composition-related disease risk. Additionally, the high amount of exercise group also showed improvements in lipids and lipoproteins, insulin sensitivity, and %BF, emphasising the potential role of fat loss in ameliorating metabolic disease risk.

1.3.3.1 Assessment methods of body composition

There are many methods to assess body composition, which vary in sensitivity, cost and accessibility. Body fat percentage can be estimated by manual callipers, densitometry (hydrostatic/underwater weighing), bioelectrical impedance analysis and dual-energy X-ray absorptiometry (DXA). DXA is currently the gold-standard for measuring whole-body composition, providing estimates of percentage body fat, lean muscle mass, and bone mineral density. Regional analyses by DXA can isolate areas of interest, such as the trunk for abdominal adiposity or a specific limb in response to an intervention or injury. This method can be used to determine changes in body composition in response to interventions (e.g. diet and exercise), as well as assessing the effects of chronic disease on body composition,

such as bone mineral density and muscle wasting in osteoporosis and sarcopenia, respectively (reviewed in Andreoli *et al.* 2009).

1.4 "Exercise resistance"

1.4.1 Heterogeneity in exercise training responses

As highlighted in section 1.2, physiological adaptations to exercise in humans are highly heterogeneous in nature and the expected responses to EET (aerobic capacity) and RET (muscle hypertrophy and strength) are not always achieved to the same degree between individuals. Mechanistic differences in capacity to respond to exercise training are difficult to elucidate, as they are determined by complex gene-environment interactions, and it is a challenge to determine exercise-induced changes from hereditary components of performance.

Adding to the complexity of exploring "exercise resistance" is the hypothesis that exercise adaptation is likely to be specific to the training mode investigated, as responses to both EET and RET have distinct physiological endpoints. For example, RET is associated with influencing networks involved in hypertrophic responses such as the phosphatidyl-inositol-3 kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTORc1) pathway in which mTORC1 regulates the efficiency of translation as well as translation capacity via ribosomal RNA production (Iadevaia *et al.* 2012). Conversely, EET is more commonly associated with activation of pathways regulating mitochondrial biogenesis, such as peroxisome proliferator-activated receptor gamma (PPARγ) or coactivator alpha (PGC-1α) signalling (Wenz *et al.* 2008).

Further complexity is added when it is considered that exercise responses can be highly pleiotropic in nature. For example, EET and RET can produce similar patterns of signalling in humans, such as protein signalling regulation of muscle growth (Camera *et al.* 2010). Molecular responses to training may overlap regardless of exercise mode, such as with cellular networks associated with satellite cell activation and cell differentiation. For example, Phillips *et al.* (2013) showed that All-*trans* retinoic acid (ATRA) and aryl hydrocarbon receptor (AhR) are activated with both RET and EET. ATRA is associated with myogenic cell differentiation and ECM remodelling, as well as angiogenesis, which relates to the vascular remodelling observed with both of these exercise training modes (Green *et al.* 1999; Laughlin and Roseguini 2008). AhR is a ligand-activated transcription factor, associated with tumour suppression and angiogenesis. Regulation of these two factors are therefore important for the appropriate tissue development and remodelling in response to both modes of chronic exercise training.

1.4.2 "Exercise resistance" and health

The proposition exists that the "exercise resistance" phenotype (i.e. low responder status) for expected primary physiological adaptations (VO_2max for EET and skeletal muscle hypertrophy for RET), is associated with reduced health improvements in response to exercise training (e.g. insulin resistance and adiposity). Essentially, if true, these individuals may not respond to exercise in the expected manner nor improve health status, potentially due to inabilities in their metabolism to adapt due to "metabolic inflexibility" (Galgani and

Ravussin 2008). In healthy populations, it is unclear of the precise mechanisms underlying responder status.

Gene expression changes with RET and EET are likely to be greater in high responders compared with low responders for the respective exercise mode (Raue *et al.* 2012). With respect to health, Lessard *et al.* (2013) developed an "exercise resistant" outbred rodent model by selectively breeding rats to be "high" and "low" responders to aerobic exercise training, whereby low responders failed to improve exercise capacity, and high responders improved by ~54%. Interestingly, in this model, low responders exhibited metabolic dysfunction, such as insulin resistance and increased adiposity and impaired exercise-induced angiogenesis in skeletal muscle. They did, however, have normal increases in mitochondrial capacity exercise training, implying that impaired vascular remodelling is at least partially responsible for exercise resistance. Additionally, the low responders had increased inflammatory signalling and altered TGF-β signalling.

As fitness and health benefits of exercise are undeniably linked via relevant tissue adaptations, such as the role of vascular remodelling in both VO_2max and BP, it is speculated that failed activation of key signalling pathways controlling metabolic endpoints (i.e. MPS and angiogenesis) underlies exercise resistance.

1.5 Study design & aims

Aim 1: To assess the heterogeneity of physiological adaptations to exercise training

This pilot study involved a 14-wk programme, which included 4 weeks of RET and 4 weeks of EET in a cross-over design, as shown in Figure 6 (section 1.6.2). A 6-week detraining period between training modes aimed to allow subjects to establish a new baseline for the subsequent training mode. The first goal was therefore to validate the use of this short-term (4-week per mode) exercise training programme for improving aerobic function with EET and hypertrophy with RET. It was also necessary to validate the detraining period, i.e. whether the "wash-out" period was sufficient in duration to abolish training-induced effects of the first training mode, so that the training modes could be independently assessed. Furthermore, as significant heterogeneity has been observed in response to both EET and RET in a variety of clinical populations (as described earlier in this chapter), we aimed to investigate heterogeneity in a cohort of young healthy males. For this pilot study, a sample size of 10 was chosen to demonstrate the specific physiological responses to the two training modes after 4 weeks of training, and to demonstrate heterogeneity in inter-individual responses in this cohort, with less emphasis on identifying statistically powered relationships. Thus, inter-individual variation in training responses for expected primary physiological adaptations (aerobic capacity with EET, and muscle strength and hypertrophy with RET) was investigated herein.

Aim 2: To assess the mode-specificity of responder status to exercise training

A novel aspect of this study was the investigation of both EET and RET responses in the same individuals. Using this model, we aimed to investigate whether, within each individual, if response magnitude to one mode of exercise training was associated with response magnitude to the alternative mode of exercise training (for the expected primary physiological adaptations). In essence, can high responders to one type of exercise training be low responders to the alternative mode? Similarly, we also asked the same question but

for expected health outcomes. Primarily, investigated herein were parameters of metabolic health, vascular health, and body composition.

Aim 3: To determine the relationship between "fitness" and health adaptations to exercise training

This aim was based around determining whether the magnitude of physiological "fitness"-based adaptations to exercise training (i.e. muscle hypertrophy, increased aerobic capacity) was related to the magnitude of health-based (i.e. lowered blood pressure, improved glycaemic control) adaptations within each individual. Does exercise make you healthier even if "fitness"-based adaptations are not being achieved? Does exercise resistance also apply to health outcomes? Is responder status for health improvements mode specific? For example, if "exercise resistance" is a truly global phenomenon (not mode or adaptation-type dependent) then all health and "fitness" outcomes may be unaffected/adversely affected by exercise training in low responders regardless of exercise mode. In contrast, if exercise resistance is specific to the training mode and/or a type of adaptation (health vs. "fitness", for example), then personalised exercise prescription would ensure some benefit of exercise training, simply based on adaption of the right mode for the individual in question.

1.6 Experimental methods

1.6.1 Subject recruitment

The study was approved by the University of Nottingham Medical School Ethics Committee. Participants were recruited via demographically (age and gender) targeted posted letters to those residing close to the study site (Appendix I) and subsequent word of mouth. Subjects were healthy (free from disease: diabetes, hypertension, asthma, heart disease, stroke, or epilepsy) and presented with habitually active lifestyles.

Seemingly suitable candidates were invited for a study screening session, comprising informed consent and a health check. Volunteers were provided with study information in writing (via an information sheet; Appendix II) prior to the screening session, as well as verbally during the screening session. Written informed consent was given at the screening session by the volunteer. The study overview can be visualised in Figure 6 (section 1.6.2).

The screening session included a blood test, ECG, BMI calculation (from height and weight measurements), blood pressure measurement, and heart rate monitoring. Specifically, subjects characterised as obese (BMI >30) and hypertensives (blood pressure >140/90 mmHg) were excluded from the study. Any current medications or allergies, as well as radiation exposures, were taken noted, and suitability for the study confirmed by a medically qualified fellow. Subjects specified their pre-existing activity levels, however, were excluded if they were currently involved a structured exercise training programme. Herein, 'structured exercise training' was defined as either strength or aerobic training >2 times per week, for the improvement of physical athleticism. Subject characteristics of the included participants are shown in Table 1.

The blood samples were checked for coagulation, full blood count, urea and electrolytes, liver function, thyroid function, and random glucose and insulin (12 ml in total); the samples were sent to the Pathology department at the Royal Derby Hospital for analysis. Blood and

ECG results were assessed by a medical fellow, prior to subjects being invited to enrol in the study.

Table 1. Subject characteristics for the participants included. Values are mean ± SEM. The exclusion criteria are shown in red.

	Participants included	Exclusion criteria
Age (years)	23.6 ± 1.61	>40
Height (m)	1.78 ± 0.03	
Body weight (kg)	78.1 ± 4.80	
BMI (kg/m^2)	24.5 ± 1.07	>30
Systolic BP (mmHg)	122.8 ± 2.90	>140
Diastolic BP (mmHg)	72.8 ± 2.26	>90
Resting HR (mmHg)	66.2 ± 3.31	

1.6.2 Exercise training

Subjects were randomised into one of two groups, consisting of a 4-wk period of endurance training (EET), a 6-wk wash-out period (no training), then a 4-wk resistance training (RET) period, or vice versa (RET before EET). Before each training session, subjects were asked to fast for 2 hours (*water ad lib*) and were offered a Lucozade Sport isotonic drink (non-compulsory) after completion of each training session. As is the nature of human research studies, the 6-wk wash-out period had to be extended in a few (four) instances due to personal circumstance, however, the longest "wash-out" was 12 weeks.

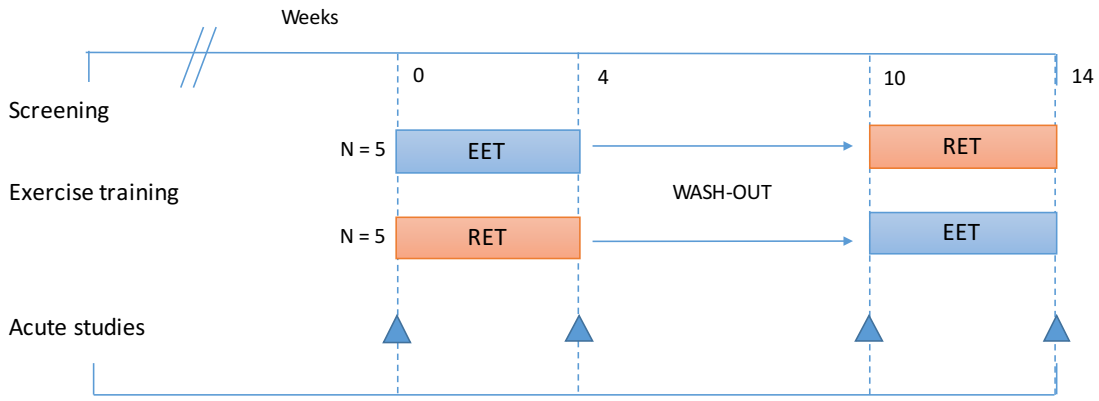


Figure 6. Study overview. Exercise training for 4 weeks of each of two exercise modes with a wash out period between training modes. RET: whole body resistance training at 70% 1-RM (12 reps × 2 sets × 6 exercises). EET: 60 min cycling at 60-70% wattage achieved at VO_2max. Wash-out: 6–12 weeks normal activity levels.

1.6.2.1 Resistance exercise training (RET)

In this study, RET consisted of six different exercises, three of each upper and lower body exercises, with no particular emphasis on either concentric (shortening) or eccentric (lengthening) motions, as each of these may differentially induce morphological adaptations in skeletal muscle (Franchi *et al.* 2014). Repetitions were performed using moderate velocity contractions (1–2 s CON; 1–2 s ECC) over the full range of motion for the individual for each apparatus.

RET was carried out at 70% of the 1-RM for two sets of 12 repetitions on each of the six exercises: leg press, chest press, leg extension, “lat” pull-down, leg curl, and seated row (in that order), with a 2-min rest between sets (exercises are demonstrated in Figure 7). Prior to the first training session, 1-RM assessments were made for each of the exercises, in a maximum of 3 attempts with a 2–3-min rest between attempts. RET was performed three times a week for six consecutive weeks. The 1-RM assessments were repeated 2 weeks into training to maintain the intensity of the training for the 4-week period.

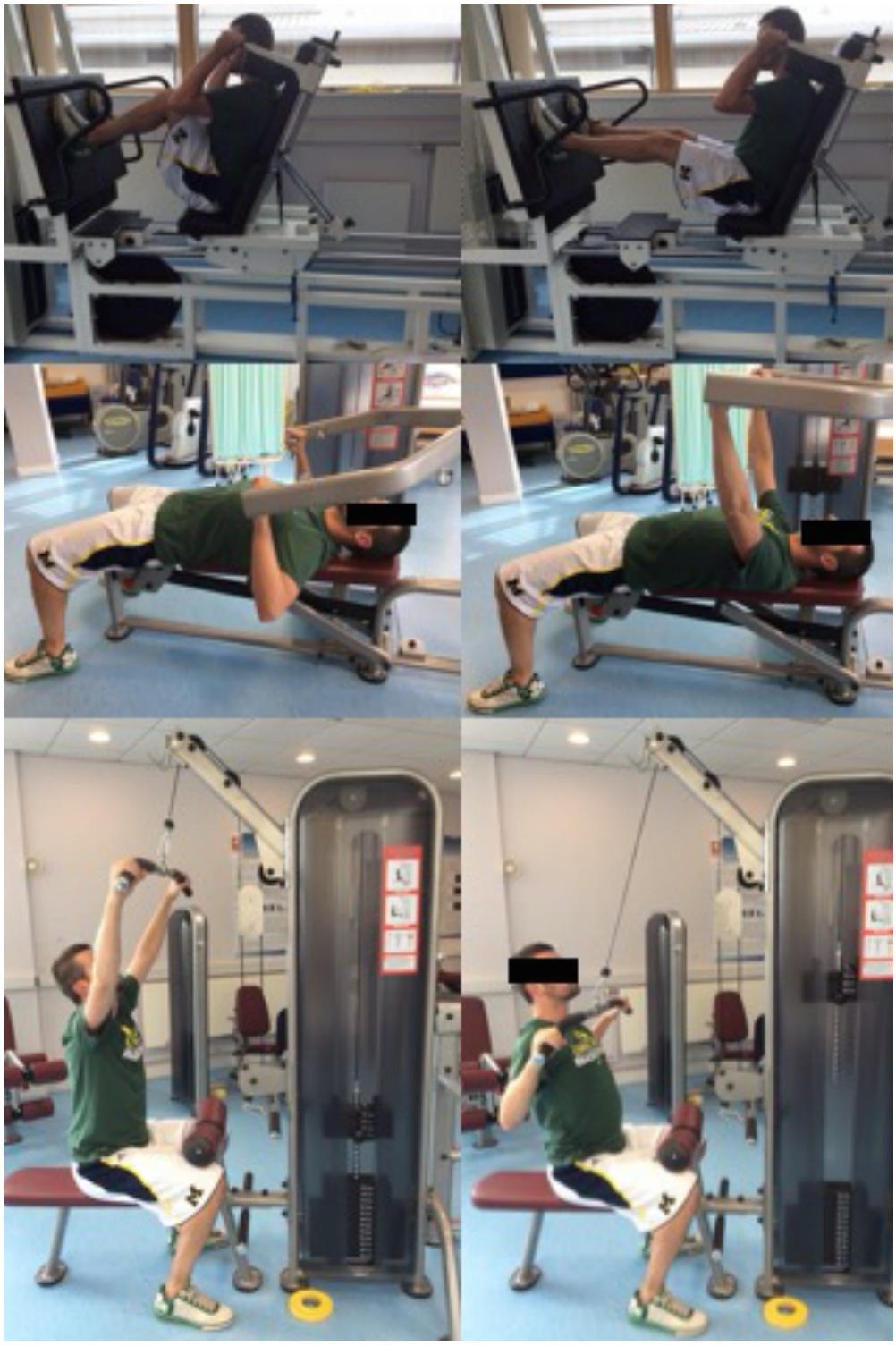




Figure 7. RET training protocol.

As subjects recruited were of mixed ability, a relatively high-repetition 4-week strength training programme (12 reps at 70% of 1-RM for 2 sets, 3 days/week) was used, as opposed to emphasising heavy loading. This design was used based on ACSM recommendations to achieve hypertrophy and strength gains in our chosen cohort (ACSM, 2009). Repetitions were at moderate velocities (1–2 seconds concentric; 1–2 seconds eccentric) which (along with fast velocities (<1:1)), compared with slower velocities, have been shown to be more effective in terms of enhancing muscular performance and strength gains (ACSM 2009). Exercise session frequency and intra-session rest periods were also based on current recommendations.

1.6.2.2. Endurance exercise training (EET)

EET was performed at percentages of the wattage achieved at pre-training VO_{2max}, (determined by CPET) three times per week for four consecutive weeks. This wattage was programmed into a Lode cycle ergometer with the intensity of training constant (watts) irrespective of cadence (rate of pedal revolutions per minute; rpm). For the first week of EET, sessions consisted of 45 min cycling, with 60 min sessions for the subsequent three weeks of EET. Intensity of training for weeks 1, 2, 3, and 4 was 60%, 60%, 65%, and 70%, respectively. This progressive intensity was designed to maintain the intensity of the exercise stimulus to maximise potential performance results in the 4-wk training period (Hoier *et al.* 2013).

1.6.2.3 "Wash-out" phase

During the "wash-out" phase subjects were requested to return to normal physical activity levels and were not required to attend our facility. Dietary intake was not controlled for in subjects, however, subjects were asked to maintain a consistent diet throughout the course of the study. It was also requested of subjects to maintain consistent habitual physical activity levels throughout the training and wash-out periods.

1.6.3 Acute studies

Acute studies were performed in all subjects at the beginning (0 wk) and end (4 wk) of each training period, for both RET and EET (four in total). Subjects attended our facility fasted overnight or >6 h, based on the start time of their acute study; which was consistent within each individual for each of their four visits. The acute studies consisted of a six-hour series of clinical and metabolic tests, which included a blood test, an OGTT, DXA, HR and BP measurements, Doppler ultrasound of LBF (femoral artery), muscle structural ultrasound, and CPET (Figure 8). On a separate visit (also before and after each training period), subjects attended the facility for assessment of maximal strength. Measurements of MVC around the knee joint were taken to assess unilateral isometric strength. Values of 1-RM were used to calculate the training intensity for RET, whereas pre-training CPET test VO_{2max} results were used to calculate EET intensity.

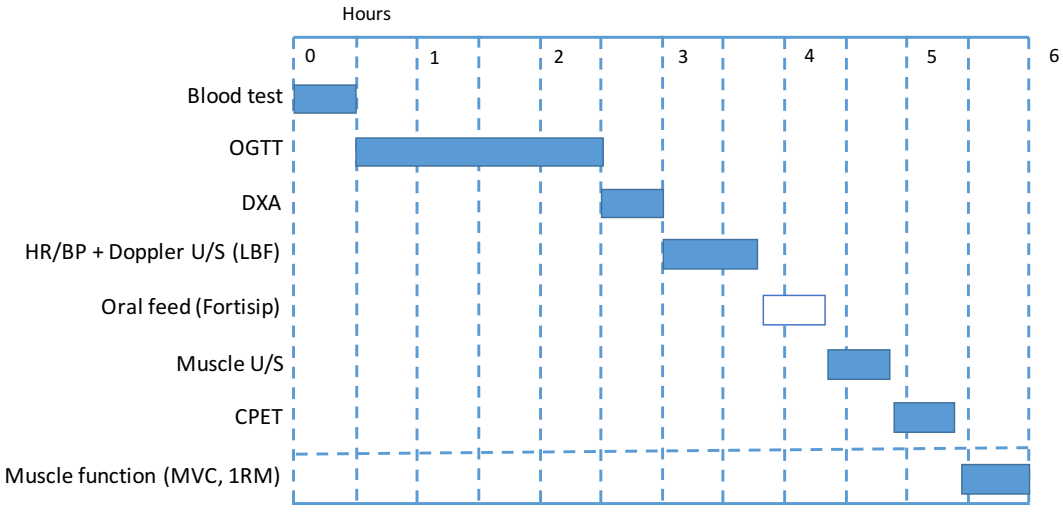


Figure 8. Acute study day protocol. The sequence of clinical tests on the acute study day is specified sequentially. The horizontal dotted line represents parameters assessed on a separate day.

1.6.3.1 Blood tests

A total of 20 ml blood was taken from subjects and mixed in anticoagulant and anti-glycolytic coated plasma separating tubes (lithium heparin and EDTA). Blood was spun down by centrifugation and the serum was extracted and stored at -80°C for later analysis. In addition to the blood plasmas stored in our laboratory, blood samples were also sent to the Royal Derby Hospital for quantification of fasting glucose and insulin, a lipid profile (e.g. triglycerides), a cholesterol profile (e.g. TC, HDL-C, non-HDL-C) and an inflammatory profile (e.g. CRP). Fasting glucose and insulin values facilitated the calculation of the homeostatic model assessment of insulin resistance (HOMA-IR) (Wallace *et al.* 2004):

HOMA-IR = [Glucose] × [Insulin] / 22.5)

1.6.3.2 Oral glucose tolerance test (OGTT)

An oral glucose tolerance test (OGTT) was performed to indirectly measure insulin sensitivity of subjects, reflecting beta cell function, as a measurable health outcome in response to each exercise training mode. The OGTT was performed for 2 h, taking venous blood via a retrograde cannula in the hand, inside a heat box at 57°C. The heat box creates a shunt, allowing arterial-like blood to be sampled from a vein near the capillary bed in the hand (as described in Moore *et al.* 2000). The cannula was connected to a saline bag on a constant drip setting with the cannula port flushed after each withdrawal.

Blood was sampled every 15 min for for 120 min in total. The first measurement was a measure of fasting blood glucose, before consumption of anything other than water from midnight the previous night, or >6 h prior to the OGTT sampling time. After this, 75 g of anhydrous glucose (Dextrose, PURE SERIES) in ~250 ml water was orally ingested and this was recorded as time 0 min. Blood was drawn from the cannula port (three-way tap) using a syringe, which involved drawing off waste (saline and ~2 ml cannula blood) before sampling ~3 ml blood. Whole-blood was initially sampled using a YSI 2300 STAT PLUS glucose analyser to measure blood glucose at each specific time point. Remaining blood was mixed in an EDTA tube and stored on ice until samples were spun down by centrifugation (Thermo Electron Corporation) at 3200 rpm for 20 min and aliquoted plasma was stored at -80°C. An ultra-sensitive enzyme-linked immunosorbent assay (ELISA; DRG Diagnostics) was performed on the plasma samples for determination of insulin concentrations determination during the OGTT. The baseline sample for the OGTT was run undiluted, with all other samples diluted in 'Calibrator 0' at 1:5 as per manufacturers instruction. The ELISA plate was read using Multiskan Ascent software (Thermo Electron Corporation).

1.6.3.3 Dual energy X-ray absorptiometry (DXA)

A whole-body DXA scan was performed on subjects in order to measure total body weight, (fat and bone-free), lean mass, fat mass and bone mineral density (GE LUNAR II) to accurately measure training-induced changes in whole-body composition. Scans were analysed using integrated DXA software, with all regions manually checked by a trained DXA operator for accurate demarcation.

1.6.3.4 Blood pressure (BP) and heart rate (HR)

BP was measured via a non-invasive blood pressure cuff (Datascope) after a prolonged period (~2 h, but >30 min) resting in a supine position. Measurements were repeated three

times, and the mean value recorded, with all measurements required to be within 10% of each other for the measures to be valid. Heart rate was also taken in this period using an ECG (3-lead; displayed on Phillips iU22).

1.6.3.5 Vascular reactivity

Doppler ultrasound was used to measure LBF in the femoral artery. Vasodilatory stimuli include food intake (Skilton *et al.* 2005) and exercise (Poole *et al.* 2003), and were therefore controlled for in this study. As outlined above, prior to the study day, subjects had been rested for >48 h (no intense exercise) and fasted from midnight the night before (or >6 h).

A Doppler ultrasound L9-3 probe and gel was used to detect arterial blood flow (Phillips iU22). Once the artery was located, using anatomical landmarks to try ensure repeat positioning on all visits, blood flow was recorded in real-time over a pulse wave of 5–10 cardiac cycles. Vessel diameter was set at the visible width of media-to-media borders for peak blood flow measurement. These measurements were taken before and after a series of unilateral leg extensions, standardised at 8 reps of 20 kg for all subjects. This vasodilatory stimulus markedly increased leg blood flow, therefore it was LBF response to this stimulus that was measured as an index of vascular reactivity.

1.6.3.6 Nutrient provision

After the LBF measures and before the CPET, subjects were given an oral mixed macronutrient feed (Fortisip, Nutridrinks). Fortisip is a liquid drink with equivalent composition to a normal mixed-meal (16% protein, 49% carbohydrate, and 35% fat), and was provided as a single bolus with the time point relative to the CPET recorded. The dose (flavour and amount) consumed was decided by the participant; however, the amount and time were replicated on all subsequent acute study days (pre- and post-EET and RET) for that individual. Nutrient provision was deemed necessary to ensure that participants were able to perform at maximal physical effort in CPET, ~1 h later.

1.6.3.7 Cardiopulmonary exercise testing (CPET)

CPET was performed on a Lode Corival cycle ergometer using an inline gas analysis system (ZAN 600 USB OXI, nSpire Health). Before the test began, a mask was fitted to ensure that no air escaped from the sides of the mask during the test. The subjects' height, weight and age were entered into the programme to estimate values for expected physiological measures, such as HR, respiratory exchange ratio (RER), and VO_2. The test was planned to last for 8–12 min, therefore an estimated increment was set based on the subjects age, weight and pre-existing activity levels. Participants were told what to expect from the test, such as the rest and warm up periods, when the load was to come on, and to keep cycling until they reached the point of volitional exhaustion (maximum effort). Subjects indicated (by pointing) their effort on a modified Borg scale (1–10) every minute, indicating their rate of perceived exertion (RPE) at that time point.

During the course of the test, the subject was monitored with a 12-lead ECG system, connected to the CPET system. Peripheral capillary oxygen saturation (SpO_2) was measured with a pulse oximeter measured by finger clip. At rest, the participant sat on the bike and measures of resting HR, breathing rate, ECG (electrical activity of the heart) and BP were taken for 3 min, during which time subjects were requested not to talk. After this, a 2-min

warm-up period began whereby participants carried out unloaded cycling at 60 rpm. Following the warm-up, the pre-determined load was applied to the bike and the CPET testing phase began. For the subjects in this study, the ramp protocol was between 20 and 30 W/min, with test durations of between 7 and 12 min.

An essential criterion for the determination of VO_2max is an observed plateau of VO_2, whereby at the end of the test VO_2 is longer increasing despite an increase in work rate; this criterion determined that true VO_{2max} had been achieved in the test. In addition to this criterion for gas exchange and ventilatory variables, other criteria to be met to classify a VO_2max test were achieving >3 out of 4 of the following: HR >95% of maximum, RER >1.1, RPE of >9 (modified Borg scale), and failure to maintain 60 rpm at the end of the test despite verbal encouragement. VO_2peak was measured by averaging VO_2 values from the last 30 s of maximal cycling. AT was measured by the conventional V-slope method, as well as by respiratory equivalents and $PETO_2/CO_2$ (Albouaini *et al.* 2007); a mean value of these three parameters was used as previously reported (Boereboom *et al.* 2016), which were manually determined by two blinded independent assessors.

1.6.3.8 Muscle architecture and quadriceps cross-sectional area (CSA)

VL muscle architecture was visualised as *in vivo* images using a structural ultrasound standardised protocol (Franchi *et al.* 2014). The VL muscle was measured in length, from the greater trochanter of the femur to the mid-patella, and was marked on the skin surface at 50% of the femur length. Using a 100 mm 10-15 MHz linear-array probe (LA923), the borders of the VL muscle were determined and 50% of the VL width was marked manually on the skin surface. These overlapping marks gave a reference point for the VL mid-muscle belly.

At the VL mid-muscle belly, *in vivo* images allowed pennation angle (PA) and fascicle length (Lf) to be retrospectively measured using ImageJ (NIH Software). As the VL muscle is superficial, the fascicles and tendon aponeuroses can be identified, allowing for measurements of PA, Lf and muscle thickness (MT), shown in Figure 9. Identification of the deep tendon aponeurosis and the superficial aponeurosis, with clear fibre visibility, was necessary to get accurate measurements of these parameters. Mid-belly MT was determined by averaging three measurements between aponeuroses at the same 50% mark. Images were taken in triplicate for each parameter for reliability.

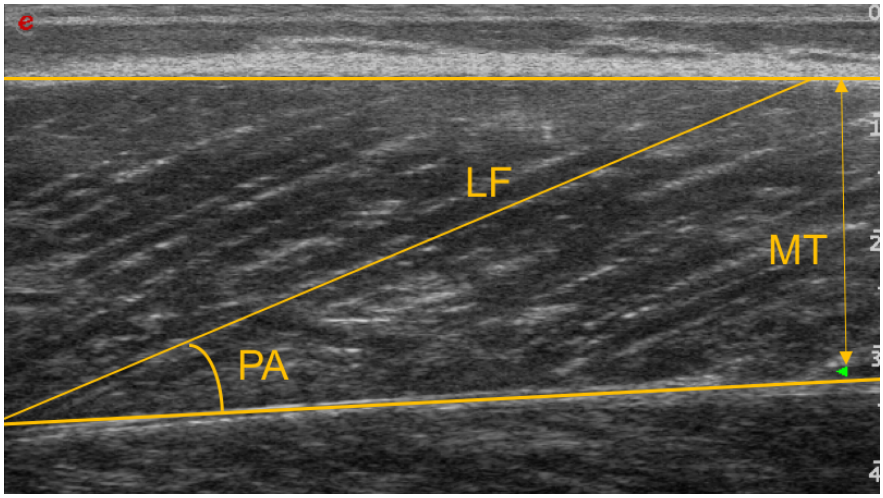


Figure 9. Muscle ultrasound at the VL mid-belly. Representative lines show pennation angle (PA), fascicle length (Lf) and muscle thickness (MT). Original image from the present study.

The quadriceps anatomical cross sectional area (CSA) was measured using a smaller probe (LA523). The maximum CSA of the quadriceps is found at 50% of the femur length (Narici *et al.* 1996). At the 50% mark, the quadriceps were first identified from the furthest border of the VL, across the *rectus femoris* (RF), to the furthest border of the *vastus medialis* (VM), and were marked superficially on the skin. Starting from the VM border, the probe was translocated laterally, recording CSA using the VPAN setting to create an overlapping 3D image. The overall image was considered valid if all four muscles of the quadriceps – VL, RF, VM, and *vastus intermedius* (VI) – were clearly visible.

For image collection, the same operator performed all of the muscle scans, both pre- and post-training for both exercise modes (RET and EET). The depth was recorded on the first scan and the same depth was used pre- and post-training for the same individual. Three images for each CSA scan were taken for reliability.

1.6.3.9 Dynamometry

Measures of muscle function were taken prior to the first training session, and again after the post-training acute study days, for both RET and EET. Using a Cybex Norm dynamometer, maximal voluntary contraction (MVC) of the quadriceps was performed pre- and post-training to assess changes in isometric extensor strength, and the torque produced was sampled into an analogue to digital acquisition system (Biopac System, Inc., Aero Camino Goleta, CA, USA). The dynamometer chair was set up with the dynamometer arm in line with the mid-patella (knee pivot). Position settings were recorded and used for the post-training test. The range of motion was set and the leg was weighed. Using a pre-set programme, MVC was measured twice for each of joint angles 60°, 70°, and 80° unilaterally (right leg pre-RET and left leg pre-EET). Prior to each angle, a single test repetition was performed to accommodate to the motion of the task. After two separate repetitions at an angle, a rest period of 1.5 min was set automatically before the next angle. Subjects were verbally encouraged to use maximum force, and they were able to view the scores on the computer screen during the test. Only the highest (peak) values obtained for each angle were recorded, taken from the two attempts at each angle. MVC results at 70° were used as representative for peak isometric strength of the quadriceps.

1.6.3.10 One repetition maximum

One repetition maximum (1-RM) testing was performed for each of the six exercises performed in the RET period of the study (Figure 7, section 1.6.2.1). The subject was familiarised with the movement by doing 5 repetitions of the apparatus with a load ~30–40% of their predicted 1-RM. After each test weight, the subject rated their effort on the modified Borg scale (Mahler and Horowitz 1994) to determine subsequent loading. The subjects' 1-RM was determined as the maximum weight a completion of a movement could be performed at; achieved within 3–4 attempts, with 2–3 min rest between attempts. These measurements were valid for moderate velocity apparatus-specific contractions (1–2 s concentric; 1–2 s eccentric) over the full range of motion of the apparatus. Subjects were verbally encouraged to use their maximum force in their attempts. Whole body strength was calculated as the sum of the 1-RMs multiplied by 0.9807 (described in Phillips *et al.* 2012b) to present the data in Newtons (N).

1.6.4 Statistical analysis

Means training effects were analysed by paired Student's *t*-test. Results are presented as mean ± SEM. The Pearson correlation coefficient was used to measure the degree of association between variables of interest. Data was also presented per individuals in order to illustrate the inter-individual response to the intervention. For both means and correlation analyses, in cases of expected physiological changes in a single direction, one-tailed tests were used; otherwise, two-tailed tests were used by default. A trend was defined as a *P* value of less than 0.1 (greater than 0.05), for both mean and Pearson correlation analyses.

Chapter 2: Exploring the heterogeneity in exercise responses

It is undeniable that there are certain physiological responses that are associated with different training modes. For example, it is well known that EET leads to improvements in cardiorespiratory function, and that RET leads to improved (increased) muscle mass and function. Universally, for both EET and RET, significant *mean* improvements are reported. However, what is beyond doubt is the vast spectrum of inter-individual exercise responses that can be observed with chronic exercise training, mechanisms of which are still to be fully elucidated. The HERITAGE Family Study identified a significant gradation of high, low and even negative responses in VO_2max with EET (Bouchard and Rankinen 2001), despite significant mean improvements. Additionally, great variation exists between individuals' hypertrophic responses to RET (Phillips *et al.* 2013). In these aforementioned studies, the results were obtained from large-scale, 20-week EET and RET programmes, respectively. Although in the present study the training periods (EET and RET) were just four weeks in duration, recent work has shown that exercise adaptation predominates in the early phases (0–3 weeks; Brook *et al.* 2015). Therefore, the first aim of this study was to ensure that mean improvements in the expected "fitness" parameters for both training modes could be achieved over this time period and accordingly identify the degree of response heterogeneity. In addition, we aimed to validate that the 6 week (minimum) "wash-out" period was sufficient for our subjects to return to pre-training values for our primary responder criteria (AT and whole-body muscle mass).

2.1 Does heterogeneity exist in response to EET?

It is well known that low aerobic (or cardiorespiratory) capacity (maximal: VO_2max; submaximal: AT) is associated with an increased risk of metabolic and cardiovascular disease. VO_2max is indicative of cardiorespiratory fitness, and therefore an increase in either of VO_2max with training represents a positive improvement in this parameter. On average, EET leads to improvements in VO_2max and cardiopulmonary fitness, with limited associated with baseline VO_2max values (Bouchard and Rankinen 2001). Investigated herein are cardiorespiratory (VO_2max, AT, and VO_2peak) responses following 4 weeks of EET, in addition to inter-individual response variation.

RESULTS

As expected, there were significant improvements (increases) in VO_2max (+4.02 ± 1.09 $ml \cdot kg^{-1}min^{-1}$; $P<0.01$), AT (+4.41 ± 0.86 $ml \cdot kg^{-1}min^{-1}$; $P<0.001$), and VO_2peak (+3.92 ± 0.91 $ml \cdot kg^{-1}min^{-1}$; $P<0.01$) in response to four weeks of EET, which are all markers of cardiorespiratory fitness (Figure 10). A clinically relevant improvement in VO_2 (at AT) has been defined as +2.0 $ml \cdot kg^{-1}min^{-1}$ (Kothmann *et al.* 2009) or +2.77 $ml \cdot kg^{-1}min^{-1}$ (West *et al.* 2014); therefore, using the minimum classification, these were clinically significant mean improvements, even with the inclusion of low responders identified amongst the group. The prevalence of low or non-responders to EET (10–20% dependent upon parameter) is in accordance with the reported responder ratios in the HERITAGE family study. It is also of note that for AT, two of the nine participants (in addition to the identified low responder) are only just above the threshold for a clinically significant improvement.

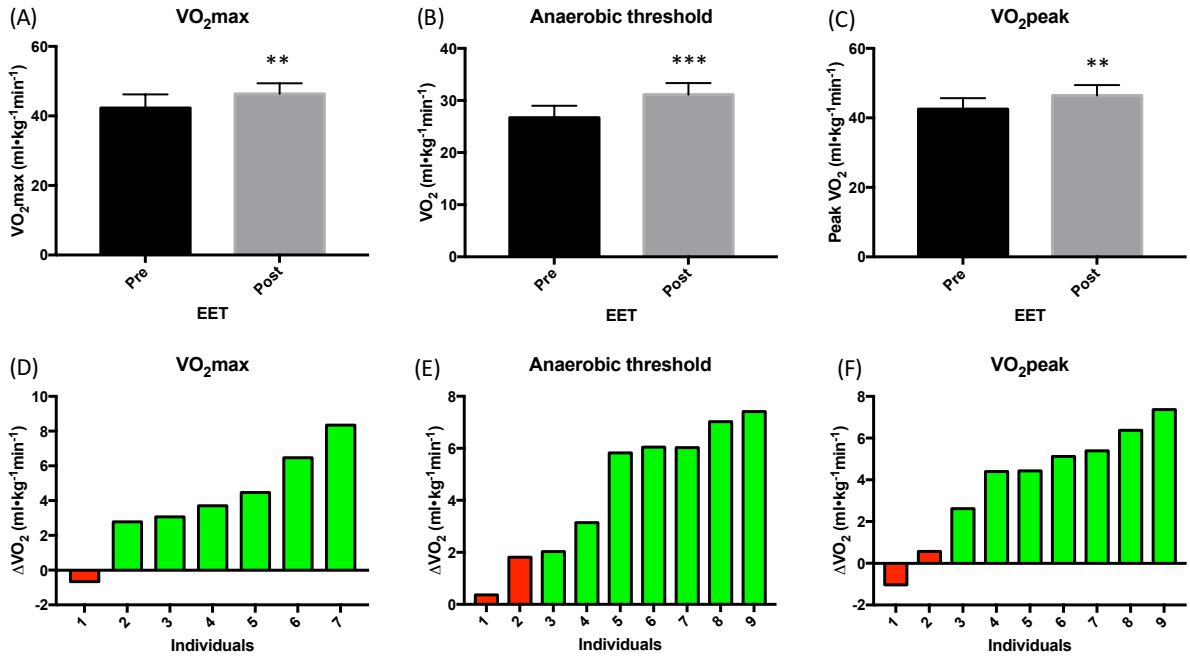


*Figure 10. Mean (A–C) and individual (D–F) improvements in cardiorespiratory parameters in response to EET. VO_2max: n=7; AT and VO_2peak: n=9. Error bars are shown as mean ± SEM. ***P<0.001; **P<0.01. Green: positive aerobic responses to EET; Red: Low or negative aerobic responses to EET.*

VO_2max was defined as a plateau in VO_2, or >3 of the 4 following criteria: RER >1.10; HRmax >85% of age predicted; volitional exhaustion (Borg score >9.5/10); inability to continue cycling. In this group, 7 of the 9 subjects reached VO_2max during the CPET test, therefore AT has been selected to represent aerobic capacity in the subsequent text for greater subject inclusion and to minimise bias from subject effort. With regard to aerobic responses to RET, no significant improvements were shown in VO_2max, VO_2peak, or AT (Figure 11). This is in line with previous work; although response heterogeneity is still apparent, improvement in some individuals maybe due to non-specific responses to exercise training, day-to-day variation in activity, and/or CPET limitations.

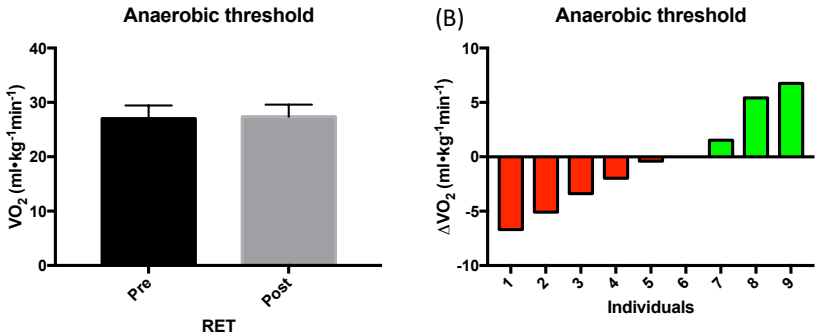


Figure 11: Mean (A) and individual (B) changes in cardiorespiratory fitness (AT) in response to RET. Error bars are shown as mean ± SEM; n=8. ns, non-significant change. Green: positive aerobic response to RET; Red: Low or negative aerobic response to RET.

2.2 Does heterogeneity exist in response to RET?

RET is associated with improvements in muscle strength and skeletal muscle hypertrophy. Muscular strength is important for day-to-day functionality and mobility, as well as athletic performance. Adequate lean mass is required for muscular strength as well as metabolic function, therefore changes in lean mass (hypertrophy) has been assigned the primary outcome in response to RET for this study. Lean mass is also the most quantifiable factor,

free from errors associated with subject effort/motivation, in contrast to that of strength measures of MVC and 1-RM.

Heterogeneity has been reported for hypertrophic responses to RET; for example, Raue *et al.* (2012) reported quadriceps CSA changes ranging from -1.2 to +10.4 cm^2 and strength increases of +5.7 to +41.3 kg in response to a 12-week progressive RET programme, in both young and old participants. There are many causes for an improvement in strength with RET, whereby muscle hypertrophy is the most well understood. In this case, hypertrophy is a consequence of MPS of contractile material. It is therefore expected that this contractile material is able to be used to overcome forces of resistance imposed by the initial stimulus, i.e. RET, leading to adaptive improvements in muscle function. However, a number of other factors contribute to muscular strength such as muscle activation (i.e. how much of the muscle an individual able to use), which encompasses neurological factors. Furthermore, RET is associated with changes in muscle architecture: structural adaptations that may improve muscle function. When lean muscle is increased, can this directly be used to improve strength? Or conversely, do some individuals improve in strength without the associated hypertrophy?

RESULTS

2.2.1 Hypertrophic responses

In the present study, the group of participants *on average* showed a trend for increased lean muscle mass by DXA analysis (+0.75 ± 0.49 kg, P=0.081; Figure 12A) and significant increases in quadriceps CSA by ultrasound analysis (+4.11 ± 1.91 cm^2, P<0.05; Figure 12C), but not total lean leg mass assessed by DXA (Figure 12B). These CSA results are in line with those obtained from Raue *et al.* (2012). Furthermore, as expected, there exists marked heterogeneity in hypertrophic responses to RET with 10–30% (for total LM and CSA) of individuals displaying low or non-responder profiles for adaptation to RET (Figure 12D–E).

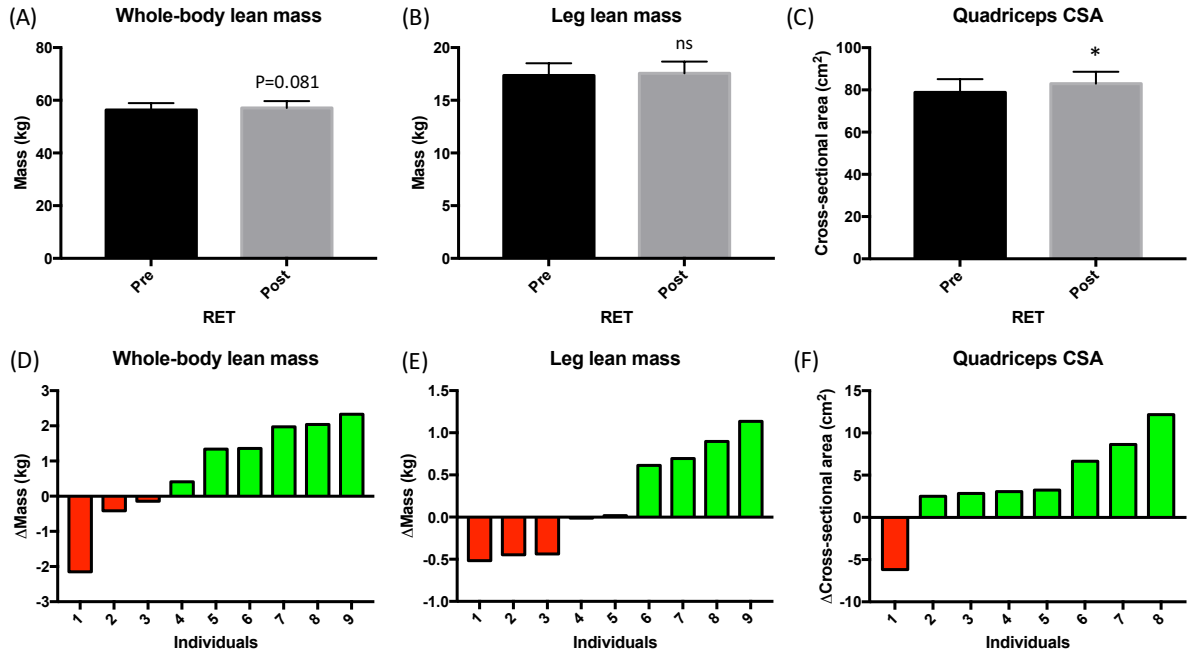


*Figure 12. Mean (A–C) and individual (D–F) hypertrophic responses to RET. (A–C) One-tailed paired t-tests; error bars show mean ± SEM. *P<0.05; ns, non-significant change. Green: positive hypertrophic responses to RET; Red: Low or negative hypertrophic responses to RET. CSA, cross-sectional area.*

In contrast to the improvements in hypertrophy with RET, EET did not result in mean improvements in hypertrophy post-training (whole-body or leg), represented here by total leg lean mass (Figure 13). Both DXA of the upper leg region and ultrasonography of quadriceps CSA were used to measure quadriceps muscle mass, and these two methods were positively correlated (R^2=0.27, P<0.05; Figure 13A). However, ultrasound is known to be a more sensitive assessment of regional lean mass (Menon *et al.* 2012), therefore this method was used for subsequent analyses of upper leg lean mass (ULLM). ULLM assessed by ultrasound (quadriceps CSA) showed significant improvements with EET (+4.65 ± 1.07 cm^2, P<0.01), as well as RET (+4.11 ± 1.91 cm^2, P<0.05). This may be due to the relatively intense nature of the cycling training, specific to the quadriceps muscle group.

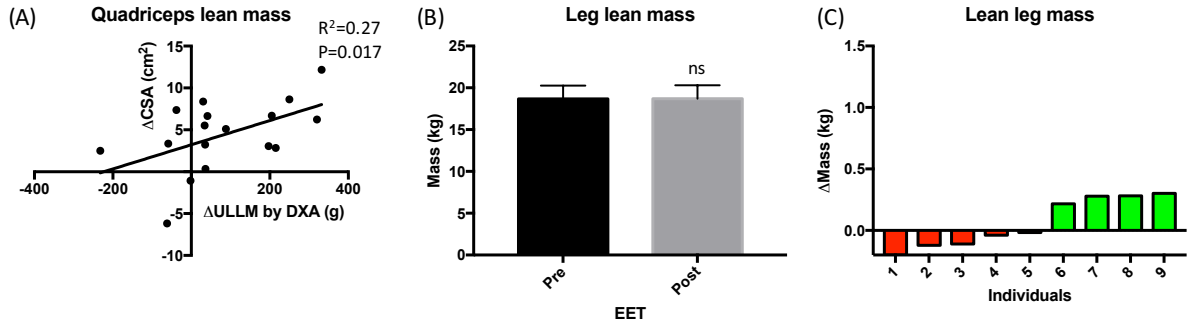


Figure 13. Responses to leg lean mass changes with EET. (A) Assessment of quadriceps lean mass was correlated by methods of DXA and CSA (RET and EET combined plots); one-tailed correlation. (B) Total lean leg mass did not show mean average improvements with EET; two-tailed paired t-test. Error bars show mean ± SEM. ns, non-significant change. (C) Individually, total lean leg mass was not positively affected to a significant degree by EET. B–C are assessed by ultrasonography of quadriceps CSA. CSA, cross-sectional area; DXA, dual x-ray absorptiometry; ULLM, upper leg lean mass.

2.2.2 Strength responses

Strength was improved significantly after RET, in terms of whole-body 1-RM assessment and MVC (Figure 14). After RET, 1-RM strength showed mean improvements of +1074 ± 182.2 N

from 0 to 4 weeks (P<0.0001), and +728.6 ± 115.1 N from 2 to 4 weeks on average (P<0.01). 1-RM strength did not significantly improve between 0 and 2 weeks of RET. Isokinetic strength by MVC also significantly improved with RET (+39.4 ± 11.0 Nm, P<0.01), assessed at a knee angle of 70°. In contrast to the largely heterogeneous hypertrophic responses in this group, all participants showed a numerical improvement (increase) in muscle strength assessed by 1-RM, whereas some heterogeneity was observed for MVC quadriceps strength responses. As expected, the same improvements were not shown with EET in 1-RM strength (lower body) or MVC (Figure 14C, F), validating the specificity of these responses to RET.

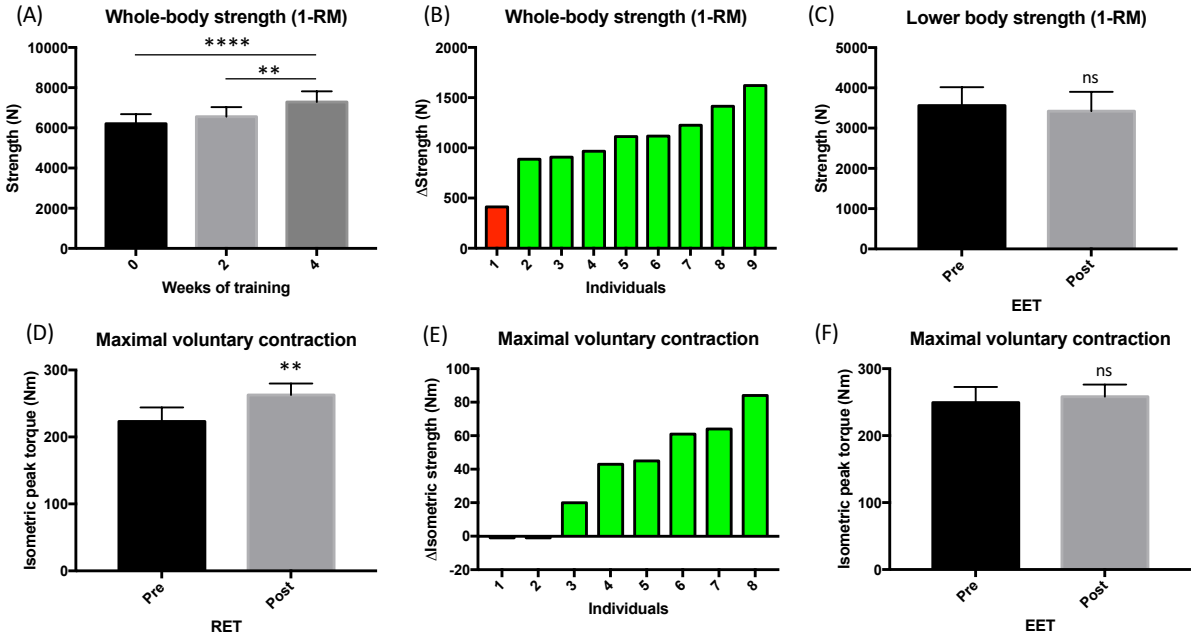


*Figure 14. Strength responses to training. (A) Whole-body strength by 1-RM showing mean results from 1-RM assessments at 0, 2, and 4 weeks of training; Tukey's multiple comparison test, one-tailed, n=9. (D) Mean average results by MVC assessment, one-tailed paired t-test, n=8. (B,E) Individual responses by 1-RM assessment. (C) Lower body strength changes with EET, n=7. (F) MVC assessment of quadriceps strength after EET, n=8. ****P<0.001; **P<0.01; ns, non-significant change. 1-RM, one-repetition maximum. Error bars are shown as mean ± SEM.*

In order to link strength improvements to lean mass changes with RET, muscle activation and muscle architectural changes were also assessed before and after training (Figure 15). Improvement in quadriceps MVC was related to an improvement in quadriceps CSA (R^2=0.41, P<0.05), indicating that improved muscle size and therefore hypertrophy was at least partially responsible for gains in muscle strength (Figure 15A).

Isometric torque per unit of quadriceps CSA was assessed, by methods described in Narici *et al.* (1996). This parameter improved (increased) on average after RET (+0.37 ± 0.12 Nm/cm^2; Figure 15B), implying greater activation of the quadriceps after RET. Furthermore, when muscle architecture was assessed after RET, mean increases in muscle fibre pennation angle were observed (+2.78 ± 1.41°; Figure 15C), implying that the VL muscle had adapted to RET anatomically. Previous studies have shown increases in pennation angle following RET measured by ultrasonography, such as in the VL (Aagaard *et al.* 2001) and the triceps brachii (Kawakami *et al.* 1995), which showed relationships with quadriceps strength and volume.

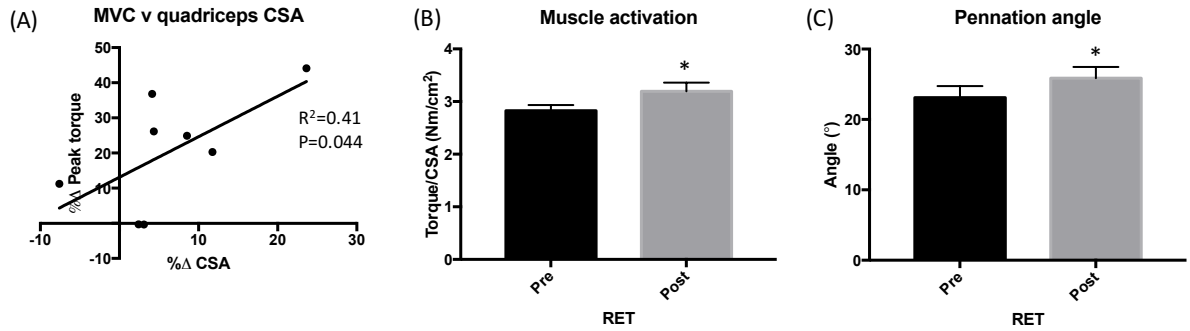


*Figure 15. Adaptive responses to RET. (A) Linear regression analysis of RET-induced changes in MVC and quadriceps CSA; one-tailed correlation. (B) Muscle activation assessed by isometric torque per unit of quadriceps CSA; one-tailed paired t-test. (C) Muscle architectural change in pennation angle after RET; one-tailed Student's t-test. *P<0.05. MVC, maximum voluntary contraction; CSA, cross-sectional area. Error bars are shown as mean ± SEM.*

2.2.3 "Wash-out" period

The "wash-out" period was designed as a 6-week period whereby the first training mode (e.g. EET) should not affect the response to the subsequent training mode (e.g. RET). Of the subjects that completed both training modes (n=8 of 10), this group was randomly divided into two subgroups: Group 1 (n=5) began with EET and Group 2 (n=3) began with RET. Responses to the two training modes are illustrated in Figure 16. Observably, EET lead to an improvement in AT, and this response returned to a similar baseline value (Figure 16A). Conversely, EET did not lead to improvement in lean mass, but lean mass showed a trend for an improvement with RET (Figure 16B). For AT, although a significant mean improvement was shown after EET (section 2.1), the improvement did not statistically return to baseline, although it was observable. The subgroups are not presented here, as subject numbers were too low to show statistical changes; additionally, as heterogeneity exists within the group (high and low responders to EET and RET), this may not be representative of a larger sample size. Overall, this section outlines the training specificity of responses to EET and RET, and thus a larger sample size will allow for subgrouping to determine a statistical effect of the wash-out period.

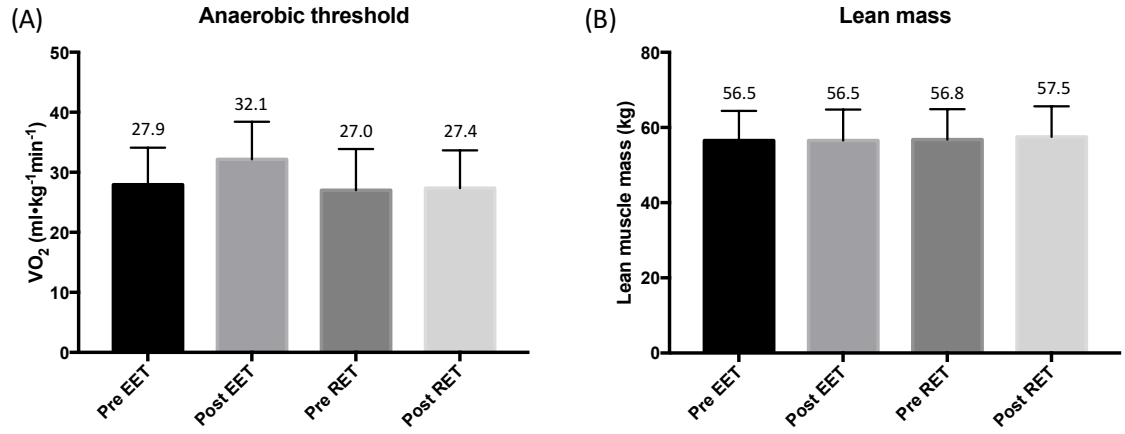


Figure 16. Effect of the "wash-out" period on responses to the two training modes. (A) AT responses assessed by CPET. (B) Whole-body lean mass responses assessed by DXA.

2.3 Summary

Overall, this chapter has demonstrated mean improvements in cardiorespiratory fitness for EET, and strength and lean muscle mass for RET, the expected mode-specific positive

outcomes for these respective training modes. Furthermore, as previously demonstrated in larger and longer-term chronic exercise studies, we were able to replicate the spectrum of heterogeneous responses to exercise after 4 weeks, for both EET and RET in our small group of healthy males. For RET, some of the individual low responses in the expected outcomes could be explained by neurological or architectural adaptations in skeletal muscle, leading to functional improvement by other means. Training-specific responses are undoubtedly shown, with an observable effect of the 6-week wash-out period on abolishing previous training status.

Chapter 3: Is "responder" status dependent on exercise modality?

High and low responses to training, i.e. "responder" status, have previously been identified and described in response to a number of different exercise modes, including EET (Bouchard and Rankinen 2001), RET (Raue *et al.* 2012), and high intensity interval training (HIIT; Higgins *et al.* 2015). A novel element of the present study is the investigation of training mode-specific responses to EET and RET within individuals. Will high responders to one training mode be high or low responders to the other? Furthermore, health-based parameters are also investigated in response to these training modes; can low (or indeed non) responses in these health-based parameters be overcome by adopting a different training mode? The results presented herein will give valuable insight into the role of exercise prescription to improve fitness and health.

RESULTS

3.1 Are fitness responses mode dependent?

To investigate the mode-specific nature of responder status, the expected primary physiological endpoints for the distinct training modes were used to determine the "fitness" responses to EET and RET. The primary fitness outcome for EET was AT (assessed by CPET) with increases in whole-body lean muscle mass (hypertrophy, assessed by DXA) used for RET. These specific outcomes were chosen (vs. VO_2max and muscle CSA, for example) due to the limited interference of subject and/or operator effort/consistency on these results. For example, AT can be measured as a physiological parameter at a submaximal endpoint during an incremental cycling test (CPET). In contrast, VO_2max and VO_2peak responses are measured at the end of the CPET where there may be various reasons why the subject may not achieve the expected plateau in VO_2. For RET, lean mass was chosen as the primary outcome as this change can be reliably measured by DXA, whereas strength by 1-RM or MVC may be subject to day-to-day variation in subjects' feelings of well-being and psychological factors such as subject motivation. Ultrasound measures of muscle, such as CSA may be subject to operator consistency both in scanning and analysis.

Here, it was shown that hypertrophic responses to RET showed no statistical relationship with cardiorespiratory fitness responses to EET assessed as AT (R^2=0.08, P=0.50; Figure 17A). However, strength improvement with RET assessed by quadriceps MVC showed a trend for a negative relationship with AT responses to EET (R^2=0.49, P=0.08; Figure 17B). On the other hand, whole-body strength by 1-RM assessment showed no relationship with cardiorespiratory responses to EET (R^2=0.22, P=0.20; Figure 17C).

Observably, when lean mass responses to RET were compared to AT response to EET (where no significant relationship was observed), it appears that there are groups: higher responders to RET, and higher responders to EET (Figure 18A). However, there is still considerable heterogeneity here. Alternatively, when lean mass responses to RET were compared to VO_2peak response to EET (where no significant relationship was observed), the group could be divided into clusters of responses to the two training modes (Figure 18B), with individuals presenting in four segments: high EET-high RET (HH), low EET-high RET (LH), high EET-low RET (HL), and low RET-low EET segment (LL). Due to limited subject numbers, statistical principal component (PCA) analysis was not performed to validate this observation. However, this figure does highlight the complex nature of identifying

responder status, the mode-specificity of responder status, and the potential role personalised exercise prescription has to play in achieving "fitness" improvements with exercise training. Importantly, it must be emphasised that due to the limited subject numbers, just one or two different responses can tip the scale between significance of correlations, thus some degree of error must be taken into account on interpretation of the present results.

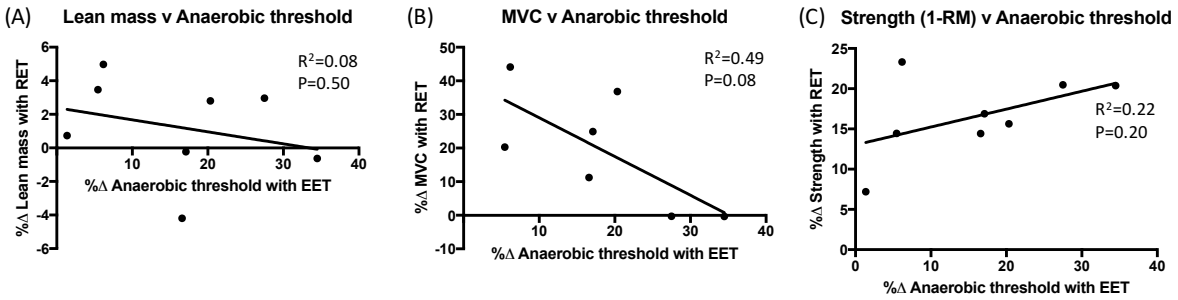


Figure 17. Relationships between fitness outcomes of RET and EET. (A) Linear regression analysis of whole-body lean mass with RET against anaerobic threshold; two-tailed correlation. (B) Linear regression analyses of quadriceps MVC with RET against anaerobic threshold with EET; two-tailed correlation. (C) Linear regression analysis of total strength from 1-RM tests with RET against anaerobic threshold with EET. 1-RM, one-repetition maximum; MVC, maximal voluntary contraction.

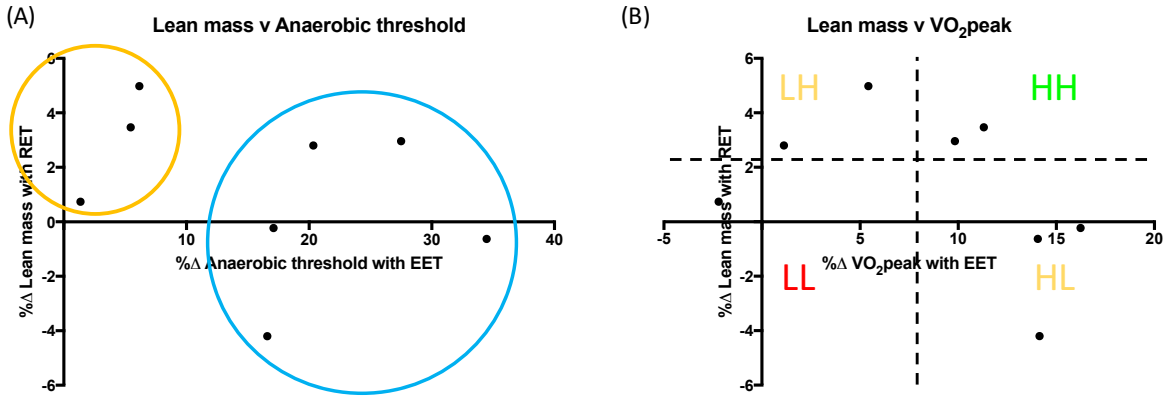


Figure 18. Illustrative presentation of the relationship between whole-body lean mass and VO_2peak responses to RET and EET, respectively. Orange: predominant response to RET; Blue: predominant response to EET. (B) Cluster presentation of the relationship between whole-body lean mass and VO_2peak responses to RET and EET, respectively. HH, high response to both EET and RET; LH, high response to RET, low response to EET; HL, high response to EET, low response to RET; LL, low response to both EET and RET.

3.2 Are health responses mode-dependent?

The notion that exercise training improves health *on average* is universally accepted. However, it is currently unclear whether health reponses are training-mode specific or global across the two exercise modes. What is the relationship between the two training modes for the same health-based parameters? Described here are measures of metabolic health, vascular health, and body composition, which are all associated with positive clinical outcomes and general health and well-being. Selected parameters are chosen for graphical representation of metabolic and vascular health, and body compostion. This section therefore displays relationships between health parameters, comparing responses between the two training modes.

RESULTS

3.2.1 Metabolic health

Insulin sensitivity is key to metabolic health and risk of developing T2DM. Fasting glucose and fasting insulin concentrations, which are regulated in tandem to control carbohydrate metabolism, are used to calculate HOMA-IR (Glucose (mmol/L) × Insulin (µU/L)/22.5), a validated measure of insulin resistance. HOMA-IR was measured in response to exercise training as a negative function of the insulin sensitivity response to training, therefore indicating the effects of exercise on this measure of insulin resistance. HOMA-IR was chosen as representative of metabolic control as it encompasses both fasting glucose and insulin. However, for both EET and RET, there was no response to training, and there was no relationship between training-mode-specific responses (Figure 19A–C). Insulin AUC is also represented here, and unexpectedly, this parameter showed a mean increase (negative) change (+44.1 ± 17.65 µU·min·mL^{-1}; $P<0.05$) following RET (Figure 19D–F). It was investigated whether this response was training-mode-specific for individuals. However, although there was a numerical reduction (positive change) in insulin AUC for EET (-29.6 ± 36.6 µU·min·mL^{-1}; ns), there was no relationship between EET and RET for this metabolic parameter. Overall, although there are differential responses in metabolic parameters between the two training modes, there is no relationship between these responses with EET and RET.

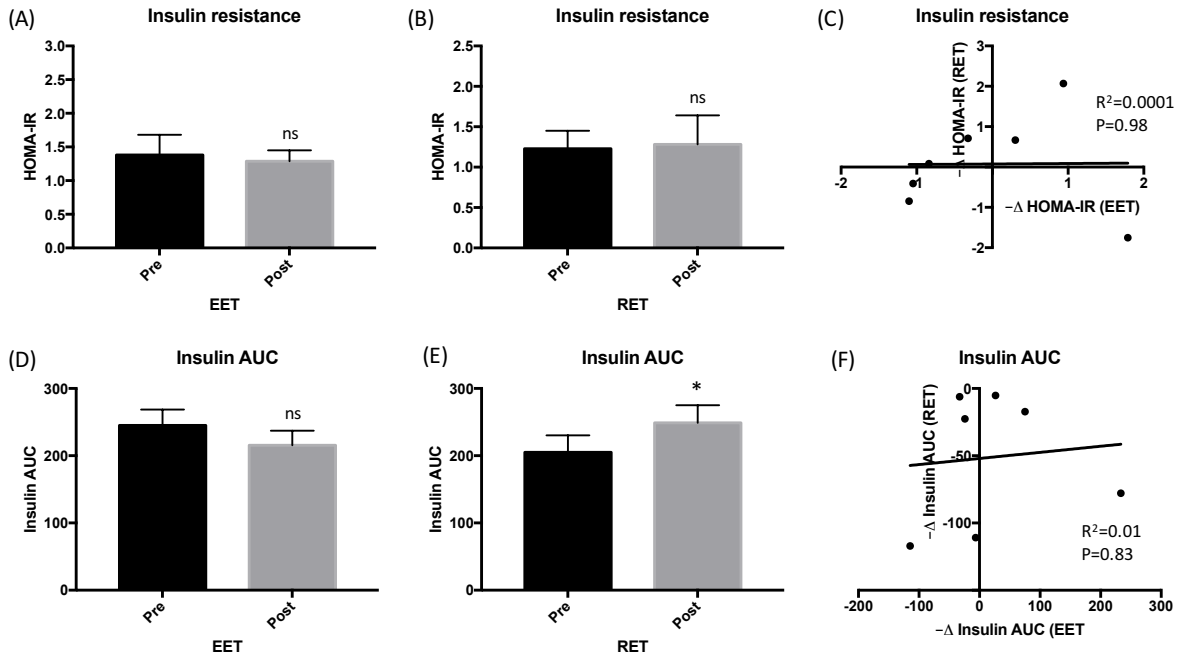


Figure 19. Response in the homeostatic model assessment of insulin resistance (HOMA-IR) to each training mode. (A) HOMA-IR response to EET; two-tailed paired t-test, n=7. (B) HOMA-IR response to RET; two-tailed paired t-test, n=7. (C) Linear regression analysis for the relationship of HOMA-IR responses to EET and RET; two-tailed correlation, n=6. (D) Insulin AUC response to EET; two-tailed paired t-test, n=9. (B) Insulin AUC response to RET; two-tailed paired t-test, n=9. (C) Linear regression analysis for the relationship of Insulin AUC responses to EET and RET; two-tailed correlation, n=8. N.B. HOMA-IR and insulin AUC changes in (C) and (F) shown as positive values indicate positive responses for these parameters.

3.2.2 Vascular health

Various biological factors have an influence on vascular health, such as physiological parameters of BP and HR, and biochemical markers of cardiovascular risk, such as blood plasma levels of lipids and cholesterol, and inflammatory status. Blood pressure and markers of inflammation/haemostasis are reported to significantly contribute to exercise-mediated cardiovascular risk reduction, whereas lipids, BMI, and HbA1c may contribute to a lesser extent (Green *et al.* 2008). Inflammatory markers, such as CRP and IL-6, are important risk factors for cardiovascular disease (Zhang *et al.* 2008), as well as development of metabolic syndrome and sarcopenia (in later life).

3.2.2.1 Haemodynamic factors

Haemodynamic parameters displayed here are those of diastolic blood pressure (DBP) and resting heart rate (HR). DBP was used to represent blood pressure results as DBP has previously been reported as the BP parameter most affected by both EET and RET (Rakobowchuk *et al.* 2005; Cornelissen and Smart 2013), and was, in agreement with this work, and the blood pressure parameter most affected by the exercise training presented herein.

DBP and resting HR relationships with EET and RET are shown in Figure 20. Contrary to previous work and our expectations, DBP showed no change with EET, however, there was a trend for a reduction with RET (-2.38 ± 1.56 mmHg, P<0.1; Figure 20B), with no significant relationship for DBP changes between the two training modes. There was no significant change in resting HR after EET or RET. Again, no significant correlation was observed for this

parameter between the two training modes. Resting HR showed no improvements with either training mode, and the responses were not related (Figure 20D–F).

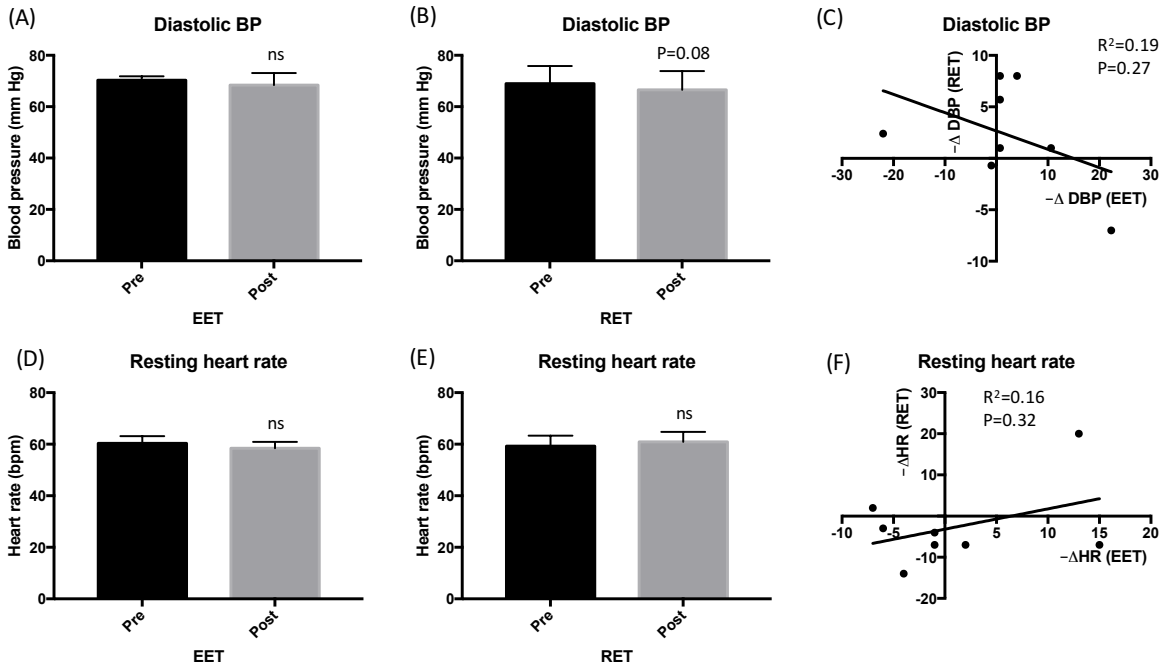


Figure 20. Physiological markers of cardiovascular risk in response to EET and RET. Mean changes in DBP with EET (A) and RET (B); two-tailed and one-tailed t-tests, respectively. Mean changes in HR with EET (D) and RET (E); two-tailed t-tests, n=8. Linear regression analysis for the relationship of DBP (C) and HR (F) responses between EET and RET; two-tailed correlation, n=8. N.B. DBP and HR changes shown as positive values indicate positive responses for these parameters.

3.2.2.2 Biochemical factors

Biochemical parameters displayed here are those of HDL-C and CRP. HDL-C, known as "good" cholesterol, is positively associated with cardiovascular health and has been shown to increase with exercise training (Williams *et al.* 1994). CRP is an inflammatory marker, and high levels are related to a chronic inflammatory state, indicative of heightened atherosclerotic risk.

The present results demonstrate that although numerical improvements (increases) in HDL-C were achieved with both EET (+0.047 ± 0.061 mmol/L, ns; Figure 21A) and RET (+0.051 ± 0.051 mmol/L, ns; Figure 21B), neither of these reached statistical significance. There was no correlation in HDL-C responses between EET and RET (Figure 21C). CRP did not show improvement (reduction) with either EET or RET (Figure 21D and E, respectively), and CRP responses showed no relationship between the two training modes (Figure 21F).

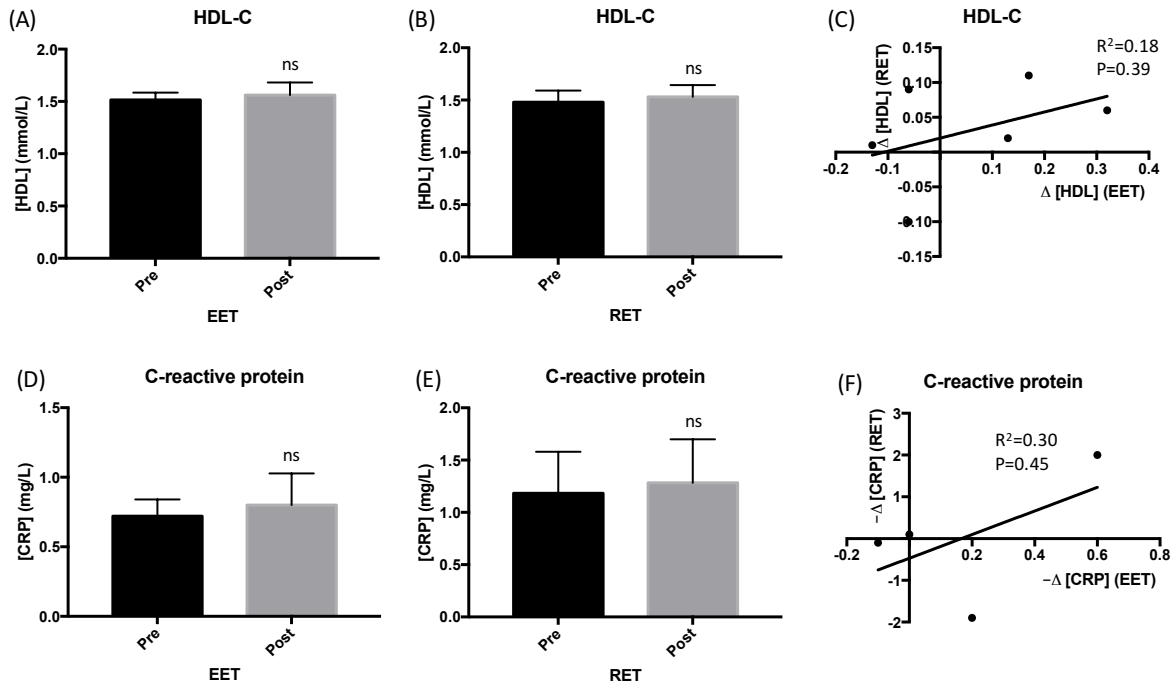


Figure 21. Biochemical markers of cardiovascular risk for EET and RET. Mean responses in HDL-C to EET and RET; two-tailed paired t-tests. (C) Linear regression analysis for HDL-C between EET and RET. Mean responses in CRP to EET (E) and RET (F); two-tailed paired t-tests. (F) Linear regression analysis for CRP between EET and RET. N.B. CRP changes in (F) shown as positive values indicate a positive response for this parameter.

Overall, these results imply that parameters of vascular health are not specific to the training mode, as mean improvements in the various parameters and vascular health were not improved with either EET or RET and there was no relationship between responses to the two training modes within individuals.

3.2.3 Body composition

Body composition, such as a high percentage body fat, has a great impact on health with implications for obesity, T2DM, and cardiovascular disease and the metabolic syndrome, plus other comorbidities. Low percentage body fat (within 'normal' limits) confers a low risk for obesity and T2DM, and the other aforementioned comorbidities, and is therefore a positive marker of health. Excess abdominal fat in particular is an indicator of risk for these comorbidities. Often presented as an android-to-gynoid (A/G) fat ratio, central adiposity is strongly related to poor health outcomes (St-Pierre *et al.* 2007). Therefore, exercise-induced body composition changes with exercise are of great importance for health, as well as the commonly appreciated aesthetic benefits.

There were numerical reductions in adiposity with both EET (-0.53 ± 0.40 g, ns; Figure 22A) and RET (-0.78 ± 0.31 g, P<0.05; Figure 22B), which reached statistical significance. However, there was no correlation between reductions in adiposity with EET and RET (Figure 22C). The A/G ratio significantly improved with RET (-0.092 ± 0.027, P<0.01; Figure 22E), whereas EET showed no significant effect on this (Figure 22D). There was no correlation for this parameter between the two training modes (Figure 22F).

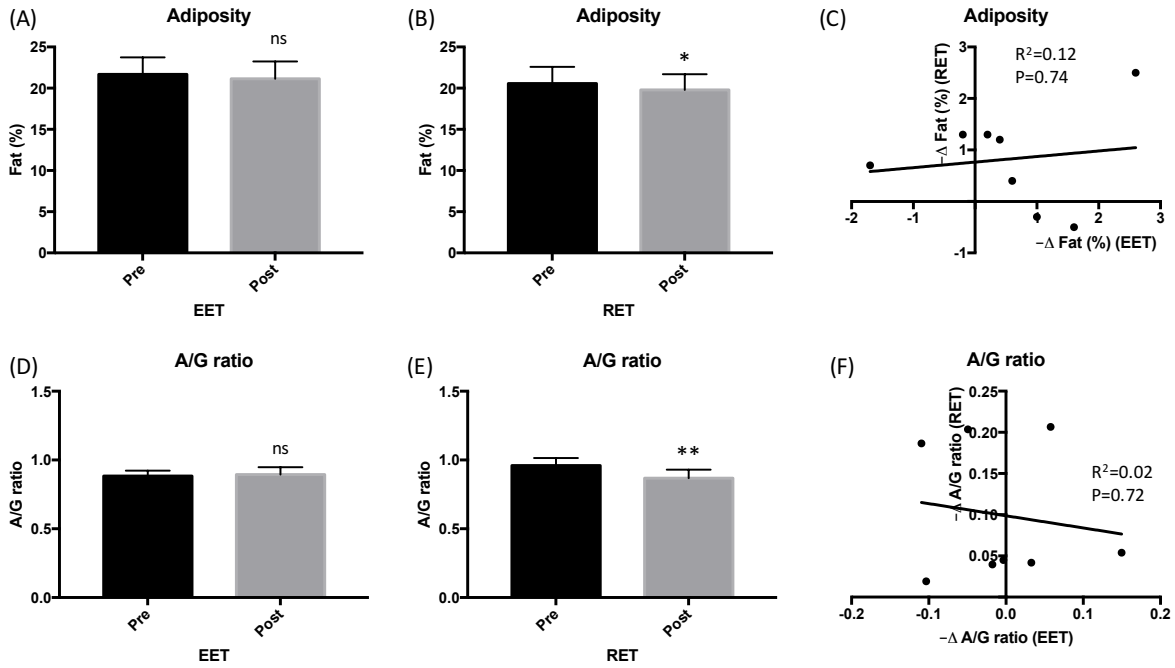


Figure 22. Body compositional parameters in response to EET and RET. Mean responses in abdominal adiposity to EET (A) and RET (B); two-tailed paired t-tests, n=7. (C) Linear regression analysis for abdominal adiposity between EET and RET. Mean responses in A/G ratio to EET and RET; two-tailed paired t-tests, n=8. (F) Linear regression analysis for A/G ratio between EET and RET. N.B. Fat percentage and A/G ratio changes in (F) shown as positive values indicate positive responses for these parameters.

3.3 Summary

From the data presented, it appears that a complex relationship exists for various elements of health benefits with exercise training. For all of the health parameters assessed – those of metabolic and vascular health and body composition – although some mean improvements or trends were found specific to a training mode, none of these parameters were improved in a linear training-mode-specific manner. Specifically, for cardiovascular health parameters, BP (DBP) and A/G fat ratio improved (reduced) with RET but not EET; conversely, EET showed no statistically significant improvements in the health parameters assessed. Overall, no clear relationships were found between training modes for the same parameter. Improvements in some parameters appear to be more commonly improved by a particular training mode, but the responses for an individual are not specific to the mode of training. This leads us into the next chapter, wherein health responses are described in relation to primary outcomes of "fitness" responses to exercise training.

Chapter 4: Are fitness- and health-related responses linked?

Following on from Chapter 3, in which health responses were compared between the two training modes investigated, this chapter describes relationships between responses to "fitness" parameters, i.e. cardiorespiratory fitness (AT) responses to EET and lean mass changes with RET, and various health parameters. Results presented herein aimed to provide insight as to whether fitness (indicative of specific physiological adaptation) can improve health after just four weeks of training. Are high responders for fitness also high responders for health? Furthermore, from a public health perspective, can non-responders to fitness can still improve health with exercise? For example, could motivation for exercise adherence be given to an individual who does not appear to be getting "fitter" with their training regime by informing them that they will still be achieving health benefits?

4.1 Cardiorespiratory fitness and health

4.1.1 Metabolic health

It is known that good cardiorespiratory fitness is correlated with a healthy metabolism (Hamer and O'Donovan 2010). However, investigated herein was the relationship between improvements in cardiorespiratory and improvements in metabolic parameters in healthy young males. In the present study, glucose and insulin responses to a glucose challenge (75 g dextrose) were assessed by OGTT. Insulin sensitivity was calculated from fasting blood glucose and fasting plasma insulin concentrations to produce the negative function, HOMA-IR. Shown here are responses of carbohydrate metabolism in relation to AT (Figure 23). Carbohydrate metabolism is presented as glucose area under the curve (AUC) for the OGTT, insulin AUC for the OGTT and HOMA-IR. Glucose AUC represents the glucose tolerance for an individual to a standardised glucose load from a fasting concentration, and represents the absolute glycaemia of an individual over 120 min (in the present case). Conversely, insulin AUC represents the insulin response to this glucose load, which is concurrently secreted to remove excess glucose from the blood to the tissues. HOMA-IR based on fasting values only, as a validated measure of whole-body insulin sensitivity. In this data set, no relationships were observed for changes in glucose AUC compared with AT responses, to EET. These results were in keeping with some previous studies; for example, Ball *et al.* (2004) that showed that VO_2max was not independently related to insulin sensitivity or secretion in overweight Hispanic youths, and although this study did not assess exercise responses, implied that improvement in cardiorespiratory fitness may influence insulin action indirectly through reductions in fat mass. Furthermore, Bouchard *et al.* (2012) showed that adverse responders for health outcomes (e.g. no improvement in fasting insulin) to 20 weeks of EET were not related to VO_2max responses (i.e. not the adverse responders for this parameter). These data, in addition to our findings imply that a more complex mechanism exists for the amelioration of insulin resistance with EET than improvements in cardiorespiratory function.

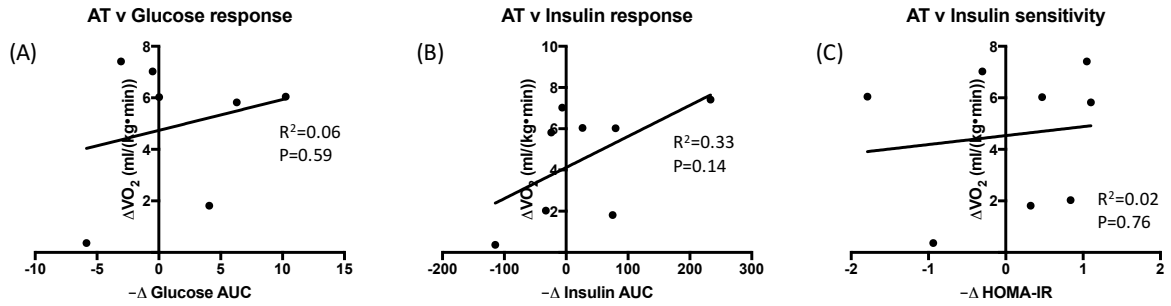


Figure 23. Relationships between metabolic responses and cardiorespiratory responses to EET. (A) Glucose response (AUC) v AT responses; n=7. (B) Insulin response (AUC) v AT responses; n=7. (C) Insulin sensitivity (HOMA-IR) v AT; n=8. Positive responses are shown in a positive direction. Analysis by Pearson's correlation. Units are glucose AUC: mmol·min-^{1}L^{-1}; insulin AUC: µU·min-^{1}L^{-1}.

4.1.2 Vascular health

It is known that a high VO_2max is positively related to cardiovascular health, due to the anatomical relationship between the heart, lungs and circulatory system. It has previously been shown that EET (>8 wk) can increase in HDL-C with training, associated with increases in VO_2max and decreases in body weight also induced by EET (Kelley *et al*. 2005a). Furthermore, both EET and RET have been shown to reduce IL-6 levels (Donges *et al.* 2010), and RET has been shown to also reduce serum CRP levels (Donges *et al.* 2010). However, how and whether improvements in cardiorespiratory fitness are related to improvement in direct parameters of vascular health is less well defined. Reported here are the responses of vascular parameters in relation to AT responses that showed mean average improvements with EET (see Chapter 2).

As shown in Figure 24, there was a significant correlation between AT improvements and MAP improvements with EET (R^2=0.56, P<0.05). Furthermore, there were significant correlations between AT improvements and improvements in the individual BP components used to calculate MAP, i.e. DBP (R^2=0.40, P<0.05) and SBP (R^2=0.43, P<0.05; graph not shown) with EET. Similarly, changes in resting HR were significantly positively correlated with AT (R^2=0.34, P<0.05), however, vascular reactivity did not show significant relationships with changes in AT. These are positive findings, indicating that cardiorespiratory fitness may directly lead to improved blood pressure and resting heart rate in response to EET, thus have application to hypertension therapy. In contrast, previous studies have indicated that HR and BP changes with EET may not be directly related to VO_2max responses (Vollaard *et al.* 2009; Bouchard *et al.* 2012). Parameters of biochemical cardiovascular risk (fasting HDL-C, non-HDL-C, and triglycerides; Figure 24D–F) showed no relationships with improvement in cardiorespiratory fitness with EET.

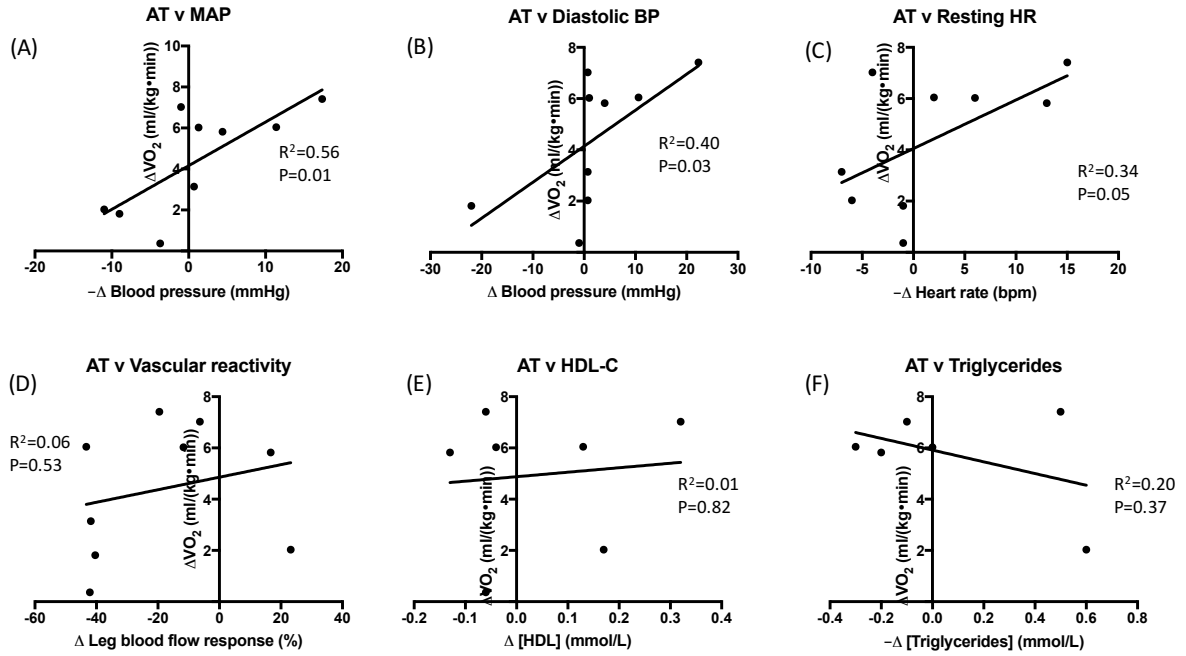


Figure 24. Relationships of vascular responses with EET responses. (A–F) Linear regression analyses of responses mean arterial pressure (MAP), diastolic blood pressure (DBP), resting heart rate (HR), vascular reactivity (leg blood flow response), HDL-C, non-HDL-C, and fasting triglycerides, respectively, in relation to anaerobic threshold responses to EET; two-tailed correlations. Positive values indicate positive responses for all parameters.

4.1.3 Body composition

Whole-body adiposity (total % body fat) and abdominal adiposity (regional % fat) were assessed by DXA analysis. One postulated mechanism for EET-induced reductions in body fat, is an increased energy expenditure, which results in a negative caloric balance, which can thus lead to fat loss provided that the diet is maintained (Nordby *et al.* 2015).

As shown in Figure 25, improvements in AT showed trends for positive correlations with improvement of both whole-body adiposity (R^2=0.43, P<0.05) and even more so with abdominal adiposity (R^2=0.52, P<0.05). These results are interesting as it was shown in Chapter 3 that total percentage body fat or abdominal adiposity did not reduce significantly on average with EET. A correlation of improvement in cardiorespiratory fitness (AT) and percentage body fat therefore shows that individuals that gained greater cardiorespiratory fitness from EET (high responders) lost more fat with the training, implying that they also had enhanced energy utilisation capacities. This could be due to increased peak fat oxidation that is known to occur with EET (Nordby *et al.* 2015). Moreover, it appears that this increased fat loss was specific to the visceral region, a target area for cardiometabolic risk. Furthermore, in accordance with results showing lack of relationship of AT with metabolic health in section 4.1.1, the data implies that improvement in cardiorespiratory fitness with EET may be more directly related to improvements in body composition than improvements in carbohydrate metabolism.

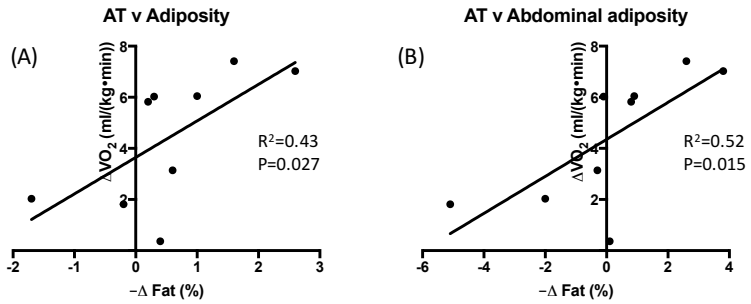


Figure 25. Relationships between body compositional and cardiorespiratory responses to EET. (A) Whole-body adiposity (total % body fat) responses v AT. (B) Abdominal adiposity (regional % body fat) responses v AT. Positive values indicate positive responses. n=9. Analysis by Pearson's correlation.

4.2 Hypertrophic responses and health

4.2.1 Metabolic health

Enhanced lean muscle mass is associated with metabolic health, with low skeletal muscle mass a risk factor for insulin resistance. Improving lean muscle mass has been reported to improve insulin sensitivity and glucose tolerance in healthy as well as overweight and diabetic populations (Srikanthan and Karlamangla 2011; Fukushima *et al.* 2016). In the present study, lean mass responses to RET were investigated in a healthy population of young males in response to just 4-weeks of RET.

To assess hypertrophic responses to RET, whole-body lean mass changes were assessed by DXA. Oral glucose tolerance and insulin responsiveness was measured by oral glucose challenge (OGTT), and HOMA-IR was calculated from fasting plasma glucose and insulin concentrations. The results showed that changes in lean mass with RET were not correlated with glucose tolerance (AUC), insulin response (AUC) to the OGTT, or HOMA-IR (calculated from fasting glucose and insulin concentrations) (Figure 26). In section 3.2.1 it was shown that insulin AUC significantly increased after RET, however, these results suggest that this response was not attributable to lean mass changes with RET. Furthermore, high responders to lean mass may not be the same high or low responders to metabolic health with RET. These data thus highlight the complex nature of the effects of exercise training on health responses.

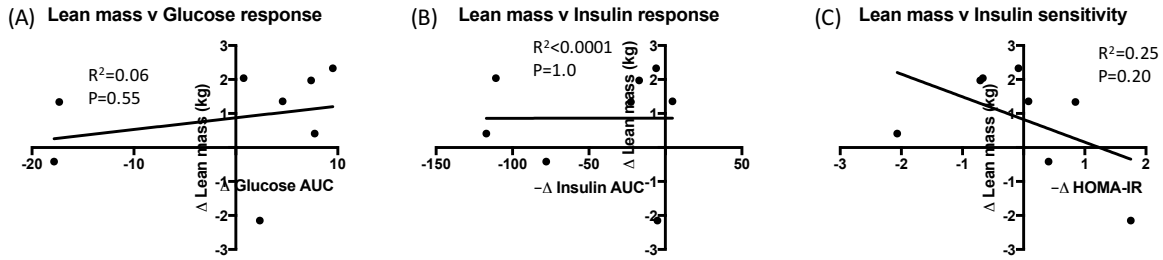


Figure 26. Relationships of metabolic responses with RET responses. (A) Linear regression analysis of glucose response (AUC) with hypertrophy (lean mass). (B) Linear regression analysis of insulin response (AUC) with lean mass. (C) Linear regression analysis of insulin sensitivity (HOMA-IR) with lean mass. Responses are shown as negative changes for all parameters, whereby positive responses are shown in a positive direction.

4.2.2 Vascular health

Vascular health is important for the efficient delivery of blood and therefore oxygen and nutrients to the different tissues of the body. For example, adequate leg blood flow is implied in good vascular health, and is reduced with ageing (Phillips *et al.* 2012b). Furthermore, vascular reactivity to a vasodilatory stimulus, such as exercise or feeding, is important for periods of demand, i.e. hyperaemia or an exercise challenge, and has been shown to be improved with 20-weeks RET (Phillips *et al.* 2012b). Other factors of vascular health include BP, resting HR, and biochemical markers of cardiovascular risk.

Results shown in Figure 27 indicate that changes in MAP, resting HR, HDL-C, and triglycerides were not related to changes in lean mass induced by RET. Unexpectedly, improvements (increases) in lean mass were negatively correlated with improvements in vascular reactivity (R^2=0.53, P<0.05; Figure 27D). These results imply that the non-responders for lean mass changes were high responders to vascular reactivity, and improvement in lean mass may not be directly related to increases in lean mass; RET may act on improving vascular reactivity by other mechanisms. Alternatively, the recovery time from the stimulus (a single bout of 8 × 20 kg leg extensions) was not assessed, but observably, subjects that showed reduced vascular activation following training appeared to be the individuals that recovered from the stimulus most quickly during assessment (who showed lower average vascular reactivity). Thus, in future, a greater or prolonged stimulus (e.g. feeding) may be necessary to capture vascular activity improvements from RET.

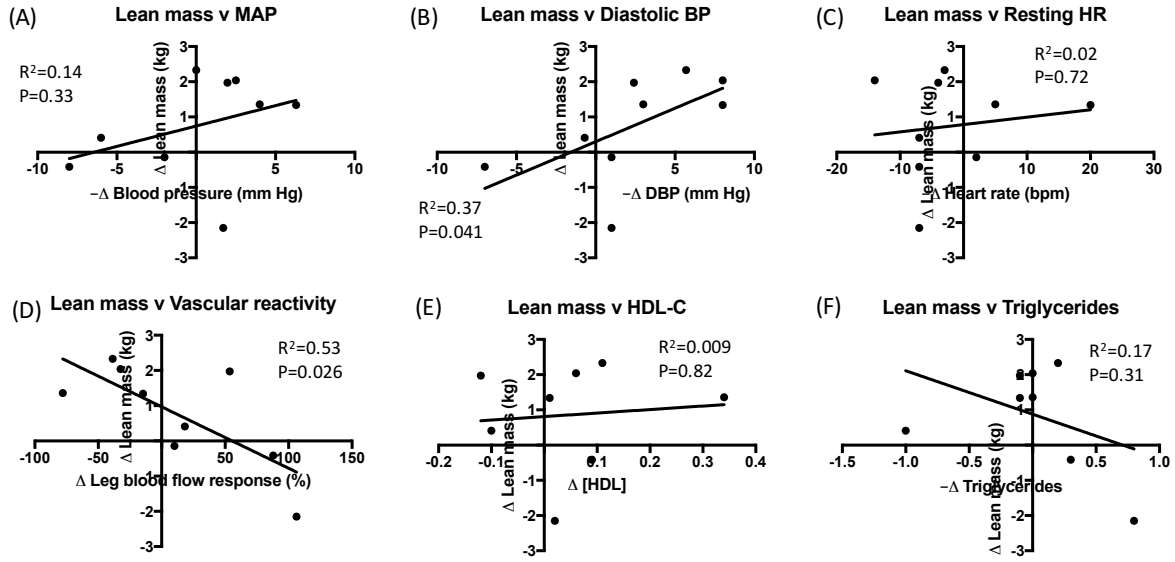


Figure 27. Relationships of vascular responses with RET responses. (A-F) Linear regression analyses of responses mean arterial pressure (MAP), diastolic blood pressure (DBP), resting heart rate (HR), vascular reactivity (leg blood flow response), HDL-C, non-HDL-C, and fasting triglycerides, respectively, in relation to hypertrophic (whole-body lean mass) responses to RET; two-tailed correlations. Positive values indicate positive responses for all parameters.

4.2.3 Body composition

Lean muscle mass and adipose tissue have a tight relationship, and relative ratios of these tissues are implied in metabolic pathologies of obesity and T2DM (Hwang *et al.* 2016). In this group of young healthy males, whole-body adiposity responses were positively correlated with lean muscle mass gains (R^2=0.50, P<0.05; Figure 28A); however, changes in abdominal adiposity showed no relationship with lean mass changes (R^2=0.03, P=0.86; Figure 28B). These results show that high responders to hypertrophy with RET were also

high responders to fat loss with RET, however, imply that fat loss losses were not specific to the abdominal regional. Gains in muscle mass with RET may therefore contribute to fat loss with this intervention, posing a dual benefit of RET for high responders. One possible explanation for this is is that the increased lean mass in the high responders, results in a higher day-to-day metabolic rate (as muscle is a metabolically active tissue), which may contribute to a negative energy balance and subsequent adipose losses (Byrne and Wilmore 2001). Conversely, low and negative responders for lean mass did not achieve this health response and were thus also low responders to reducing total body fat with RET. An interesting perspective is that as lean mass and percentage body fat changes are positively correlated, it must be distinguished which of these responses to RET induces the associated responses in health parameters to RET.

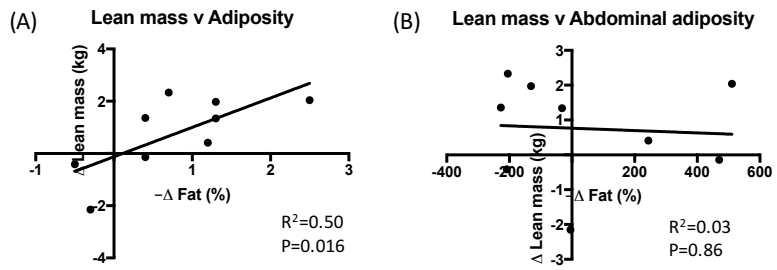


Figure 28. Relationships of body compositional responses with RET responses. (A) Whole-body adiposity (total % body fat) responses in relation to anaerobic threshold with EET. (B) Abdominal adiposity (regional % body fat) responses in relation to anaerobic threshold with EET. Both are two-tailed correlations. Positive values indicate positive responses for both parameters.

4.3 Relationships between health parameters

On average, improving fitness has positive effects on a number of health parameters. However, are these parameters independently regulated? Are high responders to certain health parameters high responders to other health parameters? This section will thus address the relationship between different aspects of improvements in health with exercise training.

Percentage body fat improved with both EET and RET (section 3.2.3), thus primarily investigated in this section are body compositional effects of EET and RET, in relation to other health parameters. Excess fat has many negative implications in metabolism and cardiovascular health, such as pathogenesis of obesity and diabetes. For example, visceral fat has been reported to be highly correlated with poor insulin response to a glucose challenge, elevated fasting triglycerides, BP (SBP and DBP) and Chol/HDL-C ratio (Slentz *et al.* 2005). Relationships with health parameters investigated here are those for parameters that showed numerical improvement with the associated training mode.

4.3.1 EET

For EET responses, changes in percentage body fat were related to HOMA-IR (metabolic parameter) and MAP (haemodynamic parameter), and the relationship was assessed between haemodynamic parameters (MAP and HR) (Figure 29). Improvement (reduction) in body fat was not correlated with changes in HOMA-IR, a negative function of insulin sensitivity. For vascular haemodynamic parameters, reduction in body fat showed a trend for a positive correlation with a reduction (improvement) in MAP (R^2=0.36; P=0.088; Figure 29B). Furthermore, resting HR and MAP responses to EET were significantly positively correlated (R2=0.50; P=0.034; Figure 29C). These data imply that improvement in adiposity

is not related to improvement in metabolic health in young healthy males, however, reduction in fat mass may result in improved blood pressure. Additionally, improvement in haemodynamic parameters may be regulated via similar mechanisms.

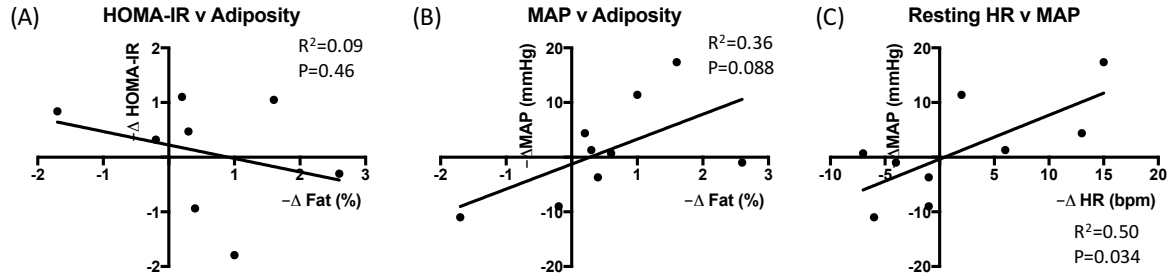


Figure 29. Relationships between health parameters with EET. (A) HOMA-IR v adiposity responses; n=8. (B) MAP v adiposity responses; n=9. (C) Resting HR v MAP responses; n=9. Positive responses are shown in a positive direction. Relationships were assessed by Pearson's correlation; all two-tailed correlations.

4.3.2 RET

Health responses to RET in relation to associated significant improvement in percentage body fat are shown in Figure 30. After RET, mean percentage body fat was not related to responses of insulin response to an oral glucose load (insulin AUC; Figure 30A), a metabolic parameter. DBP showed a significant positive relationship with improvement in whole-body adiposity (R^2=0.52, P<0.05; Figure 30B), and vascular reactivity (haemodynamic parameter) showed no significant relationship with improvements in body fat (Figure 30C). These results suggest that RET improves DBP in association with improving body fat, however, not all vascular parameters are affected in the same way following RET. Interestingly, as improvements in lean mass and adiposity are positively correlated (section 4.2.3), it is thus difficult to distinguish in this study whether it is body compositional changes of lean mass or percentage fat that leads to these patterns of inter-individual health responses.

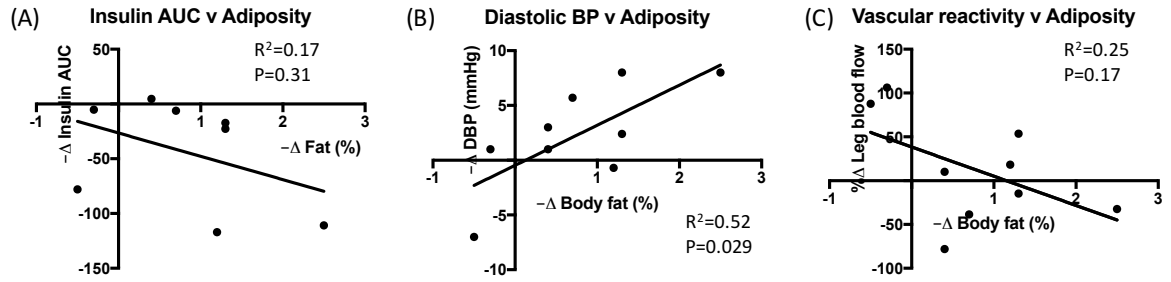


Figure 30. Relationships of body compositional parameters with other health parameters. Linear regression of insulin sensitivity (HOMA-IR; A), diastolic BP (B), and fasting triglycerides (C) were assessed against change in percentage body fat; two-tailed correlations. Positive responses are shown in a positive direction.

4.4 Summary

In summary, EET responses of AT were not related to changes in metabolic health, whereas blood pressure responses of vascular health, as well as body compositional responses to training, were positively related to improvement in cardiorespiratory fitness. In contrast, RET responses were also not related to parameters of metabolic health, however, the haemodynamic parameter vascular reactivity was negatively affected by lean mass response to RET. Reduction in adiposity was positively correlated with increases in lean mass, highlighting the tight relationship between skeletal muscle and fat mass responses.

Furthermore, relationships of other health parameters with RET (e.g. DBP and vascular reactivity) showed similar correlations with increases in lean mass and fat loss, muddying the waters on distinguishing the cause of individual health responses to these parameters. Overall, it appeared that improvement in lean muscle mass with RET had more of a relationship with health responses than improvement of cardiorespiratory fitness with EET. Improvements in the primary outcomes of both training modes appeared to be positively related to some blood pressure improvements and fat loss, both of which are key indicators of improved health.

Chapter 5: Discussion & Conclusions

5.1 Study design

The study presented herein assessed the responses of healthy male individuals to two standardised training programmes, independently assessing responses to 4 weeks of EET and 4 weeks of RET in the same individuals. Previous studies have validated the use of 4 weeks as a sufficient training duration to produce responses in both aerobic function with EET (Hoier *et al.* 2013) and strength and hypertrophy with RET (Brook *et al.* 2015). Heterogeneity has been reported to exist in these responses to the associated training modes (Bouchard and Rankinen 2001; Raue *et al.* 2012), much of which cannot currently be explained by known mechanisms. Previous studies have indicated that these training-induced responses are not related to, age, sex, race, pre-training or habitual fitness levels, although a significant hereditary component exists (Bouchard *et al.* 1999). What has not been defined in the present literature is whether the responses for an individual to these two different training modes is universal; i.e. is a low responder to one mode also a low responder for the other? Furthermore, it is unclear whether non/low-responders in 'fitness' parameters (e.g. aerobic capacity or hypertrophy), can be high-responders for health-benefits associated with exercise training. Therefore, the overall aim of this work was to identify heterogeneity in training responses in young, healthy males (to minimise confounding factors associated with age and disease profiles), and to explore the relationship between responses ("fitness" and health) to the two exercise training modes.

5.2 Heterogeneous responses to exercise

5.2.1 Variation in cardiorespiratory responses to EET

It is well known that aerobic exercise training (e.g. EET and HIIT) improves cardiorespiratory fitness, *on average*. In the present study, cardiorespiratory fitness significantly improved following 4 weeks of EET as assessed by multiple CPET parameters (VO_2max, AT and VO_2peak), validating the use of short-term, moderate-intensity aerobic exercise training as a model to explore improvement in cardiorespiratory fitness in healthy males.

In keeping with the literature, heterogeneity in inter-individual cardiorespiratory responses to EET was observed in this study. It is undeniable that there exists a largely hereditary component to the VO_2max response phenotype. Indeed, to explain heterogeneity in cardiorespiratory responses to EET, the HERITAGE Family Study was undertaken; identifying a significant hereditary component, accounting for ~47% of gains in VO_2max (Bouchard *et al.* 1999). A possible explanation for this is that, in high responders to EET, differentially regulated genes (including those related to proangiogenic and tissue developmental networks), may lead to enhanced skeletal muscle and aerobic adaptation compared with low responders to EET (Keller *et al.* 2011). In the present study, methodologies presented herein did not assess the hereditability component of the EET response, however, muscle biopsy samples were taken for future RNA analysis, which hopefully, when combined with the wide degree of physiological measures, will provide a high degree of understanding for the contribution of genetic factors in relation to the physiological outcomes reported here.

Importantly, much of the heterogeneity in the other ~50% of the trainability of VO_2max has not been elucidated. Bouchard and Rankinen (2001) reported in the HERITAGE family study that heterogeneity could not be accounted for by age, as subjects were 17–29 years, similar

to the age range of the present study (18–32 years). Furthermore, similar heterogeneity was observed between men and women, thus sex was unlikely to be a cause, and this study controls for sex, as the cohort is all males. The HERITAGE cohort involved hundreds of participants, thus the fact that similar responder ratios were reproduced in this small cohort agrees with Kohrt *et al.* (1991), implying that the cohort size was not an issue with respect to identifying heterogeneous responses. Race (black and white) only accounted for 1% of the variance in HERITAGE. Overall, the HERITAGE study showed that age, sex, race and baseline VO_2max values accounted for only 11% of the variance in VO_2max responses, leaving ~40% undetermined.

Other factors specific to the present study may have impacted on VO_2max responses. Most importantly, we expressed difficulty in pushing all subjects to VO_2max. This could be due to psychological or physiological factors, as some subjects may not have been comfortable pushing themselves to maximal exercise tolerance (Hall *et al.* 2005). Furthermore, it has been reported that ~50% of subjects cannot achieve the expected plateau in VO_2 observable at the end of a "true" max test (Howley *et al*. 1995; Smirmaul *et al.* 2013). The VO_2 plateau at an apparent VO_2max may also be questionable as there are many submaximal plateaus in VO_2 during a CPET (Noakes 1998; Edvardsen *et al.* 2014). To account for this VO_2peak was determined to assess the maximal VO_2 achieved at the end of the test; this is often used as a clinical parameter of cardiorespiratory fitness as opposed to athletic performance (Balady *et al.* 2010). A submaximal parameter for aerobic performance is AT, which is not subject to individual effort. Responses to AT herein were all in a positive direction, albeit heterogeneous, in comparison to reported negative responses for VO_2max in the HERITAGE Study. Thus heterogeneity in VO_2max responses could be attributed to some degree to lack of achievement of "true" VO2max during a CPET, perhaps due to insufficient subject effort, something that is unlikely a possible explanation for the heterogeneity we observed in AT.

Characteristics of the training programme may contribute to heterogeneity in cardiorespiratory fitness responses. This may include how individuals respond to homeostatic stress; thus, this would impose a different stimulus on different individuals to a standardised exercise programme (Mann *et al.* 2014). To design the standardised training programme, maximum wattage achieved during CPET (at VO_2peak) was used to calculate training intensity. Wattage standardised at a percentage of VO_2peak or HRmax is the convention for EET prescription; however, this training intensity is not directly related to metabolic parameters, such as AT, thus individuals may respond differently to the stress imposed on the body by EET (Mann *et al.* 2013). Thus, it would appear that EET standardised to a percentage of AT would provide more homogenous perceived training intensities and EET responses. Moreover, other factors affecting training responses could be related to sleep, psychological stress, habitual physical activity, and perhaps diet (Mann *et al.* 2014). There must be balance between stress and recovery with an exercise training programme, and imbalance may lead to fatigue that may lead to maladaptation (Kreher and Schwartz 2012). In the present study rest, sleep and accurate determination of habitual physical activity was not recorded, thus these factors may have led to variation in training-induced responses.

It has been suggested that improvements in high-intensity aerobic performance may not be directly related to maximal oxygen transport capacity (Vollaard *et al.* 2009). Thus,

submaximal parameters such as AT may be regulated independently of maximal parameters (VO_2max). In the present study, subjects showed more positive responses in AT than they did in VO_2max supporting this suggestion. Positively, these data imply that submaximal aerobic capacity may be able to be improved with EET regardless of lack of improvement in maximal parameters. This relates to the positive impact of EET on improvement of physical function and activities of daily living as opposed to emphasising improved athletic performance.

VO_2max responses are thought to be beyond measurement error, however, it cannot be ruled out that another factor accounting for variability in VO_2max responses may have been reliability of parameters assessed during CPET. In contrast to other studies (Bouchard *et al.* 2012), only one CPET was performed before and after training, thus the technical error and day-to-day variability was not able to be assessed in this cohort. Additionally, there may have been a familiarisation effect in which people improved their CPET performance as they were more familiar with the test procedures.

5.2.2 Variation in hypertrophic responses to RET

The RET programme herein aimed to achieve strength and hypertrophy, and corresponded to ACSM recommendations of CON and ECC motions over single and multiple joints using large muscle groups (ACSM 2009). Dynamic CON muscular strength improvement is greatest with the combination of CON and ECC actions are trained, as exercised in this study. In the present study, in response to RET, all subjects increased in 1-RM strength and MVC strength assessed by peak isometric torque, as well as peak isokinetic torque. To enhance the RET programme investigate herein, it may have been beneficial to include a component of ISOM exercise and/or supramaximal ECC actions, which have been reported to provide an added benefit; all of CON, ECC, and ISOM muscle actions are recommended for RET progression in novice, intermediate, and advanced individuals (ACSM 2009). However, the primary aim of this study was not to achieve optimal functional improvements, but to deliver a stimulus that was sufficient to induce hypertrophy, *on average*, and explore the heterogeneity of this response. Although the training period duration was short, significant hypertrophy could be observed following RET (quadriceps CSA), reflecting the efficiency and specificity of the 4-wk RET element of the programme. Muscle hypertrophy with RET increases linearly with time, exceeding 6 months of training (Narici *et al.* 1996). In the present study, training was just four weeks, thus the duration of the training may not have been enough to produce as large gains in lean muscle mass as those reported for longer RET studies. Additionally, some participants may take longer to achieve significant gains in lean mass, and often longer studies (e.g. 20 weeks) do not report lean muscle mass changes after just four weeks. Increases in fibre areas are reported to be a delayed response to training and may not be observable until seven weeks of training (Green *et al.* 1999). However, Brook *et al.* (2015) reported that hypertrophic gains from lower-body RET peaked after 3 weeks of training, assessed by VL MT (and matched by peak MPS responses at this time). Therefore, the hypertrophic responses in the present study may not be optimal in all individuals.

Herein, there existed an undeniable heterogeneity in responses to parameters of hypertrophy (total lean mass, quadriceps CSA) and strength (1-RM and MVC), consistent with findings from Raue *et al.* (2012). In this cohort, 661 genes were identified to be responsive to RET, correlating with training-induced muscle size and strength adaptations,

thus compiling a “transcriptome signature” of RET-induced adaptations. Furthermore, the young male participants showed a significant transcriptome response to RET in both the trained and untrained states, suggesting that previous training status does not explain the heterogeneous responses. Although in this study, participants did not perform structured RET or EET, their historical training status was not thoroughly assessed. However, previous history of strength training status has been reported to have an impact on strength and hypertrophic responses to RET with a general acceptant that added improvements in muscular strength and hypertrophy are more limited in previously trained individuals (Hakkinen *et al.* 1994). Indeed, Ahtiainen *et al.* (2003) reported a differential hormonal response to 21 weeks of strength training between strength-trained and untrained (no previous strength training history) individuals. The group confirmed that MVC and 1-RM strength increased more in the untrained than the previously strength-trained individuals, although both groups showed statistical improvements. Furthermore, muscle hypertrophy (quadriceps) significantly increased more in the untrained group than the trained. Hormonal changes were not assessed in the present study, which may be another factor contributing to heterogeneity in responses, but thus there is scope for future work in this population. Combined, these data present inconclusive effect of pre-training status on hypertrophic and strength responses to RET, although this factor should be tightly controlled for in future studies to ameliorate any added contributions in variation.

Other factors may contribute to heterogeneous responses to RET such as neurological adaptive factors. The results herein show that individuals that showed lack of improvement in hypertrophy still improved strength with training (1-RM and MVC). This could be explained by significant mean improvements in muscle activation (torque/CSA) shown in this cohort. Neural factors largely contribute to early responses to RET, whereas muscular hypertrophy comes later in the training process, both of which contribute to the strength phenotype (Moritani and deVries 1979). Ahtiainen *et al.* (2003) reported that after 21 weeks of RET, subjects (previously trained and untrained) increased in 1-RM-to-CSA ratios, analogous to torque/CSA improvements described in the present study. Some of the increased quadriceps torque produced by MVC may be due to reduced antagonist muscle activity brought about by RET (Narici *et al.* 1996). Thus, greater 1-RM-to-CSA ratios in strength-trained individuals, compared with previously untrained individuals, may be due to a greater ability of strength-trained individuals to produce greater maximal voluntary activation of the quadriceps and thus have a greater ability to increase neural drive with RET (Ahtiainen *et al.* 2003); this improvement may also be due to beneficial architectural adaptations (Aagaard *et al.* 2001). In the present study, participants had mixed prior training history. In this respect, heterogeneity in strength responses to RET in the present study may be impacted by previous training status. However, regardless of hypertrophic responses, the fact that all individuals were able to improve 1-RM strength shows that functional capacity is a universal benefit to RET, reflecting improved physical capability even in the absence of hypertrophy.

Not accounted for in most studies assessing responses to RET is muscle architecture. Hypertrophy has been reported to lead to an increase in the pennation angle of muscle fibres, and greater pennation angles are observed in hypertrophied human *triceps brachii*, compared with normal muscle (Kawakami *et al.* 1993). Indeed, in the present study, pennation angle was significantly increased in response to RET. Furthermore, training-

induced changes in PA were positively correlated with changes in MT, therefore it is likely that changes in PA are responsible for changes in MT and thus VL hypertrophy. Increased pennation angle is thought to decrease the useful vector of force running along the tendon, however, it allows for packing of a greater number of contractile elements in parallel along the tendon, generating more force per unit area (Kawakami *et al.* 1993; Narici *et al.* 1996). Mechanotransduction relays signals from mechanical stress to drive skeletal muscle damage-induced adaptation to training via intramuscular signalling proteins (Martineau and Gardiner 2001). Taken together, it could be hypothesised that low hypertrophic responses to RET may be due to failed mechanisms to improve muscle quality and induce beneficial architectural adaptations.

Another factor that may have affected individual muscle hypertrophy responses in the present study could be dietary protein to support muscle growth. Lack of dietary protein may hinder the increased rate of muscle protein synthesis from RET that leads to muscle hypertrophy (Biolo *et al.* 1995), and starvation and dieting may ultimately deplete glycogen reserves, leading to protein deficiency and therefore loss of lean muscle tissue (Wagenmakers 1998). However, 4 weeks of lower-body RET has been shown to significantly increase muscle strength (1-RM and MVC) and hypertrophy (VL MT and quadriceps CSA) in previously untrained men, with no effect of additional protein supplementation (Boone *et al.* 2015). To explain any potential error due to dietary effects on responses, subjects were asked to keep their normal habitual diet relatively consistent throughout the entire study period. We also administrated 4-day diet diaries to all participants to complete during the wash-out and training phases of the programme, which will be analysed in future work. To control for this effect, in future, the study could provide dietary guidelines uniform across the cohort, or more robustly provide meals for participants throughout the programme. However, meal provision may result in additional, perhaps unessential, cost for the study.

5.3 Mode-specificity of exercise responses

5.3.1 Performance

In the present study, fitness outcomes of EET (AT) and RET (whole-body hypertrophy) showed a trend for a negative correlation, implying that high responses to EET and RET are independent of one another, with a gradation of response, e.g. intermediate responders to both training modes. Reasons for mode-specific responses may be due to the contrasting adaptations to these two training modes, while the numerous factors mentioned in the section above (genetics, diet, concurrent activity, etc.) may favour adaptation to one mode of exercise above another.

EET and RET result in distinct cardiovascular and neuromuscular adaptations. Specifically, EET leads to central and peripheral adaptations resulting in improved cardiovascular function and oxidative capacity of skeletal muscle; conversely, RET leads to increased muscle hypertrophy, motor unit recruitment capacity, and motor unit firing rate, all of which result in improved muscular strength and power. A combination of EET and RET (i.e. concurrent training) thus seems an obvious method to gain maximal physiological benefit from exercise training; indeed, concurrent training has been reported as an efficient method to gain both cardiorespiratory and neuromuscular benefits of exercise, shown in elderly populations (Wood *et al.* 2001; Cadore *et al.* 2014). However, in other studies, EET and RET-induced adaptation are reported to constrain each other, known as the “interference

effect" (Chtara *et al.* 2008). The link between these two training modes is therefore inconsistently demonstrated.

Out with debate about the benefits of concurrent exercise training, it is unclear, indeed to my knowledge, unexplored, whether an individual can be a high or low responder to both EET and RET (responder status is a global trait) or whether individuals have a predisposition to preferentially improving with one training mode over another (responder status is a mode-specific trait). Indeed, the definition of a "response" to training is loosely defined and is dependent on the volume, intensity, and duration of training and not least the endpoint that is assessed. Churchward-Venne *et al.* (2015) identified reported "no non-responders" to RET, despite heterogeneous responses. However, this work was not exploring the same concept as that herein, and did not classify responders based on an expected physiological endpoint; rather a non-responder was defined as an individual who did not respond for any of a number of measured parameters. Furthermore, the additional dimension of health responses was assessed herein.

With respect to muscle physiology, fibre type may have a large influence on the responsiveness to training. In simple terms, type I ("oxidative") fibres are primarily associated with adaptations to EET, whereas type II ("glycolytic") fibres area more commonly associated with muscular strength and power adaptations. Furthermore, there appears to exist a mechanism by which fibres can undergo preferential hypertrophy in response to a specific training stimulus (Takekura and Yoshioka 1990). With RET, hypertrophy can be observed for both major fibre types (I and II) and subtypes (IIA and IIB), whereas the magnitude of hypertrophic responses appears to be fibre-type-specific. RET produces increases in muscle size largely due to an increase in the size of type II fibres (Trappe *et al.* 2004), and four weeks RET has been shown to increase muscle fibre area (Green *et al.* 1999). Thus, fibre-type compositional shifts may account for training-induced specific adaptations to EET or RET, and high responses to one training mode or the other could be attributed to pre-existing fibre type composition (Gollnick and Matoba 1984), much of which has a genetic foundation.

Other qualities of skeletal muscles are their aerobic capacities, and ability to improve aerobic capacity with training, i.e. mitochondria and angiogenesis whereby oxygen utilisation and delivery, respectively can be improved. Indeed, both the absolute number and size of mitochondria increase to maintain the oxidative potential. Prolonged submaximal EET is a major stimulus for both angiogenesis and mitochondrial biogenesis (Hudlicka *et al.* 1992). RET, in contrast to EET, is not continuous, and is less reliant on blood flow demands than EET, thus RET is presumed to have a lesser effect on angiogenesis. High responders to EET may therefore have a greater capacity to improve oxidative potential with training.

Contrary to the preconceived notions of EET- and RET-specific adaptations, some individuals may improve fitness regardless of training mode. For example, quadriceps CSA improvement was correlated with both EET and RET, although EET targeted the quadriceps muscle area. This parameter was thus not training-mode specific for individuals, suggesting that individuals had a greater hypertrophic capacity regardless of training stimulus. Phillips *et al.* (2013) reported that much of the exercise-adaptive signalling networks overlapped i.e.

those associated with improvements with EET and RET. Indeed, in some studies, RET has been shown to improve absolute increases in angiogenesis and mitochondrial biogenesis (more commonly associated with EET), which may be associated with improved metabolic rate and blood flow, possibly related to the cellular hypoxaemia that occurs with this mode of training (Gassmann and Wenger 1997). Indeed, cardiorespiratory responses to RET were heterogeneous, and although most individuals showed no change, one participant showed a greater change in VO_2max and AT with RET than EET (data not shown). Ozaki *et al.* (2013) reviewed cardiorespiratory responses to RET, concluding that RET was able to concurrently improve VO_2max and hypertrophy in both young and old participants. These observations highlight that training responses may be more complex than preconceived and individuals may have different limitations to improving both cardiorespiratory fitness and hypertrophy.

5.3.2 Health

The mode-specificity of health responses were assessed for primarily for parameters of metabolic health, vascular health, and body composition. Overall, many of the improvements were specific to either EET or RET with individuals showing no preference for a particular type of health parameter. Overall, there was no clear link between most parameters of health and their responses to the two training modes.

Metabolic health

Metabolic responses did not show mean improvement following training. Sasaki et al. (2014) did not observe an effect of 4 weeks HIIT or continuous endurance training (EET) on insulin responsiveness (insulin AUC), consistent with the present results. In fact, RET led to a reduction in insulin AUC. However, this response was not related to that to EET, thus implying that this effect could not be ameliorated by subsequently or concurrently taking up EET.

Physically trained individuals have been shown to have a blunted insulin response to a glucose challenge, albeit normal glucose tolerance (Heath *et al.* 1983). In the present study, although the majority of individuals (6/8) improved insulin sensitivity with RET, a small proportion (2/8) did not. These individuals were not physically trained, although they were of a healthy status. This is thought to be due to existing mechanisms of glucose responsiveness already in place for individuals with adequate lean mass, that may not require further anatomic adaptation. Thus, improved muscle quality vs. quantity may underlie heterogeneity in adaptive metabolic responses to exercise training.

Vascular health

Vascular parameters were subcategorised into haemodynamic and biochemical factors affecting vascular health. Haemodynamic factors, such as blood pressure, resting HR, and vascular reactivity were assessed in relation to the two training modes. Of all the vascular parameters assessed, DBP was the only parameter to show a trend for an improvement with RET, whereas otherwise there were no significant improvements. Biochemical parameters presented are those of plasma cholesterol (HDL-C) and CRP (inflammatory marker). HDL-C and CRP did not significantly improve with either training mode.

In 2005, Rakobowchuk *et al.* completed a 12-week progressive RET study in young males, at a slightly higher intensity (80% 1-RM, 6-12 repetitions, ~3 sets) than performed herein. This

study showed significant improvements in DBP (from 6 to 12 weeks of training) and pulse pressure (PP), whereas SBP, MAP, resting HR, and carotid intima-media thickness did not significantly improve (lower) following the training. This is in line with results from the present study, whereby no significant differences were observed in SBP, MAP, or resting HR following 4 weeks of RET compared to baseline week 0, whereas DBP showed a trend for a positive improvement.

When using correlation analysis for all of these vascular parameters, no relationships were observed between the two training modes. These data indicate that training mode specificity did not underlie differential effects for individual responses in vascular responses to training.

Body composition

Body composition was also assessed in relation to training-mode specificity of responses, specifically percentage body fat (adiposity) and A/G ratio, which are related to cardiometabolic risk. Although EET and RET (statistically) showed numerical improvement in whole-body adiposity, no relationships were found between EET and RET responses to this parameter. Furthermore, RET significantly improved A/G ratio, highlighting the multiple positive effects the 4-week RET programme on improving body fat and distribution, implying the responsiveness of these young males in body composition to RET. Differential responses between the two training modes may underlie the differential stimuli (i.e. volume and intensity) these two training modes have on the body, which may differentially affect mechanisms of energy utilisation and hormonal regulation (Ahtiainen *et al.* 2003). Between EET and RET, EET is associated with higher energy expenditure (Nordby *et al.* 2015), thus these results are somewhat unexpected. However, it may be the group on average had a particular RET-specific limitation to improving body fat. Furthermore, subjects may expend more energy inter-individually, dependent on stress imposed on that participant, for performance and recovery, which may have affected mean average values. Calorie intake and consumption were not controlled or measured in this study, thus the effect of metabolic rate at rest and during exercise was not accounted for. Thus, EET is likely to contribute to greater energy expenditure during exercise, whereas RET may lead to an increased resting metabolic rate due to increased metabolically active tissue (i.e. muscle hypertrophy).

Overall, it was hypothesised that categories of health parameters may be differentially regulated on average in response to the different training modes, such as mean improvements in metabolic parameters with EET and vascular parameters with RET. Instead, within the subgroups, EET and RET had specific effects on different parameters. These data implied that EET and RET may improve various parameters of health through vastly different mechanisms. This is not surprising due to the different stimuli the two training modes elicit on the human body in the 4-wk period; however, the mechanisms underlying these effects may be more complex than once thought.

Furthermore, it was hypothesised that different parameters of health may be differentially regulated in individuals in responses to training mode. For example, some individuals may improve in one parameter with RET and show no improvement with EET, and vice versa. Alternatively, individuals may respond to a parameter irrespective of the training mode.

Overall, on an individual level, there appeared to be no clear relationship between responses to these health parameters with the two training modes, suggesting that the parameters assessed may be independently regulated by different training modes, as well as showing inter-individual variation in the magnitude of health responses irrespective of improvement in fitness.

An important point to make is that these health effects on training were short-term and occurred after 4 weeks. What is unknown in these participants is the effect that further training on these parameters. A novel element of this study was the "wash-out" period, and in larger-scale studies, this will allow us to determine the detraining effect of both EET and RET, i.e. how long it takes for training effects to wear off.

5.4 Effect of improving fitness on improving health

5.4.1 Metabolic health

Improved cardiorespiratory fitness is associated with improvement in insulin sensitivity and glycaemic and insulinaemic responsiveness (Ivey *et al.* 2007). However, in the present study, improvement in AT with EET was not related to improvement in HOMA-IR, glucose tolerance (AUC), or insulin response (AUC) with this training mode. In accordance, Vollaard *et al.* (2009) showed that VO_2max responses to EET were not related to changes in metabolic parameters. They showed a mean average improvement in aerobic capacity, metabolic control, and greater reliance on lipid metabolism during submaximal exercise, as well as heterogeneity in responses, but importantly, they showed that low responders for VO_2max were not low-responders to other variables, and training induced adaptations in other variables of performance were no different to high responders, such as fuel utilisation and oxidative capacity. Furthermore, the individuals in the current study were of healthy status (no diabetes), and much of the impaired insulin regulation associated with diabetic risk is due to glucotoxicity (detrimentally high glucose) (Poitout and Robertson 2002), which was not present in the subjects involved in this study. Thus, improved cardiorespiratory fitness may not be directly related to improved beta-cell function, although EET is related to positive responses.

The exact mechanism of how cardiorespiratory fitness contributes to beta-cell function is not precisely known, however, work in animal models has shown that exercise enhances GLUT2 transporter content, metabolic signalling (Akt and glucokinase activity), and increases mitochondrial respiration (Kiraly *et al.* 2008; Delghingaro Augusto *et al.* 2012). EET has also been shown to increase fat oxidation (Malin *et al.* 2013). Insulin action is thus related to mitochondrial function, providing rationale to investigate the relationship between cardiorespiratory responses to EET and parameters of insulin sensitivity. Inherent defects in mitochondrial function are thought to be a causal factor in the pathology of insulin resistance (Malin *et al.* 2016) and EET is associated with responses of mitochondrial biogenic and angiogenic capacities to training. As parameters of oxidative metabolism (i.e. mitochondrial capacity/volume) were not directly assessed in the present study, muscle concentrations in these metabolic factors may differ between responders and non-responders to training. Additionally, hormone levels of oestrogen and testosterone act on mitochondrial function, which ultimately affect fuel selection; testosterone levels positively correlate with insulin sensitivity, as well as VO_2max and expression of oxidative phosphorylation genes (Pitteloud *et al.* 2005). Thus, there is a link between hormonal

regulation, mitochondrial function and insulin resistance. Hormonal responses to training were not assessed in this study, which could provide an additional explanation for training-induced responses.

Lean mass improvements in the RET programme showed no relationships with improvements in glucose or insulin fasting values or responsiveness to a glucose challenge (AUC). Many literature reports indicate that RET leads to improved mitochondrial capacity, and skeletal muscle atrophy leads to mitochondrial dysfunction in ageing (Melov *et al.* 2016). Reduced oxidative capacity is thought to impair skeletal muscle insulin action, such as in T2DM and in ageing, and lean muscle mass is proposed to be directly related to HOMA-IR (Fukushima *et al.* 2016). However, these previous study took into account means analysis and not on the level of the individuals. Thus, lean mass improvements may not be directly related to improved insulin sensitivity, and the metabolic properties of newly synthesised muscle have not been defined. The timescale of improvements in insulin sensitivity with improved lean mass are usually reported as longer than the 4 weeks in the present study, and first phase (acute) insulin secretion in response to exercise is much more well-defined than second-phase (longer lasting) insulin secretion (Malin *et al.* 2013). Furthermore, exercise responses are likely to be at least partially attributed to exercise-induced energy deficit (Hagobian and Braun 2006). Thus, different diets in different individuals (e.g. macronutrients and frequency/timing of consumption) may differentially impact on the magnitude of training-induced effects on lean mass and insulin sensitivity.

5.4.2 Vascular health

Vascular health encompassed haemodynamic responses and biochemical responses to training. Investigated here is whether improvements in vascular parameters of health related to improvement in cardiorespiratory fitness with EET or improved lean muscle mass with RET. For EET, haemodynamic parameters showed significant positive correlations with cardiorespiratory fitness, such as blood pressure (MAP and DBP) and resting HR. In contrast, RET showed a similar correlation of lean mass responses with DBP, but not MAP or resting HR responses. Furthermore, training-induced responses in vascular reactivity were negatively correlated with improvement in lean mass with RET. However, no relationships were observed with biochemical vascular parameters and AT or lean mass.

Arterial compliance has been shown to be enhanced following EET (Vaitkevicius *et al.* 1993). However, mixed results have been reported following RET; despite some reports of improved vascular responsiveness after RET (Phillips *et al.* 2012b), resistance-trained individuals have also shown reduced whole-body arterial compliance and augmented age-associated central arterial stiffening (Bertovic *et al.* 1999), as well as no change following short-term training (Rakobowchuk *et al.* 2005). These data may explain some of the results in the present study whereby improvements in lean mass were negatively correlated with health improvements such as in vascular reactivity. Furthermore, it cannot be ruled out that some subjects had an increased recovery rate from the acute exercise stimulus, thus an intervention such as feeding may produce longer lasting effects (Phillips *et al.* 2012b).

A parameter not assessed in this study is capillary density, which increases with EET and RET. Jensen *et al.* (2004) reported that four weeks of acute RET (five bouts/wk ~1 h knee-extension exercise) caused an increase in leg muscle capillarisation, shown by $VEGF_{165}$

(isoform) mRNA expression. It is hypothesised that exercise training-induced increases in capillary density are to maintain or increase mean oxygen transit time, enhancing oxygen delivery by maintaining oxygen extraction (a-v O_2 difference) even at high rates of muscle blood flow. To explore this hypothesis, total RNA can be extracted from a skeletal muscle biopsy to determine physiological mechanisms of adaptation to exercise. For example, when relative VO_{2max} rate is measured by cycle ergometry, it shows a strong correlation with the number of capillaries per fibre of the *m. vastus lateralis*. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that induces endothelial cell proliferation and is involved in vascular network formation. VEGF expression is therefore an indicator of capillarisation, and exercise has been shown to increase VEGF mRNA expression. This is consistent with the notion that exercise-induced capillary growth occurs by angiogenesis.

Furthermore, the training mode may affect the extent of exercise-induced muscle capillarisation. For example, Hoier *et al.* (2013) reported that moderate-intensity EET leads to an increase in mRNA of several angiogenic factors, greater than the increase with high-intensity intermittent training. Furthermore, moderate-intensity training lead to greater VEGF secretion than high-intensity intermittent training. The relationship between capillarisation and exercise responder status was consolidated by Lessard *et al.* (2013), who showed that capillary density was 50% higher in HRT compared with LRT rates following EET. Further analyses on muscle biopsy samples collected during this study on capillarisation and VEGF gene expression may therefore further explain training-induced vascular responses and the relative contribution of this to whole-body physiological responses (e.g. VO_2max and/or hypertrophy).

It is known that the impact of exercise is greater, *on average*, in subjects with raised cholesterol, BP, or glycated haemoglobin (Green *et al.* 2008). Furthermore, the HERITAGE study showed that improvements in some parameters correlate to some degree with baseline values, such as HDL-C and SBP (Bouchard and Rankinen 2001). As all subjects recruited in the present study were of healthy status, baseline levels of these parameters may indicate no improvement, when hypertensive or hypercholesterolaemic subjects may show greater responses. Additionally, improvements may not have been great enough to construct accurate correlations of training effects, and there may not have been sufficient subject numbers to demonstrate a significant effect. Furthermore, the exercise training may not have been a long enough stimulus to produce significant vascular improvements, such as vascular responses of vascular reactivity.

Exercise training is known to produce mean improvements in inflammatory health, such as reducing proinflammatory markers and increasing anti-inflammatory markers. Lessard *et al*. (2013) showed that an altered inflammatory response to training (negative) was present in low aerobic response to training (LRT) rat model. Responder status did not correlate with baseline plasma inflammatory markers, whereas after exercise training (48 h) in LRT, TNF-α (inflammatory) was 85% higher, and TGFβ (anti-inflammatory) was 50% lower, compared with high aerobic response to training (HRT) rats. This indicates that these cytokines are regulated differently in LRT and HRT, and suggests that inflammatory signalling may play a role in the exercise training response phenotype. Unfortunately, we were not able to replicate these results in the present study using CRP as an inflammatory marker; however,

future analyses will include bioassays on plasma samples for IL-6 and TNF-α to contribute to the data regarding inflammatory responses in relation to fitness responses.

5.4.3 Body composition

Adipose tissue is a highly dynamic organ and is metabolically and hormonally active (Labbe *et al.* 2016). The amount, quality, and location of excess fat are all important in cardiovascular risk induced by obesity. In the present study, reduction in both total body fat and abdominal fat were related to improvement in cardiorespiratory fitness (AT) with EET. Importantly, improvement in cardiorespiratory fitness with EET was able to reduce cardiometabolic risk indicated by loss of abdominal fat. This reflects the effect of cardiorespiratory fitness on improving health by fat loss, or vice versa, whereby fat loss may lead to improved fitness with EET. Proposed mechanisms for this potentially reciprocal relationship include the positive effect of EET on inflammatory factors and/or energy restriction (Hamer and O'Donovan 2010).

Increases in lean mass with RET was also related to improvement in total body fat, but not specifically abdominal fat or A/G ratio. This may reflect the increased resting metabolic rate of those individuals that gained lean mass with RET (Byrne and Wilmore 2001). Conversely, low responders to RET may also be low responders to loss of body fat with RET. Thus perhaps the risk of obesity (and associated disorders) is increased in these 'RET resistant' individuals.

Greater weight loss is observed for exercise at higher intensities, shown in a dose-responsive manner; thus more fat loss occurs with greater exercise training intensities (Slentz *et al.* 2005). Important differences between the EET and RET periods of the study include energy expenditure. On average, the EET sessions were continuous and lasted for 45–60 min, whereas RET lasted for 30–45 min including rest periods between sets. Thus, energy expenditure is likely to be a lot greater for EET than RET. This may contribute to the amount of energy used during the training session, which will have accumulated over the four week periods. These differences may contribute to differential fat loss provided that diet was consistent. Reductions in body fat and metabolic risk factors are likely to be directly associated with enhanced aerobic function via increased mitochondrial content and/or higher energy expenditure (Nordby *et al.* 2015), therefore the RET programme herein may not accumulate enough volume to reduce body fat and metabolic risk factors in subjects in this 4-week programme.

Myette-Cote *et al.* (2016) reported improvements in fitness to EET and RET in non-responders to fat loss with training in post-menopausal women. This indicates that improvements in fitness, i.e. AT with EET or lean mass with RET, can take place without associated fat loss. Thus, fat loss may not be a mechanism for improvement in fitness parameters with training in all populations.

5.4.4 Relationships between health parameters

Additional important observations made in this study were relationships between improvements between different health parameters. For example, fat loss is related to many different physiological parameters mentioned herein, and improvements in fat loss have been reported here with both EET and RET.

Although fat loss in general has positive effects on health, adipose tissue in itself is metabolically active, and obese individuals in fact have a higher basal metabolism than individuals of normal weight (Byrne and Wilmore 2001). In obese individuals, fat accumulation is believed to be a mechanism to protect the body from insulin resistance by removing lipids and glucose from the blood and storing this energy as adipose tissue, contributing to improved insulin sensitivity in these individuals (Ferrannini *et al.* 2008). Hypothetically considering these implications, fat loss from exercise (not accounting for lean mass) may therefore lead to a reduction in basal metabolism, which may lead to the acute effects of loss of insulin sensitivity. These data may partially explain "negative" responses in insulin AUC with RET, for example. Thus, these effects may also mask the health effects of improving lean mass with RET.

5.6 Study limitations & Future directions

5.6.1 Study design

Participants

A total of ten subjects were recruited for this pilot study. The cohort consisted of young healthy males of age 18–40 with no pre-existing medical history, such as diabetes, obesity, or hypertension and none were on any medications known to interfere with their inclusion or trainability. An obvious limitation to the present study was the size of the cohort. However, within the group, it was possible to show statistically significant improvements in the primary outcomes to both EET and RET (AT and muscle hypertrophy, respectively), as well as health responses, and identify heterogeneity in individual responses. These preliminary findings provide rationale for future studies that would aim to recruit a larger cohort to increase the statistical power of the findings presented herein, including the strength of correlation analyses and allowing quartile/ principal component analysis. An important fact to consider is that in this small sample size, some correlations could have been reported by chance. Conversely, additional relationships may also be identified with a larger group. Obstacles to recruitment of subjects included the significant time commitment of 14 weeks in total (2 × 4-week training periods + 6-week wash-out period). It is clear that a greater number of recruits would give greater reliability to the conclusions presented herein; nonetheless, the sample size selected was fitting to provide sufficient physiological trends in responses to exercise training in this cohort within this short-timescale pilot study. This provides rationale to include more subjects in the analyses for greater statistical power, thus in future larger scale studies, it may be possible to scale these results up with statistical significance of inter-individual findings. Hopefully, the results presented herein reflect those that could be achieved on a greater scale, albeit with the possibility of more complex statistical analysis. Additionally, once relationships have been confirmed on a larger scale, these methodologies could be applied to different clinical populations, such as diabetic and COPD cohorts.

Exercise training

The exercise training programme was able to produce significant improvements in the primary outcomes to both EET and RET in just four weeks. This shows that short-term exercise training can produce mean average improvements using the protocols presented herein. However, longer-term studies (e.g. 20+ weeks) may amplify these results, and could produce a timescale of responses, including peak of improvements in fitness and health.

This would be beneficial to confirm responder status to the different training stimuli, as well as determine whether non-responders to training can become responders over longer periods of time.

Compliance is a large issue in many exercise training studies. In the present study, of the 11 participants initially recruited, two completed one training mode only and one did not complete a single training mode (8/11 completed the full study).

This study explored only two (traditionally the most common) exercise-training modes. However, high intensity interval training has been shown to lead to many of the positive effects observed with EET and RET (Higgins *et al.* 2015). An attractive quality of HIIT is the low volume of exercise, and therefore associated time-commitment, that is required to produce these benefits, which may lead to increased compliance. Some of the compliance issues with exercise training may be due to individuals "not having the time" to train. Future studies could therefore, also explore responses to HIIT, with a view of this being a mode with potential to increase exercise participation and training efficiency.

In a similar vein, concurrent exercise training (both resistance and endurance training together) has been shown in some cases to be an efficient method of gaining both of the positive responses to EET and RET (Wood *et al.* 2001). However, this method has also shown mixed physiological responses in terms of resistance or endurance-associated adaptations. Wang *et al.* (2011) explored molecular signalling in exercise training using one bout of endurance then strength exercise; interestingly, genes related to oxidative metabolism/mitochondrial biogenesis (PGC-1α, PGC-1-related coactivator) were upregulated, not downregulated as hypothesised. This implies that endurance training may actually enhance the effects of RET, providing insight to the overlap between the two training modes instead of contrasting adaptations. In future, it may therefore be beneficial to investigate concurrent training in relation to single mode training such as EET or RET, with the potential for this to offer a beneficial stimulus, even for those classified as a 'non-responder' for a single mode.

Wash-out phase

The 6-week wash-out phase in this study was an important element of this study. The wash-out was designed with the assumption that the physiological effects of the first phase of training – hypertrophy with RET and VO_2max gains with EET – would be abolished in this time period, before commencement of the converse training mode. Measures of primary outcomes of both EET and RET were thus assessed before and after both training modes; CPET was performed before and after both EET and RET, and quadriceps CSA and DXA measurements were taken before and after the two training phases. Observably, training responses returned to baseline values, as a mean average effect. However, standard error in values was high due to the small cohort number, thus not all individuals may have reduced in training responses (AT after EET, and muscle hypertrophy after RET) during this training period to the same degree. Therefore, the validity of the wash-out period must be confirmed in future larger studies, in order to provide statistical power to this detraining effect. With respect to the present study in which a 6-week wash-out period was used, it has previously been reported that endurance-trained individuals exhibit a significant 6% decrease in aerobic fitness (VO_2max) following 2–4 weeks of inactivity (Coyle *et al.* 1986)

and losses in training gains of AT are significant at 6 weeks (Ready and Quinney 1982). Losses of the effects of RET have been demonstrated regarding isometric strength (up to 3.1% after 6 weeks; Shaver 1975) and muscle fibre cross-sectional area (7% after 3 weeks; Bangsbo and Mizuno 1987); however, differential physiological responses in muscle have been shown with respect to different cohorts, training durations, and lengths of training cessation (reviewed in Mujika and Padilla 2001). Thus, a longer wash-out period may be necessary in future studies to confirm that all participants return to statistically significant near-baseline values to before commencement of the subsequent training mode. Nonetheless, EET and RET are distinct in their specific physiological adaptations to training, and training effects were distinguished from a new baseline taken following the wash-out period.

Habitual physical activity and diet

Habitual physical activity and diet were assessed in this study by the use of IPAQs and diet diaries, which involved self-reported behaviours during the non-training and training periods (4 completed in total), which will be analysed in further study. However, there are many flaws in using self-reported data, such as inaccurate estimates of quantities of food consumed or physical activities performed. Additionally, participants may omit some entries, which would be impossible to account for. Other ways of controlling for diet include providing standardised meals to participants or providing dietary guidance, however, these methods may require to additional costs to the study. Physical activity monitoring via validated accelerometry would be a preferential method to assess this, although is associated with cost implications.

5.6.2 Scope for future work

Molecular analyses

Parameters not assessed in the present study are muscle protein synthesis (MPS) and associated cell signalling responses to training. EET and RET may induce differential MPS adaptations, due to different signalling responses (Atherton *et al.* 2005). MPS can be induced by RET, as well as additional protein nutrition, which can be measured and associated with corresponding muscle hypertrophy. Deuterium-based stable isotope methodologies, such as D_2O, have been validated for use in muscle protein subfraction synthesis (Wilkinson *et al.* 2013). D_2O can be measured on a day-to-day basis; upon oral ingestion, D_2O equilibrates with the body's metabolic pools and can be detected after two hours in humans, due to hydrogen-deuterium exchange in biological molecules. Enrichment can be detected by gas chromatography-mass spectrometry (GC-MS) or pyrolysis isotope ratio mass spectrometry (IRMS), which is more sensitive for detection of isotope enrichment. Fractional synthetic rates (FSR) of myofibrillar, sarcoplasmic, and collagen protein fractions can be used to measure MPS.

D_2O was administered to subjects in this study and muscle biopsy samples (~200 mg) were obtained from the VL muscle. This subsequent utilisation of novel mass-spectrometry-based techniques will allow for the characterisation of the MPS response to both EET and RET (methods described in Wilkinson *et al.* 2014). These results may distinguish between subfractional MPS responses to EET and RET; furthermore, it is hypothesised that low-responders to these training modes may exhibit blunted MPS FSR in the associated signalling pathways.

5.7 Overall conclusions

Overall, it was shown that EET and RET reached with distinct physiological endpoints in response to 4 weeks of training. Fitness responses are likely to be exercise training-mode specific as well as health responses, and there is unlikely to be a relationship between health responses to the two training modes. Furthermore, improvements in health could be explained in part by improvement in fitness parameters, indicating the associated mechanisms of improvement in fitness as partially causal for health improvements. Further studies on biological samples will hopefully be able to further elucidate these mechanistic relationships.

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Appendix

Appendix I: Recruitment letter

The University of Nottingham
UNITED KINGDOM • CHINA • MALAYSIA

Faculty of Medicine and Health Sciences
School of Medicine
Division of Medical Sciences & Graduate Entry Medicine
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xx[h] October 2015

«Name»
«Address1»
«Address2»
«Address3»
«Town»
«County»
«Postcode»

Dear «Salutation»

I am a research student at the University of Nottingham Medical School campus in Derby. I obtained your address from a list, held by the Royal Mail, of people who live close to the postcode of the hospital. I am writing to ask you to consider helping us with some research that is being undertaken at the Royal Derby Hospital site. We are exploring why some people respond favourably to certain types of exercise (i.e. they get fitter or stronger) while other people see very little improvement. This work is important because improved strength and fitness can aid recovery from illnesses and operations and is associated with a reduced risk of heart, lung and muscle-related conditions.

In order to continue this work, we need the help of 16 male volunteers (aged 18-40 y). These volunteers will need to attend the Medical School three times a week for 4-weeks for a supervised exercise training program of either cycling or weight training exercise. This will be followed by 6 weeks returning to normal physical activity before 4-weeks of the second type of exercise. A study day will be conducted before and after each time of exercise where we will assess your fitness, blood vessel and function, and metabolic health. All volunteers will also take a small amount of specially labelled water, which allows us to assess your muscle building capacity. This is done by taking blood samples, as well as small muscle samples. These are very well established techniques that have been used in research for many years. Study participation is an interesting and generally enjoyable experience and a number of our volunteers have asked to return for more than one study.

Participating would also mean that you get a very thorough medical check-up. In addition, using a special X-ray machine, we measure the amount of muscle and fat in your body and assess your bone density and we are happy to give you a copy of these results. You will also find out which type of exercise you most favourably respond to. We also give you an inconvenience allowance for your time.

If you are interested and would like more information on what participating involves, please complete and return the enclosed form. I would be delighted to provide you with a leaflet that contains a lot more detail about this study. Alternatively, if you would rather complete an electronic form or discuss this study further please send an Email, or telephone myself using the details at the top of this page.

Thank you for taking the time to read this and I do hope to hear from you soon.

Yours sincerely

Elisa Crombie BSc – MRes student

Appendix II. Volunteer information sheet.

The University of Nottingham

Clinical, Metabolic and Molecular Physiology Research Group

Healthy Volunteer's Information Sheet

Title of Project: Exploring the mechanistic basis, and plasticity of "exercise resistance" to improve human health (Exercise X-over).

Names of Investigators:
Ms Elisa Crombie, MRes Student
Dr Bethan Phillips, Assistant Professor
Dr Phil Atherton, Associate Professor
Dr Ken Smith, Principal Research Fellow

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

Background

The health of our body and our muscles is very much linked to our level of physical inactivity (sedentary behaviour) or activity (exercise). For example, physical inactivity causes our muscles to shrink in size and weaken and, if sustained, inactivity is linked to poor health outcomes. In contrast, exercise is linked with improved health e.g., stronger muscles and a healthier heart. Therefore it would seem that it is straightforward that we should be promoting exercise to everyone to improve health. Indeed, if you asked anybody they would almost certainly tell you that exercise is "good for you". In addition, anyone would expect that if you were to cycle, lift weights, or go jogging regularly, you would expect to get fitter and stronger. However, some interesting and unexpected findings have been reported in recent years… In very large exercise training studies with hundreds of participants, it has been demonstrated that some individuals fail to get fitter/stronger (1/3 people). Even more alarmingly, some people don't improve on expected health parameters (blood pressure, blood sugar control etc). These results reveal that while exercise, on the whole, generates positive health benefits, one size does not fit all. So it seems that people claiming they weren't making progress at the gym in terms of, say, running ability or muscle gains may have been right all along. Therefore, this project aims to investigate this phenomenon of "exercise resistance" by exploring multiple potential mechanisms behind "exercise resistance" and then trying to understand if this is a 'global' phenomenon or if someone who is exercise resistant for one type of exercise (e.g. cycling) may be responsive to a different training mode (e.g. weight training). In addition, this study will aim to assess if 'performance' gains (i.e. muscle mass, cycling ability) correspond with health parameters (i.e. blood vessel function, insulin sensitivity). Overall this project could have large implications for understanding the control of exercise adaptation and health outcomes in relation to "exercise resistance", and moreover, will form the basis for pre-identifying "non-responders"; paving the way for personalised medicine approaches in the future.

What does the study involve?

Screening Visit

Before you formally enter the study you will be asked to attend our facility (the Medical School, Royal Derby Hospital), for a screening visit to ensure that you are eligible for participation in this study. During this visit (approximately 60 minutes), we will explain the study in full and answer any questions you may have, then (i) ask you to sign a consent form for the study, (ii) take a blood sample (10-20ml), and (iii) conduct a health assessment - including measuring your height and weight and a trace of your hearts electrical activity (an ECG).

Study Overview

Once we have received the results of your blood tests from screening we will contact you to inform you of your results and confirm your inclusion into the study. If you are happy to proceed with the study we will organise a mutually convenient day on which you will attend our facility to begin the study. The study period will last a total of 14 weeks in duration, although 6 of these weeks are "wash-out" between exercise training types and thus have little involvement. Over this period, you will need to visit the facility 3x each week for 4 weeks to perform "Exercise 1" (either cycling or weight training). You will then have a 6-week "wash-out" period where we will ask you to return to pre-study physical activity levels. After this you will have another 4 weeks where you will be

asked to train at the facility 3x each week performing "Exercise 2"- the alternate exercise type to that performed in the first 4-weeks. Before and after each type of exercise training (weeks 0, 4, 10, and 14) you will be asked to attend our unit for an acute study, which will last approximately 6 hours (see details below). In addition, at weeks -2, 2, 8, and 12 you will be asked to attend our unit for a short visit so that we can take small blood, muscle and saliva samples. You will also drink some 'heavy water' that is slightly altered from normal drinking water so that we can use it to assess muscle-building capacity (see Acute Studies section below). These visits allow us look at how your muscle is changing over the course of the exercise training regimes. Before your first exercise regime and at one point during each training period we will ask you to fill in a physical activity questionnaire and complete a 4-day diet diary- this is so we can gain information about your habitual diet and physical activity behaviours and try to ensure you return to them during the "wash-out" phase.



Figure 1. Study Overview Scheme

Acute Studies

You will need to attend for the acute studies at ~0900h fasted from midnight with water *ad lib*. The acute studies will begin with small blood, muscle and saliva samples will be taken at this time from with the blood draw from an arm vein and the muscle biopsy from your *m.vastus lateralis*- a muscle at the front of your thigh. At this time you will also be asked to drink some 'heavy water'. This water contains a stable isotope tracer (deuterium in place of hydrogen, but is not radioactive), which is slightly different from normal water, but it is perfectly safe. The muscle biopsy will be taken after an injection of local anaesthetic to numb the area. After the local anaesthetic a trained clinician will make a small (0.5 cm) cut through the skin and underlying muscle sheath then take the biopsy (a small piece about the size of an orange pip) with a special set of forceps. With the local anaesthetic you may feel pushing during this procedure but no pain. A stitch is placed in the skin afterwards to aid healing. A tight bandage will be placed around the thigh after the biopsy and should remain in place for ~12-hours to speed healing.

After this we will perform an oral glucose tolerance test (OGTT) which tests how sensitive your body is to sugars. A small cannula (thin plastic tube) will be placed into one of the veins in the back of your hand to allow us to take multiple blood samples. This hand will then be placed into a warm box for 2-hours. After we have taken an initial blood sample, we will ask you to drink 75g of glucose dissolved in 250mL water. We will then take a blood sample from the cannula every 15-min for 2-hours.

A DXA scan will measure your body composition and leg muscle mass, and an ultrasound will look at your muscle architecture (fibre length and thickness). The ultrasound is similar to the scan carried out to image babies during pregnancy and involves some jelly and a probe being placed on your thigh. A leaflet describing the DXA scan in more detail is attached. After this your resting heart rate and blood pressure will be recorded.

After this we will assess the function of your blood vessels in a number of ways: We will perform something known as flow mediated dilation (FMD)- this simply involves measuring the blood flow in your brachial artery using ultrasound (jelly and a probe) before inflating a blood pressure cuff and again measuring your blood flow when the cuff deflates. After this we will feed you a standard light lunch before giving you 30-min of rest.

After the rest period you will perform a cardio-pulmonary exercise test (CPET). This test involves cycling on a static bike with the intensity getting progressively harder until you cannot cycle anymore. During this test you will

wear a facemask so that we can measure the amount of oxygen you are using. Again this test will give us information to set the intensity of your cycling training. After this you will participate in a battery of simple muscle function tests to assess your strength, power, force, balance and gait speed. These tests will also provide us with information needed to set the intensity of your weight training.

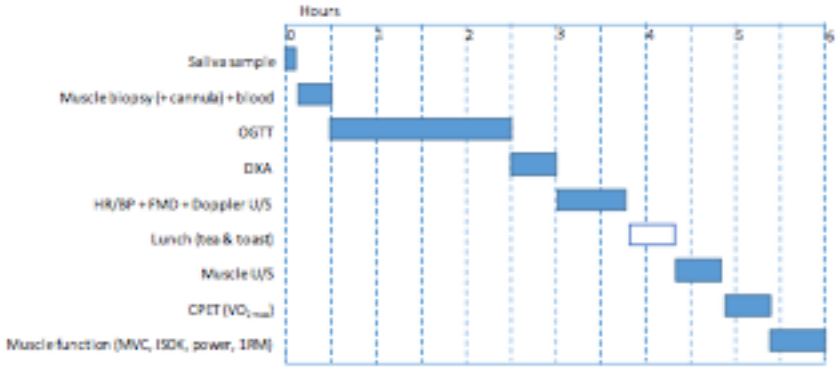


Figure 2. Acute Study Scheme

Exercise Training

Both of the exercise types will be performed for 4 weeks, 3x each week with each session last approximately 1-hour. "Exercise 1" is also known as TREND – traditional endurance training, and will comprise 60-min cycling at a moderate intensity (60% of maximal effort) as determined by the CPET test in your acute studies. "Exercise 2", also known as RET – resistance exercise training, will comprise whole-body weight training using specialised machines. You will perform 2 sets of 12 repetitions across 6 exercises (3 upper and 3 lower body) at 70% 1-RM (repetition maximum) as determined by the strength tests in your acute studies.

Why have you been chosen?

Primarily because you have expressed an interest in taking part in the study and you have met with the entry criteria and do not have on-going medical problems. We will be recruiting healthy, recreationally active males aged 18-40 years.

Do you have to take part?

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

What do I have to do?

You are requested to fast on the night before, and avoiding heavy exercise for 48h prior to your acute study visits to the laboratory. We will provide you with a recommended dietary intake for the study period that we would like you to adhere to where possible. This will follow a standard balanced diet according to current recommended daily allowances for carbohydrates, fats and proteins, and will help us to limit the impact of dietary intake upon the outcomes of study.

What is the drug or procedure that is being tested?

This study is assessing the impact of traditional endurance training (TREND) vs. resistance exercise training (RET) for improving indices of health, fitness and physical function. We are looking to assess if people who do not respond well to one type of exercise for the expected outcome (e.g. muscle mass with resistance exercise training) are able to respond well to a different type of exercise, and if level of response is linked to health benefits.

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

Research studies often involve some risks, not all of which may be currently known. In the light of our experience, muscle biopsies are well tolerated by all our volunteers. Scarring is hardly perceptible and fades with time. However, the procedure may cause mild pain in some volunteers, but with appropriate local analgesia this is minimal; indeed data from a previous audit of the procedure demonstrated that 83% of our participants experienced less than or the expected discomfort during the muscle biopsy and all respondents said that they would or may be willing to participate in a similar study in the future. Some discomfort (muscle tenderness and stiffness) may be felt for 2-3 days afterwards but mild painkillers will usually effectively abolish this. There is a slight risk of infection at biopsy sites, however all procedures are performed under sterile conditions to minimize any risk of infection. Damage to small nerves of the skin during the biopsy is possible; however these nerves grow back with restoration of normal sensation, without difficulty. We will provide you with a short, anonymised questionnaire about your experience of the biopsy procedures during this study should you wish to respond.

The risks involved in venous blood sampling are very small. Occasionally, however there may be bruising due to leakage from a blood vessel on withdrawal of the needle, but this is uncommon with good practice. The amount of blood taken during the acute studies in this project is much less than if you donate blood.

What are the exclusion criteria for this study?

We are recruiting healthy men between the ages of 18 to 40 years, who are of normal weight or slightly overweight. If you have been a subject in any other research study in the last three months which involved: taking a drug, being paid a disturbance allowance or having an invasive procedure (e.g. blood sample >50ml) you would not be eligible to take part.

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

What are the possible disadvantages and risks of taking part?

Healthy volunteers have been chosen for this study; however, it is possible that the routine tests could detect unknown health problems. Should this be the case you will be informed and advised to attend your GP practice for further management. Your GP will be notified. Be advised, however, that future insurance status e.g. for life insurance or private medical insurance, could be affected by these diagnoses.

What if something goes wrong?

In the unlikely event of a complication following the study or that you should wish to voice grievances, in the first instance please contact the clinician involved in the execution of the study or the lead investigator, Dr Beth Phillips (T: 01332 724731; E: beth.phillips@nottingham.ac.uk). If you are still dissatisfied then you should direct these concerns to the Administrator, University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee, c/o School of Medicine Education Centre, B Floor, Medical School, QMC Campus, Nottingham University Hospitals, NG7 2UH, email: louise.sabir@nottingham.ac.uk .

Will my taking part in this study be kept confidential?

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

We will follow current ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected about you will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by people authorised to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you that leaves the University will have your name and address removed (anonymised) and a unique code will be used so that you

cannot be recognised from it, with the exception of a letter sent that may be sent to your GP advising of your participation in this study if abnormal results need to be disclosed.

If you agree, your personal data (address, telephone number etc.) will be kept for 5 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality and only direct members of the research team will have access to your personal data.

What will happen to any samples I give?

We will use the blood and biopsy samples to see how your muscles respond to different types of exercise. We may also use your muscle samples to generate cell-culture samples so that we can further investigate mechanisms involved in muscle health and disease outside of the human body. We will also seek your consent for any samples remaining after analysis for this study has been completed, to be stored and used in future research. This is optional and you will be asked to give separate consent for this. The samples will be securely stored with a code unique to you at the University of Nottingham under the Universities Human Tissue Research Licence (No. 12265).

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

Will any genetic tests be done?

In the current study we will collect blood samples that can be used to obtain information about your genes (RNA and DNA), however this analysis will not give us information on disease prognosis and samples will not be subject to genetic manipulation.

Who is funding the research?

This research is funded by The University of Nottingham.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Faculty of Medicine & Health Sciences Research Ethics Committee.

Contact for Further Information

Thank you for taking part in the study. For further information you can contact Ms Elisa Crombie (Research Student) on 01332 724685 or at stxec4@nottingham.ac.uk, alternatively contactable at Clinical, Metabolic and Molecular Physiology, University of Nottingham, School of Medicine, Division of Graduate Entry Medicine and Health, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT.

Appendix II. Volunteer screening sheet

The University of Nottingham

Clinical, Metabolic & Molecular Physiology Research Group

Title of Project: Exploring the mechanistic basis, and plasticity of "exercise resistance" to improve human health (Exercise X-over).

Name of Investigators:
Ms E Crombie Dr P Atherton
Dr B Phillips Dr K Smith

Thank you for your interest in this study. I would be grateful if you would complete and return this form and a member of our research team will contact you as soon as possible.

FULL NAME (Including title):

ADDRESS:

POST CODE:

TELEPHONE NUMBER(s):

EMAIL:

DATE OF BIRTH:

Are you taking any regular medication? **YES / NO**

If yes, please list (including contraceptives/HRT):

Do you have a medical history of:

Diabetes	YES / NO	High Blood Pressure	YES / NO
Asthma	YES / NO	Heart Disease	YES / NO
Stroke	YES / NO	Epilepsy	YES / NO

Any other medical problems (please list below)? **YES / NO**

Do you consider yourself to be sedentary: **YES/ NO**

HEIGHT: **WEIGHT:**

Signature: **Date:**

Please note: Information submitted on this form will be kept **strictly confidential** and will not be shared with any additional parties