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THE EFFECT OF EXERCISE TRAINING STATUS DURING ACUTE HYPOXIA ON SUBSTRATE METABOLISM

(Dissertation submitted under Physiology and Health)

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ABSTRACT

Substrate metabolism fluctuates at rest and during exercise, with further changes as exercise intensity increases. During hypoxia, the reduction in available oxygen (O_2) will cause further alterations to substrate metabolism from the challenge that is placed on the metabolic pathways. It is currently unclear whether training status influences changes in substrate metabolism during exercise in hypoxia. The purpose of the study was to investigate the effect of training status during hypoxia on substrate metabolism at rest and during exercise. Twenty healthy male participants were used for the study; 8 untrained (20 ± 1.5 yr, 1.78 ± 0.08 m, 82.1 ± 11.2 kg, VO_{2max} 32.6 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$), 8 endurance trained (28 ± 5 yr, 1.81 ± 0.06 m, 73.3 ± 7.5 kg, VO_{2max} 46.4 ± 7.2 $ml \cdot kg^{-1} \cdot min^{-1}$) and 4 sprint trained (22 ± 3.3 yr, 1.83 ± 0.01 m, 74.8 ± 3.1 kg, VO_{2max} 45.6 ± 1.9 $ml \cdot kg^{-1} \cdot min^{-1}$). Participants attended two separate laboratory visits. During the first visit a VO_{2max} was completed on a supine tilt bike. The second visit comprised of four exercise stages lasting five minutes; rest, 30%, 40%, and 50% Wmax. Each of which was performed in normoxia (21% O_2) and hypoxia (12% O_2), which were separated by 45 minutes of rest in normoxia. Data for respiratory exchange ratio (RER) and minute ventilation (VE) were collected throughout each stage using breath by breath gas analysis on the Oxycon pro. A blood sample to measure lactate was taken in the last minute of each stage. A two-way repeated measures ANOVA was used to determine significance and a paired t-test was used to determine differences of RER, VE, blood lactate and heart rate between exercise conditions and training status groups. Analysis identified significant differences between the exercise intensities ($p < 0.05$) of all variables in both normoxia and during hypoxia, however no significance was found between the groups in any of the variables in either normoxia or hypoxia ($p > 0.05$). Differences between normoxia and hypoxia were seen in RER at rest ($p = 0.001$; $p = 0.041$) and at 50% Wmax ($p = 0.0006$; $p = 0.016$) in endurance trained and untrained individuals respectively. There was a significant interaction for VE in normoxia ($p = 0.018$) and hypoxia ($p = 0.005$) with differences at 50% Wmax in untrained ($p = 0.005$) and enruance trained individuals ($p = 0.002$). A significant interaction was found for blood lactate during hypoxia ($p < 0.0001$) and during normoxia and hypoxia there were significant differences at rest and at 50% Wmax in all groups ($p < 0.05$). The findings indicate that RER increases during hypoxia at rest, and during exercise due to the greater challenge that is inflicted on metabolism.

CHAPTER 1

INTRODUCTION

The intensity of exercise dictates which energy system that is primarily used to resynthesize ATP (Baker *et al.,* 2010; Alkhatib *et al.,* 2015). Although there are elements of both aerobic and anaerobic systems working at all times, there will be a primary substrate being metabolised which will be depicted by the intensity of the exercise that is performed (Henderson *et al.,* 2005). Aerobic exercise is usually associated with longer distance and more endurance based activities. A typical example of aerobic exercise is a marathon or triathlon, where there are prolonged periods of steady state moderate intensity exercise. Conversely anaerobic exercise is usually associated with short or repeated bouts of high intensity activity. Long distance events may also have elements of anaerobic exercise as fatigue progresses (Henderson *et al.,* 2005). During anaerobic exercise the body uses Oxygen (O_2) at a greater rate, therefore requiring a greater turnover of energy and O_2, which stimulates the onset of fatigue (Beidleman *et al.,* 2008). Both aerobic and anaerobic training intensities have been found to enhance VO_2 max and oxidative capacity, with greater intensities resulting in greater improvements (Kubukeli *et al.,* 2002; Gormley *et al.,* 2008).

Carbohydrates (CHO) in the form of glucose and lipids in the form of free fatty acids (FFAs) are the primary substrates metabolised by the human body to synthesise energy in the form of ATP during exercise (Gollnick, 1985; Van Loon *et al.,* 2001). The substrate metabolised for energy varies depending on several factors, including; exercise intensity, lipid and glycogen stores, the environment exercise takes place, and fitness level (Goedecke *et al.,* 2000). O_2 and carbon dioxide (CO_2) expiratory rates depict the energy source metabolised, and are used to calculate respiratory exchange ratio (RER). VCO_2 divided by VO_2 at a given time will provide a score for RER (Mezzani *et al.,* 2003; Katayama *et al.,* 2010). At rest and lower exercise intensities lipids are metabolised as the main source of energy. The primary oxidation of FFAs is signposted by an RER score of between 0.7 and 0.85 (Liversey & Elia, 1988; Goedecke *et al.,* 2000). An RER score of between 0.85 and 1 indicates that CHO is the main substrate being metabolised (Liversey & Elia, 1988; Huso *et al.,* 2002). CHO can be metabolised into energy at a faster rate than FFAs, allowing a greater rate of ATP turnover, however CHO provides a low energy yield (4.1 kcal/g; Katayama *et al.,* 2010; Kenney *et al.,* 2011). At higher exercise intensities CHO is primarily utilised because of this greater rate of turnover, which is critical in meeting the energy demand of high intensity exercise (Goedecke *et al.,* 2000). Lipid oxidation provides a greater sum of energy (9.4 kcal/g) than CHO, however energy is produced at a slower rate, which cannot meet the

demand of intense exercise (Liversey & Elia, 1988; Kenney *et al.,* 2011). FFA's are therefore primarily metabolised at rest and during low intensity exercise.

Changes in exercise intensity cause a cascade of physiological adaptations to cope with the change in demand on the body. The substrates metabolised are strongly related to these changes, in order for the body to continue to exercise. As heart rate, ventilation and blood lactate concentrations all increase, metabolism will adapt to allow energy to be continually produced in respect to the O_2 and CO_2 present in the body (Goedecke *et al.,* 2000). During steady state exercise, the aerobic metabolism pathway is primarily used to resynthesise ATP, through the metabolism of CHO and FFAs (Westerblad *et al.,* 2010). RER is used to measure aerobic metabolism through indirect calorimetry (Katayama *et al.,* 2010). At greater exercise intensities the use of anaerobic metabolism increases and therefore the breakdown of phosphocreatine and muscle glycogen are the primary substrates metabolised for ATP resynthesis (Westerblad *et al.,* 2010).

At high altitude the partial pressure changes which restricts the amount of O_2 that the body can utilise; this is known as hypoxia (Mamede *et al.,* 2013). In hypoxic conditions both the working muscles and brain have limited O_2 supply, and therefore lead to an earlier onset of fatigue during exercise (Subudhi *et al.,* 2007; Subudhi *et al.,* 2009). Cerebral Oxygenation was found to play a critical role in the decrement in performance associated with exercise in hypobaric hypoxia (Subudhi *et al.,* 2007). Hypoxia has been seen to cause changes to the substrates metabolised at rest, and during exercise (Katayama *et al.,* 2010). During exercise a shift towards increased CHO utilisation has been found (Katayama *et al.,* 2010), whereas post exercise recovery in hypoxia leads to an increase in FFA oxidation (Workman & Basset, 2012).

Endurance trained have a greater contribution of energy from aerobic metabolism in comparison to untrained and sprint trained individuals (Henderson *et al.,* 2005) and with a greater aerobic capacity the metabolism of FFAs increases due to a reduction in absolute intensity (Goedecke *et al.,* 2000). However, the greatest decrement in performance during hypoxia has been found in endurance athletes with high aerobic capacity (Woorons *et al.,* 2010). Although other findings have found that highly trained individuals VO_2max was not significantly reduced, however in untrained individuals VO_2max was significantly lower (Benoit, 2003). Sprint trained individuals would be expected to have a greater preference towards anaerobic metabolism due to muscle fibre type and training adaptations, including greater resistance to blood lactate accumulation (Goedecke *et al.,* 2000; Westerblad *et al.,*

2010). However the metabolism of sprint trained individuals is currently relatively unknown, particularly during hypoxia.

The primary aim of the study was to investigate the effect training status during hypoxia at rest and during three different exercise intensities on substrate metabolism. The secondary aim is to highlight differences in substrate metabolism between untrained, endurance trained and sprint trained individuals. The hypothesis of the current study is that hypoxic conditions will increase RER regardless of training status and therefore induce greater CHO metabolism at rest and during exercise.

CHAPTER 2

LITERATURE REVIEW

Substrate metabolism

The intensity that an individual exercises at and the muscular contractions involved ultimately dictates the energy source that is primarily oxidised during exercise (Van Loon *et al.,* 2001; Janyacharoen *et al.,* 2009; Heinonen *et al*., 2011). Indirect calorimetry through breath by breath analysis is considered the gold standard measure of RER and substrate utilisation estimations (Infante-Vázquez *et al.,* 2013). Indirect calorimetry is used to examine ventilation, an RER value can be calculated from carbon dioxide production (VCO_2) divided by the oxygen consumption (VO_2) of an individual. From RER the contribution of substrates being metabolised for energy expenditure can be determined (Ramos-Jimenez *et al.,* 2008; Infante-Vázquez *et al.,* 2013). RER is an indirect measure of the oxidative capacity of the working muscle during steady state exercise, however, it is not used as a predictor of fitness in preference for other measures such as minute ventilation (VE), lactate threshold and VO_2 alongside heart rate (Ramos-Jimenez *et al,* 2008; Gallagher *et al*., 2014). RER can only measure the oxidation of FFAs and CHO during aerobic metabolism, therefore it is only appropriate for steady state exercise and exercise intensities less than 60% Wmax (Wmax is the % of peak power output; Janyacharoen *et al.,* 2009).

Aerobic exercise is usually primarily associated with low to moderate intensities and therefore higher levels of lipid oxidation whereas anaerobic exercise is associated primarily with high intensities and therefore CHO is predominantly oxidised (Van Loon *et al.,* 2001). This is related to the availability of O_2 in the working muscles, when O_2 availability is reduced, RER increases and so reliance on glucose oxidation increases (Heinonen *et al*., 2011). RER is most effective and appropriate as a measure during steady state exercise, due to the calculation through gas analysis, it is important that there are consistent stages of a steady state in order for VO_2 and VCO_2 to plateau and remain consistent (Ramos-Jimenez *et al.,* 2008). Consistent measures allow substrate metabolism to be indirectly measured (Ramos-Jimenez *et al.,* 2008).

There are several factors that contribute towards substrate metabolism during exercise; pre-exercise nutrition, training status, ventilation and hormone levels. The substrates utilised adjust in line with the intensity of work completed, therefore at rest a lower RER would be expected in healthy individuals (Fig. 1), due to lesser demands on the body (Goedecke *et al.,* 2000). Typically at rest and during low intensity exercise RER is at its lowest, indicating

greater oxidation of FFAs (Liversey & Elia, 1988) however there is often a large variance between individuals' resting RER (Fig. 1). As exercise intensity increases, reliance on CHO utilisation increases (Fig. 2) to meet the demands of exercise (Goedecke *et al.,* 2000). Protein metabolism makes up less than 5% of total energy expenditure in endurance activities (Tarnopolsky, 2008), under severe metabolic stress, such as in a state of starvation, protein is converted into glucose to be oxidised (Emery, 2015); ketones have to be produced to maintain brain function when CHO levels are extremely low (Prabhakar *et al.,* 2015).

Muscles have a well-developed system for the utilisation of CHO which increases the metabolism of CHO during exercise, whereas fat has to be broken down from its complex form, triglyceride, to FFAs in order to be used to form ATP (Kenney *et al.,* 2011). The amount of FFAs available for metabolism largely depends on the fat stores in the body, a lean person with low body fat levels will have less FFAs available for energy (Kenney *et al.,* 2011).

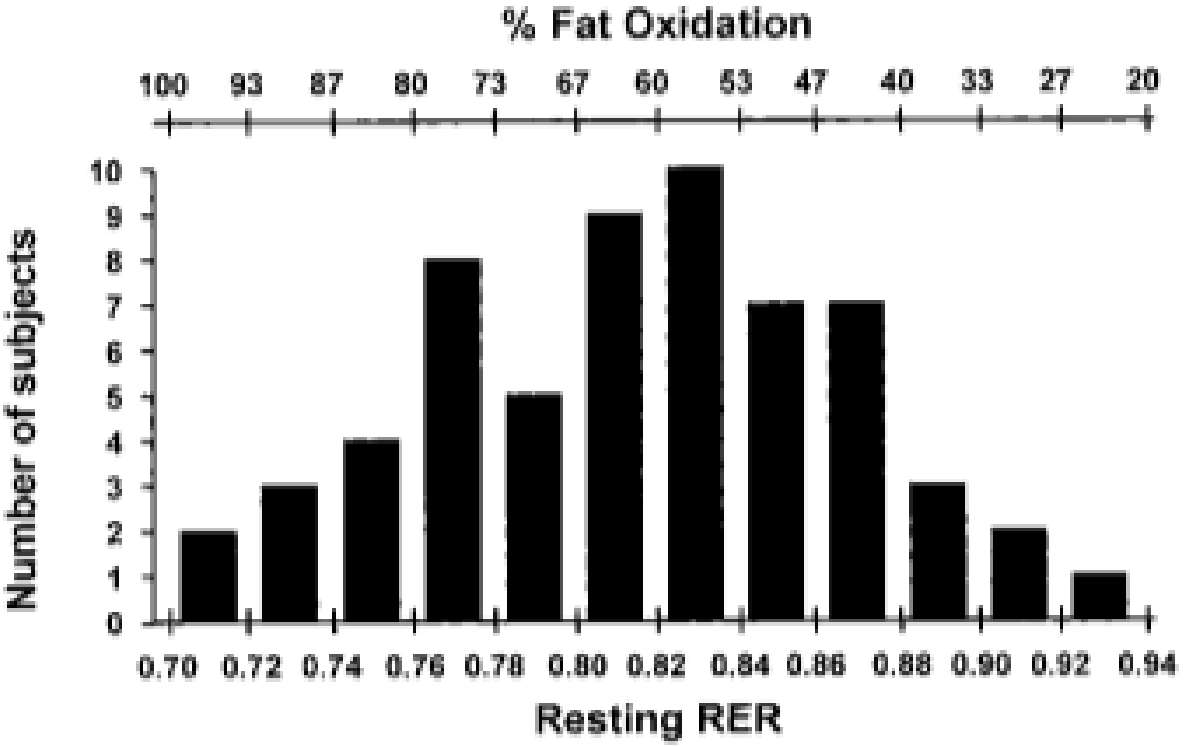

Figure 1. Distribution of fasted resting RER in males and females. Cited from Goedecke et al., 2000.

Van Loon *et al.* (2001) found that increasing exercise intensity caused changes in which substrates were utilised, FFA oxidation remained the same at intensities of 40%Wmax and 55% Wmax, however at 75%Wmax FFA oxidation decreased, whilst CHO oxidation progressively increased throughout all the conditions, reaching its peak at 75% Wmax (Fig. 2). The exact mechanisms that are responsible for the inhibition of FFA oxidation are

currently unknown, however it is understood that at higher exercise intensities FFA oxidation is reduced (Van Loon *et al.,* 2001). One suggestion towards explaining FFA oxidation inhibition at greater exercise intensity is a reduction of carnite palmitoyltranferase I (CPT 1), which leads to the prevention of entry of FFAs into the mitochondria (Van Loon *et al.,* 2001). The inhibition of FFAs may largely be due to a primitive protective reaction in order to maintain ATP resynthesis (Lundby & Van Hall, 2002). CHO therefore are primarily used at greater intensities to reduce cardiovascular strain and produce enough energy to sustain exercise intensity (Heinonen *et al.,* 2011).

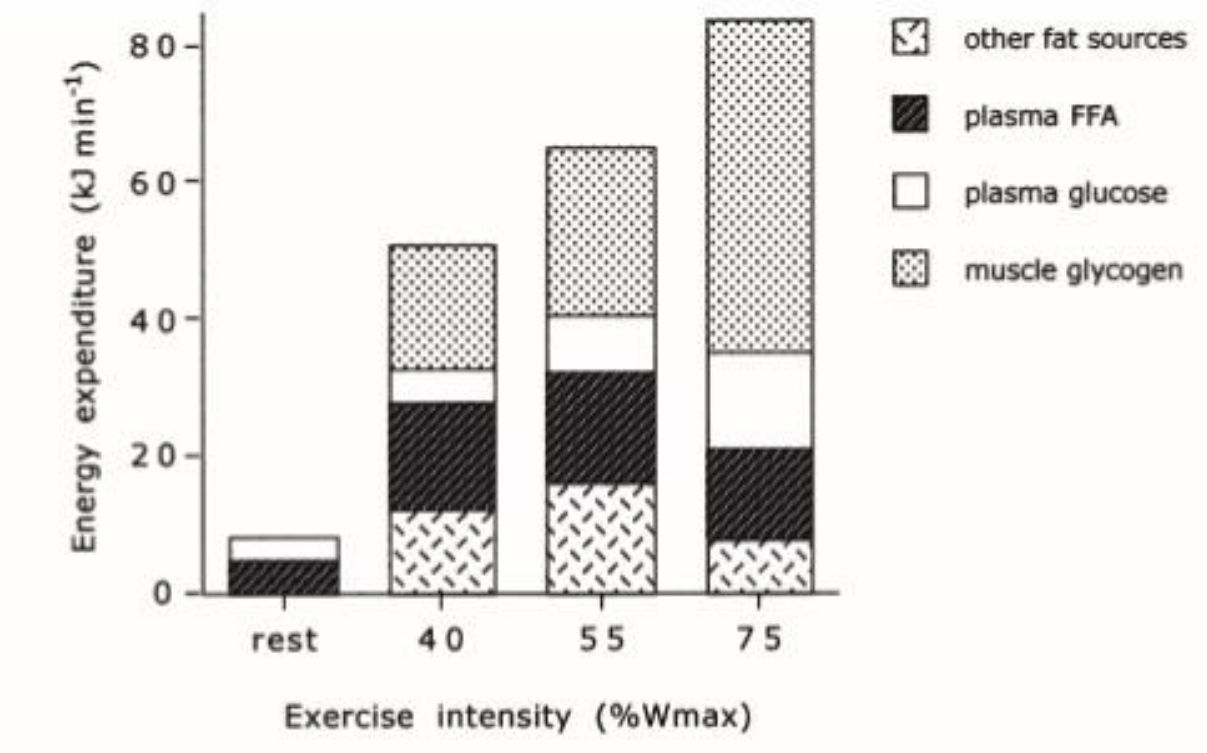

Figure 2. Substrates used at rest and at different exercise intensities in respect to energy expenditure. Cited from Van Loon et al., 2001.

Mechanisms behind substrate metabolism

Energy is released when the bonds that hold elements together are broken, substrates are converted into energy via a chemical pathway, enabling the energy to be used by the body; this process is known as bioenergetics (Kenney *et al.,* 2011). Substrates are primarily comprised of carbon, hydrogen and oxygen, the bonds between these molecules are relatively weak and therefore only create small quantities of energy (Kenney *et al.,* 2011). CHO has a better O_2 efficiency than FFAs and therefore at greater exercise intensities there is less strain on the metabolism to maintain ATP resynthesis (Lundby & Van Hall, 2002).

A review of preoperative CHO loading by Pogatschnick and Steiger (2015) looked into the mechanisms behind ingestion of CHO. In a fed state blood glucose levels increase, causing several metabolic changes; glycogen stores are no longer used for production of glucose, preventing gluconeogenesis, with excess glucose being stored as glycogen. Uptake of glucose in the muscle increases, alongside an increase in glycogen stores. Muscle glycogen levels are an important factor in the RER of an individual during exercise (Goedecke *et al.,* 2000). CHO loading has been found to increase glycogen stores in male endurance runners (Tarnopolsky, 2008), indicating that pre-exercise CHO levels are an important factor in dictating which substrates are available for metabolism, regardless of exercise intensity (Goedecke *et al.,* 2000). Therefore it is suggested that CHO loading will improve endurance performance, with increased glycogen stores providing a greater availability of CHO for metabolism, allowing a greater rate of ATP turnover to be maintained (Wismann & Willoughby, 2006). Substrate availability in the blood plasma is a major determinant of which substrate is metabolised particularly at rest but also during exercise (Goedecke *et al.,* 2000).

Measuring substrate metabolism

Roepstorff *et al.* (2002) suggest that in order to gain greater detail of substrate metabolism during exercise, examination into the working muscle rather than the whole body allows for a closer look into the local metabolism of substrates, however this method requires a muscle biopsy. Another method used to analyse which substrates are being metabolised is breath by breath analysis, where RER is representative of the whole body (Janyacharoen *et al*., 2009). Another method of measuring substrates is the use of a palmitate tracer throughout exercise to directly estimate plasma FFA metabolism, using CO_2 production from expired air (Van Loon *et al.,* 2001). The use of breath by breath analysis provides instantaneous feedback, and also allows various time breakdowns of 5 second to 60 second intervals to be assessed. Furthermore the Oxycon pro has been found to be a valid and reliable method of gas analysis (Carter *et al.*, 2002).

RER, lactate and ventilation

The anaerobic threshold is often related to exercise induced fatigue. As concentrations of lactic acid increase so does the rate of ventilation and CO_2 production, this takes place to aid the removal of hydrogen ions and minimise metabolic acidosis (Ghosh, 2004; Katayama *et al.,* 2010). During high intensity exercise (~70% Wmax) the plasma lactate concentration is key in determination of RER (Goedecke *et al.,* 2000). The accuracy of indirect calorimetry during exercise has been previously criticised due to miscalculations due to hyperventilation

causing an overestimation in CO_2 production (Katayama *et al.,* 2010), with increased blood lactate and therefore increased ventilation, particularly in hypoxic exercise, RER may be overestimated. Furthermore lactate and ventilatory thresholds are closely linked with fatigue, both are negatively affected by hypoxic conditions (Hughson *et al.,* 1995) suggesting that these may contribute to the reduced exercise capacity in hypoxia. Benoit *et al.* (2003) investigated if training status influenced the VE and blood lactate of individuals between exercising in normoxia and acute hypoxia and found that VE was higher whereas blood lactate remained the same across all training groups.

Substrate metabolism during exercise

A study conducted by Roepstorff *et al.* (2002) investigated differences in substrate metabolism during submaximal exercise in endurance trained individuals. The main substrate utilised in the legs during a 90 minute cycle at 58% VO_2peak was muscle glycogen which contributed for around 40% of total O_2 uptake. Muscle glycogen and blood glucose made up around 60% of total O_2 uptake, showing that CHO was primarily utilised in the working muscle. However 20% of the total O_2 uptake was unaccounted for in males; therefore further investigation into the substrates utilised by males is required. Findings from Van Loon *et al.* (2001) show that at moderate intensities that there is a relatively even distribution to energy expenditure of substrates, with similar contribution of fat sources and CHO sources towards energy expenditure. Greater FFA oxidation has been found to delay the onset of fatigue in trained cyclists, starting CHO oxidation was higher and the onset of fatigue was earlier when compared to cyclists that had high FFA oxidation rates (Goedecke *et al.,* 2000). FFA oxidation increases during low to moderate intensity exercise and then reduces as exercise intensity increases further to high intensity, where CHO oxidation predominantly takes place in the muscle (Janyacharoen *et al.,* 2009).

The use of RER has been criticized when used for maximal exercise, as values are only accurate when < 1.0 according to Janyacharoen *et al.* (2009), suggesting that it should only be used for sub-maximal exercise. Substrate metabolism during high intensity exercise results in greater metabolism of CHO with reports of RER values close to or greater than 1 (Janyacharoen *et al.,* 2009). Van Loon *et al*. (2001) found that during high intensity exercise (75%Wmax) muscle glycogen was primarily metabolised, with a small contribution to energy expenditure from fat sources (Fig. 2). During maximal exercise an individual will go through their anaerobic threshold with lactic acid concentrations rapidly increasing due to the rate of blood lactate production being greater than its removal (Ghosh, 2004). Accumulation of lactic acid has been linked with increased preference of CHO utilisation due to greater

production of blood lactate as a result of gluconeogenesis (Ghosh, 2004). There is a strong relationship between RER and lactic acid concentration, as concentrations of blood lactate increase so does the oxidation of CHO due to the requirement to remove hydrogen ions and minimise metabolic acidosis (Ghosh, 2004) and to maintain energy production (Lundby & Van Hall, 2002; Heinonen *et al.,* 2011).

Substrate Metabolism & Training Status

As aerobic fitness improves the absolute intensity of exercise will decrease, FFA oxidation will therefore increase contribution to energy production (Goedecke *et al.,* 2000; Carter *et al.,* 2001). A comparison between trained and untrained individuals found that FFA oxidation was greater and CHO metabolism was lower in trained individuals, even at the same relative exercise intensities, which suggests that FFA oxidation increases with training, due to a reduction in absolute exercise intensity (Goedecke *et al.,* 2000). Furthermore improved endurance fitness led to greater FFA utilisation and reductions of CHO utilisation at the same absolute intensity following a training intervention (Carter *et al.*, 2001). These changes are closely linked to augmentation of resting RER with exercise and improved training status, resulting in increased FFA utilisation at rest and during exercise, which also leads to greater fat loss (Flat, 1995). Changes in body composition, such as reduced body fat and increased lean mass will further increase exercise performance, by improving exercise efficiency and substrate availability (Goedecke *et al.,* 2000; Van Loon *et al.,* 2001).

Sprint trained individuals have a greater tolerance of lactate accumulation and therefore may predominantly oxidise CHO because of the higher concentrations of blood lactate when they exercise. Skeletal muscle is central to CHO oxidation, insulin helps to control the regulation of CHO oxidation, however during exercise, an increase in calcium in the working muscle increases CHO uptake and facilitates the entry of CHO into the muscle cells (Heinonen *et al.,* 2011). Furthermore, muscle fibre type characteristics also play a pivotal role in substrate metabolism, type I fibres have a greater oxidative capacity than type II fibres and therefore allow greater FFA oxidation during low to moderate intensity exercise (Westerblad *et al.,* 2010). Therefore endurance trained athletes exhibit a low RER due to greater FFA oxidation, whereas sprint trained individuals have greater CHO oxidation and a higher RER.

Hypoxia

An individual's aerobic capacity (VO_{2max}) is reduced at high altitude, alongside greater levels of lactic acid accumulation (Gallagher *et al.,* 2014), both of which have a detrimental effect on exercise performance, particularly in endurance based activities (Vogt & Hoppeler, 2010)

although VE was found to increase during hypoxia (Chu *et al.,* 2006). However, replicating high altitude conditions during training has been found to cause greater aerobic adaptations than seen at sea level (Vogt & Hoppeler, 2010). Recently the development of training in hypoxic conditions has grown, with the aim to improve aerobic performance (Vogt & Hoppeler, 2010). Hypoxic conditions replicate those of high altitudes, at high altitude increases in the atmospheric pressure changes, resulting in alterations to partial pressures of gasses in the air, including reduced O_2 (Mamede *et al.,* 2013).

Substrate metabolism in hypoxia at rest

At rest, the effect of hypoxia has still been found to cause physiological adaptations. One adaptation that has been observed is increased energy expenditure (kcal·min) after acute exposure, there was also an increase in energy expenditure following a short-term exposure of 3 hours a day for 7 days, this led to increased baseline energy expenditure before hypoxic exposure and further increased energy expenditure immediately after exposure on the final day (Workman & Basset, 2012). In the western world with a surge in the number of cases of obesity, hypoxia may help to reduce the weight of obese individuals that are unable to exercise, by increasing energy expenditure. CHO oxidation during hypoxia is greater than during normoxia, even at rest, due to a greater production of VCO_2 and changes in signalling pathways towards an increase in CHO reliance (Heinonen *et al.,* 2011).

Workman and Basset (2012) investigated the effect of short term and acute hypoxia on substrate metabolism and energy expenditure. They found that acute and short term hypoxia led to increased FFA oxidation and attenuated glucose utilisation after an exposure to moderate hypoxia. After 7 days of 3 hour per day hypoxic exposure, baseline FFA oxidation increased significantly, further increasing after the final exposure, the rate of increase between pre and post exposure remained the same throughout. Moroshima and Goto (2014) aimed to explore the effects of a 7-hour exposure to moderate hypoxia on postprandial metabolic responses. Postprandial CHO and FFA oxidation were not significantly altered following a 7-hour exposure to hypoxia. Although some physiological differences were recorded in VO_2 and VCO_2 between hypoxia and normoxia, there was no significant differences in postprandial metabolic responses or RER.

Substrate metabolism in hypoxia during exercise

Wadley *et al.* (2005) investigated the activation of AMPK in skeletal muscle during hypoxic exercise, comparing absolute and relative intensities. It was found that activation of AMPK, which is an important regulator of contraction-stimulated glucose uptake was more closely

linked to absolute intensities. Furthermore plasma glucose disappearance was also dictated by absolute intensities, whereas muscle glycogen breakdown and increases in muscle lactate concentrations were more closely associated with relative intensities. However the training status of the participants was not stipulated and therefore suggests it was potentially unaccounted for.

Katayama *et al.* (2010) aimed to explain the effect of hypoxic exercise on substrate metabolism. Their findings supported the proposition of a change in the regulation of the metabolic pathway towards greater CHO utilisation would help to sustain homeostasis by augmenting energy yield per unit of O_2. Furthermore they found that Insulin serum concentrations were greater in hypoxia during exercise and recovery however there was a reduction in growth hormone concentration during recovery in comparison to sea level concentrations. However diet 24-hours preceding exercise was identified as a critical factor in changes to RER (Katayama *et al.,* 2010), with previous studies revealing no differences between RER when exercising in severe hypoxia (Morishima & Goto, 2014). During hypoxia VE will be greater than during normoxia (Ghosh, 2004) which may cause the RER to increase due to changes in VO_2 and VCO_2 which may misrepresent substrate utilisation.

During acute hypoxia dependence on plasma glucose oxidation is greater than during normoxia at a given absolute intensity (Péronnet *et al.,* 2006; Heinonen *et al.,* 2011). Furthermore ingestion of a large quantity of exogenous CHO prior to exercise led to a further increase in the contribution of plasma glucose oxidation towards energy production. Although similar oxidation of exogenous CHO was seen in hypoxia and normoxia at the same absolute intensity (Péronnet *et al.,* 2006). The increase in CHO oxidation in the form of plasma glucose could be partially explained by an increased disposal of plasma glucose in the working muscle during hypoxia in comparison to normoxia (Heinonen *et al.,* 2011). Furthermore during hypoxia the use of CHO as the main source of fuel could be linked to a mechanism of efficiency when O_2 availability is low to reduce the cardiovascular strain.

An investigation conducted by Friedmann *et al.* (2004) aimed to compare the individual anaerobic threshold of endurance athletes in acute hypoxia and normoxia. They found that during acute hypoxia heart rate was significantly lower than in normoxia, as was the individuals' rate of perceived exertion; although blood lactate concentrations were found to be slightly higher in hypoxia. RER was higher throughout hypoxia, but only significantly higher at 30 and 40 minutes of a 1 hour treadmill run. In contrast to this, Calbet (2003) found that resting heart rate was higher following 9 weeks of high altitude residency, although cardiac output did not change following an exposure to chronic hypoxia. Furthermore chronic

hypoxia increased mean, systolic and diastolic blood pressure and also hypoxia induced hypertension. The severity and time of exposure were found to be important factors in the hypertensive response that is seen following chronic exposure to hypoxia (Calbet, 2003).

Subudhi *et al.* (2007) investigated the effect of hypoxia on cerebral deoxygenation. They found that in both acute and chronic hypoxia cerebral deoxygenation is a limiting factor in maximal exercise performance and that physical fatigue during acute exposure to severe hypoxic conditions was mainly influenced by factors outside the muscle. Whereas during normoxia muscle factors appear to be the central mechanism for fatigue. Furthermore hypoxia has been found to negatively influence ventilatory and lactate thresholds due to greater lactic acid accumulation and greater VE which will have a limiting effect on exercise in hypoxia, due to an earlier onset of fatigue, reaching the anaerobic threshold at a lower exercise intensity during hypoxia (Hughson *et al.,* 1995; Ghosh, 2004). During hypoxia, anaerobic metabolism pathways provide a greater contribution of energy because of the reduction in O_2 which is required for aerobic metabolism (Westerblad *et al.,* 2010).

Hypoxia & Training Status

Hypoxia had similar effects on both trained and untrained individuals in a study containing trained endurance and trained sprinters by Cleuziou *et al.* (2005). The influence of training status had little effect in hypoxic conditions on VO_{2max}, heart rate and power output at 80% or 50% Wmax. However all values irrespective of training status were reduced in respect to values during normoxia. Endurance trained individuals may have a greater resilience to hypoxia because of the fibre type they possess with better oxidative capacity to cope with oxidative stress than their untrained and sprint trained counterparts (Cleuziou *et al.,* 2005). Furthermore Benoit et al. (2003) found that VO_{2max} was significantly reduced in moderately and low trained individuals, however in highly trained individuals although there was a reduction in VO_{2max} in hypoxia it was not statistically significant, heart rate was also reduced in hypoxia for all groups. Therefore exercise capacity will be severely reduced during hypoxia, however endurance trained individuals may be able to cope with the extra demands better, although they will still experience a reduction in exercise capacity (Carter *et al.,* 2001; Benoit *et al.,* 2003; Cleuziou *et al.,* 2005).

Endurance and anaerobic athletes

Distance and duration have strong influences on the demands of exercise. Endurance running, requires greater aerobic capacity (Gormley *et al.,* 2008) whereas 400m events need a high lactate threshold in order to maintain near maximal intensity for the entirety of the

event (Saraslanidis *et al.,* 2009; Jacobs *et al.,* 2011). Therefore a higher VO_{2max} would be expected in trained endurance athletes and a high lactate threshold in anaerobically trained athletes. This will have an influence on the substrates oxidised during exercise and also how hypoxia impacts performance. Endurance trained athletes were found to have greater FFA oxidation and reduced CHO oxidation at both relative and absolute intensities when compared to untrained individuals alongside a greater VO_2 peak (Carter *et al.*, 2001). Anaerobic athletes on the other hand will use CHO as a primary source of substrate metabolism due to the demand for fast turnover of energy (Goedecke *et al.,* 2000), however currently there is limited research in the substrate metabolism of anaerobic athletes.

Acute hypoxia reduces the exercise capacity of both endurance trained and anaerobically trained athletes. VO_{2max} was significantly reduced in acute exposure to hypoxia for sprint trained athletes, although endurance trained athletes had a reduction it was not significant (Cleuziou *et al.,* 2005). Furthermore heart rate was not significantly different in either group but power output was significantly reduced in sprint trained athletes, although not in endurance athletes (Cleuziou *et al.,* 2005). This may be due to greater power output during normoxia, but a lack of aerobic capacity to maintain it during normoxia. These findings suggest that hypoxia has a less detrimental effect on endurance trained athletes in comparison to anaerobically trained athletes. An explanation for this is the muscle fibre characteristics of each training group, endurance athletes will have a greater predominance of type I fibres which have a greater oxidative capacity than type II fibres of which sprint trained individuals will possess (Westerblad *et al.,* 2010; Heinonen *et al.,* 2011).

Summary

Substrate metabolism varies greatly at rest and during exercise, at lower intensities FFAs are predominantly oxidised, at greater intensities there is a preference towards CHO oxidation (Goedecke *et al.,* 2000; Van Loon *et al.,* 2001). Muscle fire type is an important factor in determining substrate metabolism, particularly during hypoxia where oxidative capacity has great importance (Westerblad *et al.,* 2010; Heinonen *et al.,* 2011). Training status therefore will have an effect on the ability to cope with exercise during hypoxia. There is currently limited research into the substrate metabolism of sprinters, particularly in regards to substrate metabolism during hypoxia. Therefore this study aimed to investigate the effect of training status during hypoxia at rest and during exercise on substrate metabolism, using endurance trained, sprint trained and untrained individuals. It was hypothesised that RER would increase regardless of training status during hypoxia at rest and during all exercise intensities.

CHAPTER 3

METHODS

Participants

Twenty healthy males (age 24 ± 5.3 yr) were recruited into the study. This comprised of endurance trained runners (n = 8) who ran a minimum distance of > 40km per week (age 28 ± 5 yr, 1.81 ± 0.06 m, 73.3 ± 7.5 kg, VO_2max 46.4 ± 7.2 ml·kg^{-1}·min^{-1}), competent and specialised 400m runners (n = 4) (age 22 ± 3.3 yr, 1.83 ± 0.01 m, 74.8 ± 3.1 kg, VO2max 45.6 ± 1.9 ml·kg^{-1}·min^{-1}) and untrained individuals (n = 8) that completed < 2 hours of physical activity per week (age 20 ± 1.5 yr, 1.78 ± 0.08 m, 82.1 ± 11.2 kg, VO2max 32.6 ± 4.9 ml·kg^{-1}·min^{-1}). All participants were normotensive, non-smokers, with no history of cardiovascular, musculoskeletal, or metabolic disease, and at the time of testing were not taking any medication. A health questionnaire was completed by all participants prior to being accepted for any exercise procedure. Participants' age, height, weight and VO_2 max were recorded in the 1st laboratory visit.

Ethical considerations

All participants volunteered following a recruitment process using university students and athletics clubs. Each participant gave written informed consent to participate in the study after being given information of the study. The study conformed to the declaration of Helsinki and was approved by the research ethics committee of Cardiff Metropolitan University (reference number: 15/5/381U).

Experimental Design

Participants were instructed to abstain from alcohol, caffeine and vigorous exercise for 24 hours prior to each laboratory visit. Participants were given the opportunity to review the participant information and any questions about the project were answered. Participant information was collected using a health questionnaire (ACSM Pre-Participation Screening Questionnaire). Participants were considered healthy and able if they had no more than one risk factor as assessed by the health questionnaire. The protocol was then explained in person to the participant to ensure full understanding. Measurements were taken for height using a stadiometer (Holtain, Crymych, UK) and body mass using weighing scales (SECA, Hamburg, Germany).

The study protocol was made up of two laboratory visits separated by a minimum of two days. The 1st visit consisted of recording of anthropometric measurements, followed by a VO_2max test performed on a tilted supine cycle ergometer. The 2nd visit was comprised of

the collection of gas analysis, including RER and VE, blood lactate and heart rate were also recorded at rest and during three submaximal exercise intensities (30%Wmax, 40% Wmax, and 50% Wmax). Each participant completed all intensities in normoxia and hypoxia (12% $O_{2)}$, with a 45 minute rest between conditions which was blinded and counterbalanced between participants, where the 1st participant had normoxia first, then the next participant had hypoxia first.

Visit 1

A standardised incremental exercise test was performed on a tilted (45°) supine bed with a cycle ergometer (Angio, Lode, Groningen, Netherlands) with a programmable control unit (Corvial, Lode, Groningen, Netherlands) to test aerobic fitness and provide values for VO2max and maximum peak power output, heart rate was continuously monitored (Polar Electro RS4000, Polar Electro, Kemple, Finland) throughout. Prior to the test, participants were given familiarisation with the supine cycling at 25W and 50W for 3 minutes. The participant then performed incremental exercise until volitional fatigue starting at an intensity of 40W for 3 minutes maintaining 60-65 RPM, with increments of 40W every 3 minutes thereafter, using breath by breath analysis (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) VO_2 and VCO_2 were measured, and RER calculated from these values. Blood lactate was taken from the middle finger in the last minute of each stage and, immediately after volitional fatigue. Participants were encouraged to perform a best effort, at the point of volitional fatigue, data for RER, heart rate and blood lactate were recorded and the resistance was reduced to 25-50W and participants continued to pedal for a minimum of 3 minutes. VO_2max was determined by the highest VO_2 held for a 5s period. The total visit lasted approximately 1 hour.

Visit 2

Participants were asked to confirm that there had been no change to their health since their previous visit. The participants then laid on the tilted supine cycle ergometer which will be set up as it had been in visit 1. A 3-Lead electrocardiogram was then attached to provide readings for heart rate, and the participant was given either room air or 12% O_2 (equivalent of 4500m above sea level) using a McKinley altitude simulator (Higher Peak Performance, Staffordshire, UK). Air was received through a mouth piece connected to the Oxycon Pro throughout the procedure. A wash-in period of 10 minutes was completed prior to the recording of measurements to allow for the air to circulate the body and for baseline measures to settle. Once resting baseline measurements were taken, the exercise

procedure began. Exercise intensity was based upon a percentage of the maximal peak power achieved in the previous visit. The exercise procedure consisted of three consecutive 5 minute stages, at 30%, 40% and 50% of maximal peak power maintaining 60 RPM in each. Data was collected throughout each intensity, including a blood sample from the ear for measurement of lactate which was taken in the last minute of each stage. After the 1st condition is completed the participant rested for 45 minutes in normoxia before completing the same procedure in the other condition (either 21% or 12% O_2). Breathe by breath gas analysis was used throughout each stage with an Oxycon Pro attached to the mouthpiece to continuously monitor VO_2, VCO_2, RER and VE. Heart rate was also continuously monitored throughout, with mean bpm being recorded for each stage. At the end of each stage a blood sample was taken from the ear to determine lactate, following the session the blood samples were analysed using a lactate analyser (Biosen, C-Line Sport, Magdeberg, Germany).

Statistical analysis

The continuous data collected for RER, VE and heart rate was averaged for each stage to provide a mean for each intensity. Analyses were performed using graphpad prism version 6 (Graphpad, La Jolla, CA). A 2 factor (group vs time) repeated measures analysis of variance (ANOVA) was used to determine differences in RER, VE, HR and blood lactate between the training status of participants across time. Significance was expressed in relation to an alpha of $P < 0.05$. A paired t-test was performed to examine differences between normoxia and hypoxia for rest and 50%Wmax for each training status. Descriptive data was expressed as means ± SD. To meet the assumptions of the ANOVA, the Shapiro-Wilk Test was performed to determine if the data had normal distribution, to check for homogeneity the Levene test was completed. Tukey's test for multiple comparisons was used for post-hoc multiple comparisons. Which were used to make comparisons between individual time points and each training status.

CHAPTER 4

RESULTS

Respiratory Exchange Ratio

Effect of training status on RER at rest and during exercise

Normoxia

In normoxia there was a significant increase between exercise intensities ($p < 0.0001$), however there was no significant difference between the training status groups or any significant interaction. No significant differences were found from post hoc multiple comparison between the training status groups or at any exercise intensity.

Hypoxia

During hypoxia there was a significant increase between exercise intensities ($p < 0.0001$), however there was no significant differences between the training status groups or any significant interaction. No significant differences were found from the post hoc multiple comparisons between the status groups at any exercise intensity.

Effect of hypoxia on RER at rest and during exercise

Untrained

Significant differences between normoxia and hypoxia were seen in the untrained group at rest ($p = 0.041$) and also at 50% Wmax ($p = 0.016$) with greater values seen in hypoxia for both intensities (Fig. 4).

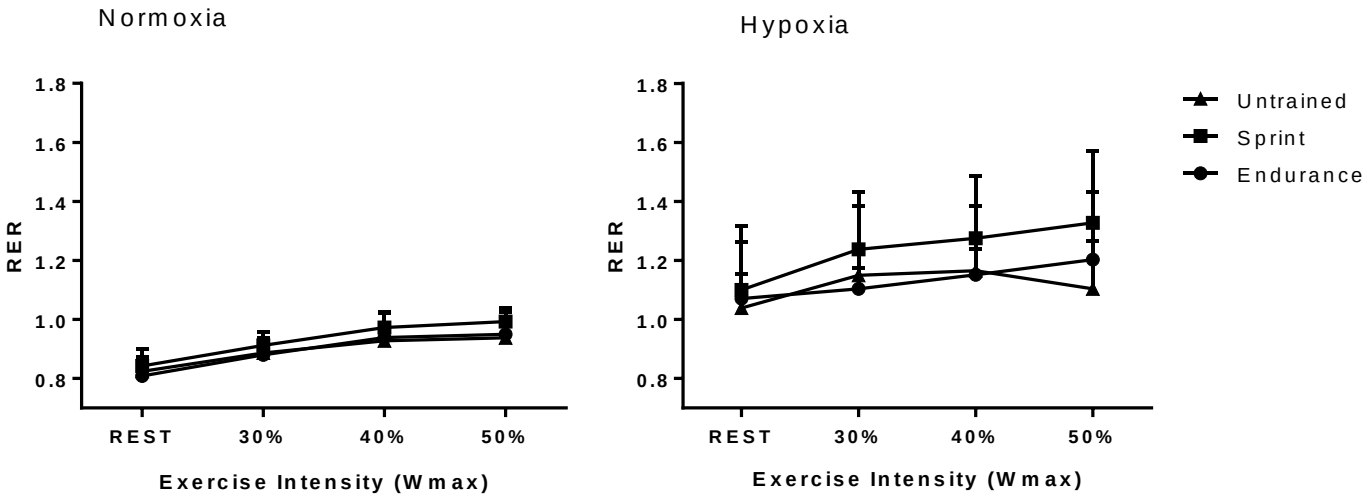

Figure 3. RER at different exercise intensities during normoxia (left) and hypoxia (right). Data presented as mean ± SD.

Endurance trained

Significant differences were seen between normoxia and hypoxia in the endurance group at rest ($p = 0.001$), and also at 50%Wmax ($p = 0.0006$) with greater values seen during hypoxia at both intensities (Fig. 4).

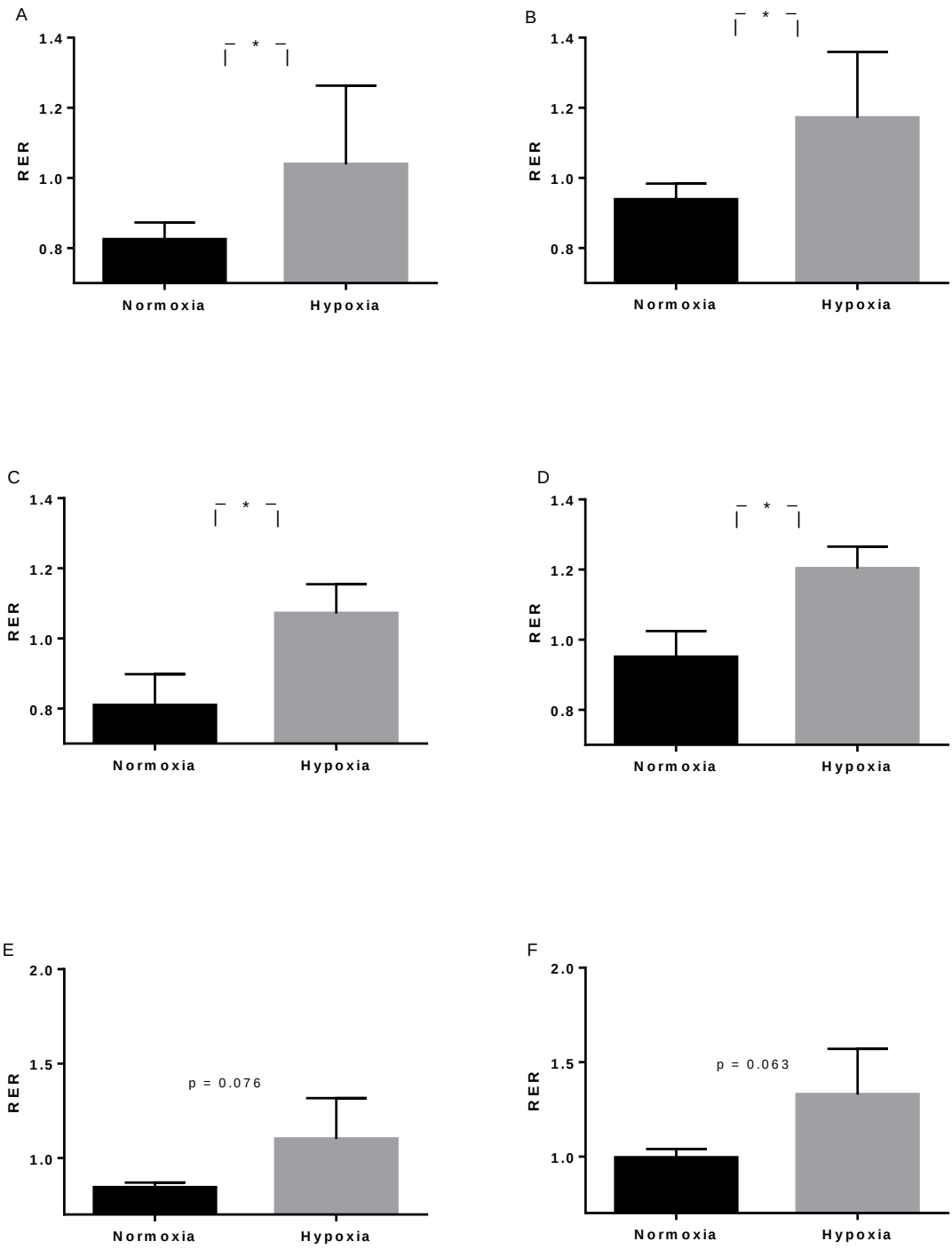

Figure 4. Comparison of RER between normoxia and hypoxia (A) untrained at rest (B) untrained at 50% Wmax (C) endurance trained at rest (D) endurance trained at 50% Wmax (E) sprint trained at rest (F) sprint trained at 50% Wmax.

Sprint trained

No significant differences were found in the sprint group at either intensity (Fig. 5), however there was an increasing trend at rest ($p = 0.076$) and also at 50% Wmax ($p = 0.063$).

Ventilation

Effect of training status on ventilation at rest and during exercise

Normoxia

During normoxia there was a significant interaction for VE ($p = 0.018$), and there was a significant increase between exercise intensities ($p < 0.0001$). There was no significant difference between training groups. Although the sprint group had a significantly greater VE at 50% Wmax in comparison to untrained individuals ($p = 0.001$; Fig. 5).

Hypoxia

There was a significant interaction during hypoxia for VE ($p = 0.005$), there was also a significant difference between exercise conditions ($p < 0.0001$). However there was no significant difference between groups. The greatest difference in VE was seen at 50% Wmax between endurance trained and untrained individuals ($p = 0.0008$; Fig. 5).

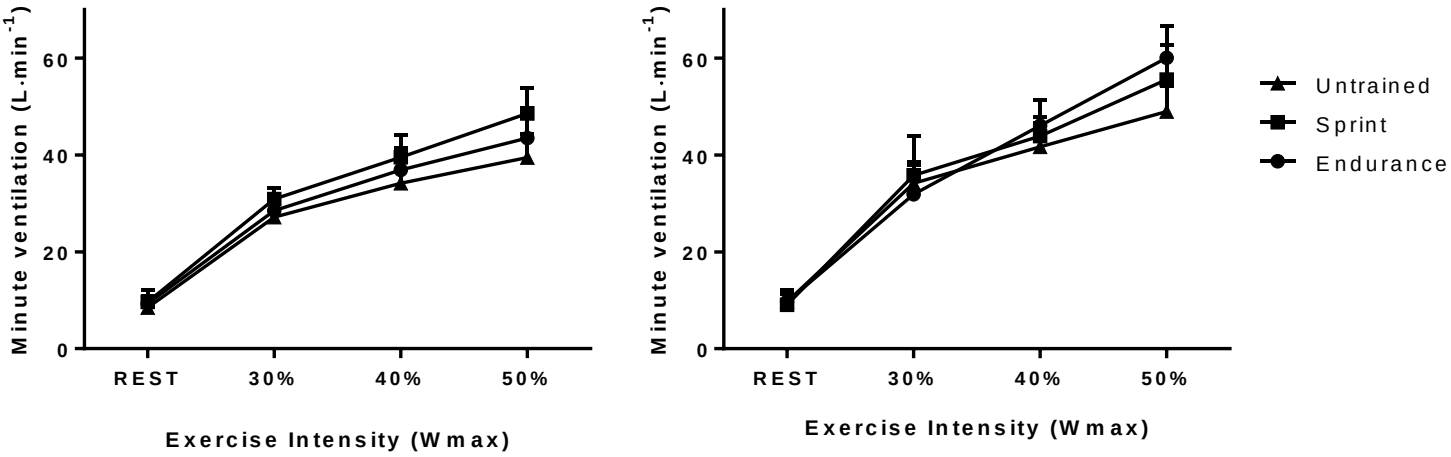

Figure 5. Ventilation (VE) during normoxia (left) and hypoxia (right). Data presented as mean ± SD.

Effect of hypoxia on ventilation at rest and during exercise

Untrained

There was no significant difference in ventilation between hypoxia and normoxia in untrained individuals at rest. However at 50% Wmax there was a significant increase in ventilation during hypoxia in respect to normoxia ($p = 0.005$; Fig. 6).

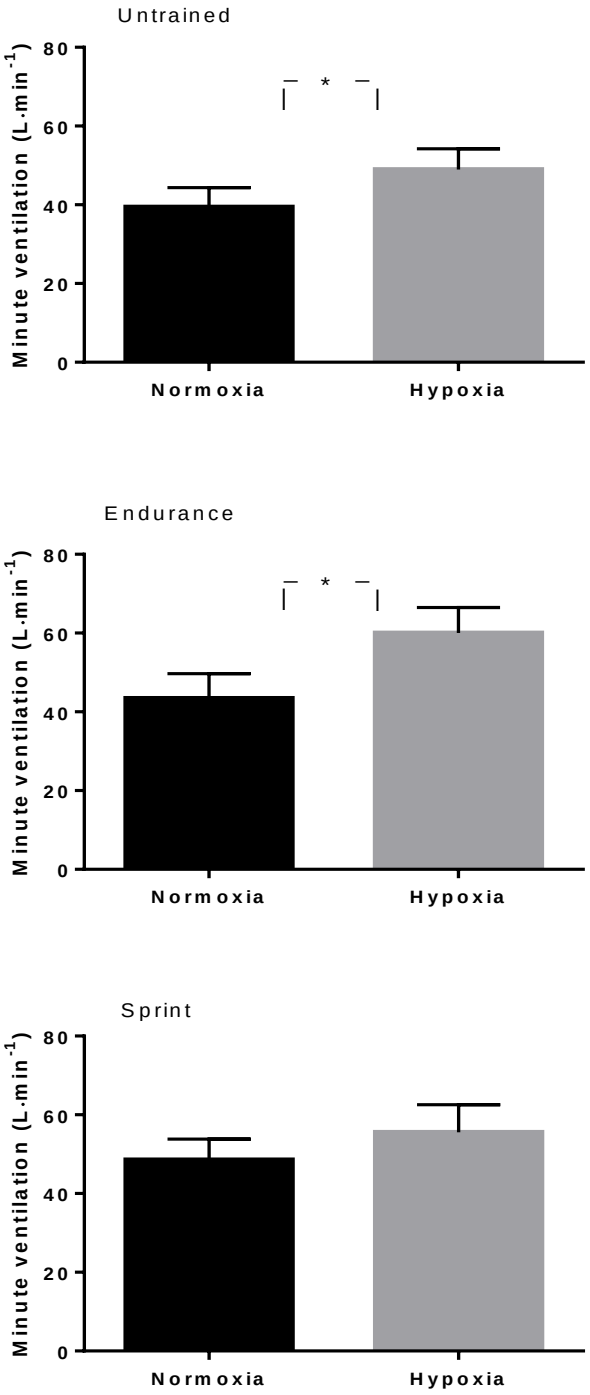

Figure 6. VE at 50% Wmax in different training status groups.

Endurance trained

There was no significant difference in ventilation between hypoxia and normoxia in endurance trained individuals at rest. However at 50% Wmax ventilation was a significantly greater during hypoxia than in normoxia ($p = 0.002$).

Sprint trained

There were no significant differences between normoxia and hypoxia in the sprint trained group at rest or at 50% Wmax.

Lactate

Effect of training status on blood lactate at rest and during exercise

Normoxia

During normoxia there was no significant interaction for blood lactate, however there was a significant increase between exercise intensities ($p < 0.0001$). There was no difference between training groups, although sprint trained individuals had significantly greater blood lactate concentrations than untrained individuals at 50% Wmax ($p = 0.033$).

Hypoxia

During hypoxia there was a significant interaction for blood lactate ($p < 0.0001$) and a significant increase between exercise intensities ($p < 0.0001$), although there was no significant difference between training groups. However there were significant differences at 50%Wmax between sprint trained individuals and endurance trained individuals ($p = 0.0004$) and sprint and untrained individuals ($p < 0.0001$; Fig. 7).

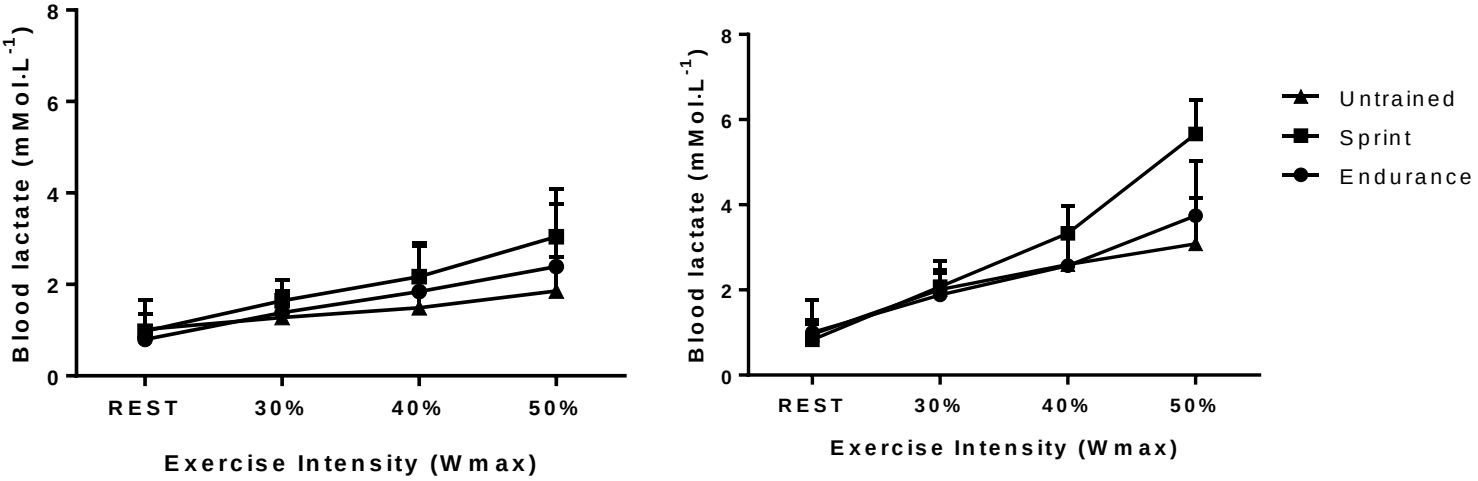

Figure 7. Blood lactate concentrations during normoxia (left) and hypoxia (right). Data presented as mean ± SD.

Effect of hypoxia on blood lactate concentration at rest and during exercise

Untrained

There was no significant difference in blood lactate concentration between normoxia and hypoxia at rest in untrained individuals. However at 50% Wmax during hypoxia, blood lactate concentration was significantly greater than in normoxia (p = 0.008; Fig. 6).

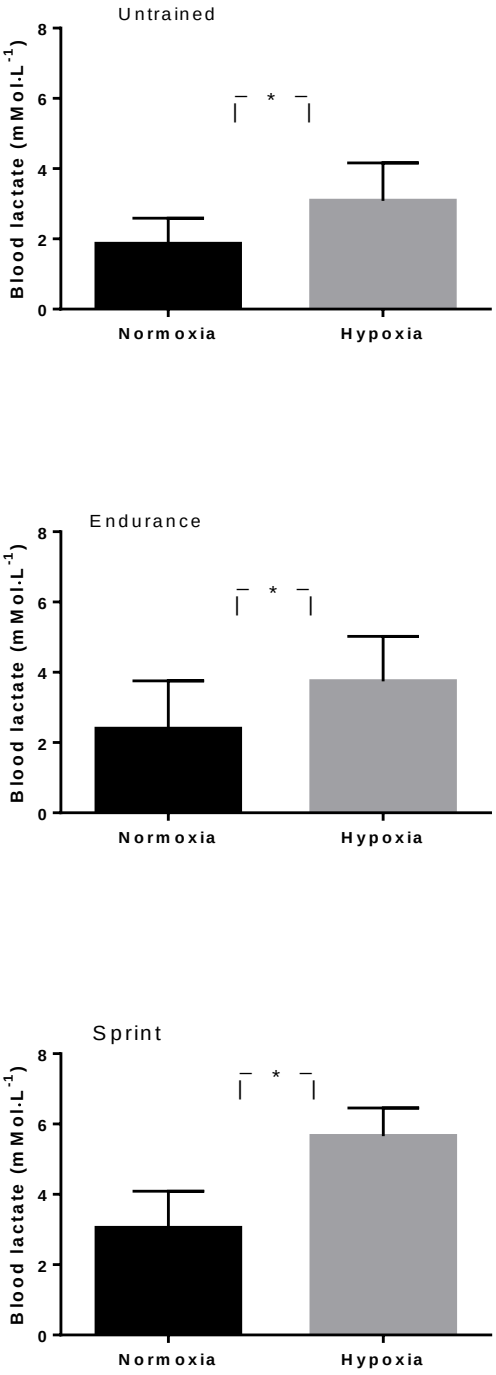

Figure 8. Differences between blood lactate during hypoxia and normoxia at 50% Wmax in different training status groups.

Endurance trained

There was no significant difference in blood lactate concentration between normoxia and hypoxia at rest in endurance trained individuals. At 50% Wmax, blood lactate concentration was significantly greater during hypoxia than in normoxia ($p = 0.011$).

Sprint trained

There was no significant difference in blood lactate concentration at rest between hypoxia and normoxia in sprint trained individuals. However at 50% Wmax, blood lactate concentration was significantly greater during hypoxia in comparison to normoxia ($p = 0.002$).

Heart rate

Effect of training status on mean heart rate at rest and during exercise

Normoxia

There was no significant interaction during normoxia, although there was a significant increase between exercise intensities ($p < 0.0001$). In normoxia mean heart rate was significantly greater in sprint trained individuals compared to untrained individuals at 50% Wmax ($p = 0.027$), whereas in the endurance and untrained groups there was no significant differences.

Hypoxia

There was no significant interaction, or difference between groups during hypoxia, however there was a significant increase between intensities ($p < 0.0001$). There was no significant differences from the post hoc multiple comparisons.

CHAPTER 5

DISCUSSION

The aim of the study was to investigate the effect of training status on substrate metabolism (derived from RER) during hypoxia at rest, and during exercise intensities of 30% Wmax, 40% Wmax and 50% Wmax. The hypothesis was that RER would be greater during hypoxia in all groups at all exercise intensities including rest. The study also examined, VE, blood lactate and heart rate at rest and during the three different exercise intensities in both hypoxia and normoxia. The main findings were that (1) during hypoxia RER was greater than during normoxia, at rest and during all exercise intensities in all the training status groups. (2) Significant interactions were found for VE during normoxia ($p = 0.02$) and hypoxia ($p = 0.005$). (3) VE was significantly greater during hypoxia than during normoxia at 50% Wmax for endurance trained and untrained individuals. (4) During hypoxia there was a significant interaction for blood lactate ($p < 0.0001$), and a significance difference during hypoxia between sprint trained and endurance trained individuals at 50% Wmax ($p = 0.0004$) and between sprint trained and untrained individuals ($p < 0.0001$). (5) Blood lactate during hypoxia was significantly greater than during normoxia in all training status groups at 50% Wmax ($p < 0.05$).

RER, as a tool for indirect calorimetry observes the aerobic metabolism of CHO and FFAs, which are shown by a value between 0.7 and 1 (Goedecke *et al.,* 2000; Huso *et al.,* 2002). Values of 0.7 to 0.85 indicate that FFAs are providing the majority of energy towards aerobic metabolism and values of 0.85-1 indicate that CHO is primarily being metabolised (Goedecke *et al.,* 2000; Huso *et al.,* 2002). The decreased O_2 availability during hypoxia leads to a greater contribution of energy from anaerobic metabolism and therefore it may be that hypoxia increases RER due to an increase in energy production via anaerobic metabolism (Westerblad *et al.,* 2010). Data collected from the current study during normoxia remained within the RER range of 0.7 to 1. However during hypoxia RER remained greater than 1 throughout suggesting that a greater contribution of anaerobic metabolism was occurring whereas during normoxia aerobic metabolism provided the majority of energy (Westerblad *et al.,* 2010).

In normoxia the RER of all three groups followed a similar pattern with incremental exercise. The sprint group maintained a marginally greater RER value throughout (Fig.3). Whereas during hypoxia although a similar increase was seen across exercise intensities, there was greater variation between the groups, with the greatest RER value in the sprint trained group

throughout. Furthermore, sprint trained individuals had the greatest increase in magnitude from rest to 50% Wmax in RER during hypoxia (Fig.3). Although the other groups also had a greater RER in hypoxia the magnitude of increment was similar to that seen during normoxia. In agreement to the findings of exercise during normoxia in the current study, Van Loon *et al.* (2001) found that during exercise in normoxia an equal contribution of FFA and CHO oxidation occurred up until ~55% Wmax, as intensity increased further to ~75% Wmax an increase in CHO oxidation occurred. Although the same relative intensity was used during hypoxia and normoxia, the greater metabolism of CHO at higher intensities suggests that, even at low intensities during hypoxia, there is a much greater challenge to the metabolic system, equivalent to much greater exercise intensities.

During hypoxia, RER was significantly greater at rest and at 50% Wmax in untrained and endurance trained individuals, however, sprint trained individuals did not show a significant difference in RER during hypoxia at the same intensities, however this was likely due to a statistical power issue due to a low sample size. The findings suggest that there was a greater reliance on the utilisation of CHO during hypoxia, similar to previous findings (Van Loon *et al.,* 2001; Katayama *et al.,* 2009). The shift towards greater CHO metabolism is likely to be linked to the reduced ability to produce energy aerobically due to a lower saturation of O_2 in the blood (Kennedy *et al.,* 2008). During hypoxia the decrease in O_2 availability increases the strain on aerobic metabolism, which usually provides energy for low to moderate intensity exercise (Mamede *et al.,* 2013). This can be seen in the data from the current study, where, during hypoxia at rest RER was greater than during normoxia at rest in all groups, as exercise intensity increased, RER also increased in both conditions, during normoxia the RER suggested that aerobic metabolism provided the majority of energy, whereas during hypoxia there was a greater utilisation of anaerobic metabolism at rest and during all exercise intensities.

During normoxic rest, FFAs are predominantly utilised, although there is great variability between individuals, particularly if untrained (Goedecke *et al.,* 2000; Janycharoen *et al.,* 2009). There are several determinants that dictate the substrate utilised at rest, hence the great variation of resting RER, including; predominant fibre type, substrate availability and resting blood lactate (Goedecke *et al.,* 2000). However in the present study CHO served to be the predominant source utilised regardless of training status during hypoxic rest (mean RER = 1.07). A study investigating the effect of hypoxia on substrate utilisation found that following acclimatisation there were no differences seen at the same relative exercise intensity (50% sea level VO_2max; Lundby & Van Hall, 2002), suggesting that the findings of

greater CHO utilisation in the current study may have been mainly due to the increased intensity rather than an effect of hypoxia alone. However the findings in the current study found a significant increase in RER alone during hypoxia at rest, whereas, VE, lactate and mean heart rate were not significantly different at rest during hypoxia, which in contrast suggests that this is not due to the exercise intensity alone.

The small contribution of FFAs during hypoxia may be partially due to an impairment of FFA oxidation from the down regulation of CPT 1, which reduces the promotion of FFAs as fuel as exercise intensity increases (Van Loon *et al.,* 2001). Furthermore the increased preference towards CHO metabolism during hypoxia, may be in order to reduce the cardiovascular strain that would be required to maintain FFA oxidation (Heinonen *et al.,* 2011). This could be explained by the greater O_2 efficiency of CHO in producing ATP in comparison to that of FFA metabolism (Lundby & Van Hall, 2002). Another mechanism that has been suggested as an explanation for increased RER during hypoxia and therefore is a greater utilisation of CHO in the exercising muscle, is that there is a greater glucose disposal in the working muscle (Heinonen *et al.,* 2011). Greater disposal of blood glucose could be due to an increased reliance on blood glucose during hypoxia, which is linked with a fuel efficiency mechanism when O_2 availability is low (Heinonen *et al.,* 2011).

Sprint trained individuals had the greatest RER in all exercise conditions during both normoxia and hypoxia, this may be due to a greater predisposition to utilise CHO during exercise. This increase in CHO metabolism could be a consequence of the training that they perform as a sprinter and the predominant muscle fibre type they possess (Goedecke *et al.,* 2000; Westerblad *et al.,* 2010). Type II fibres have a lower oxidative capacity than type I fibres which have a much greater oxidative capacity (Westerblad *et al.,* 2010; Slot *et al.,* 2014). Therefore more CHO is metabolised in the sprint trained group as they possess a greater quantity of type II fibres, which have been developed by high intensity training that they perform (Kenney *et al.,* 2011). Type I fibres are reliant on O_2 to maintain resynthesis of ATP and removal of fatiguing metabolites (Ghosh, 2004), therefore during hypoxia the reduction in O_2 availability may result in a greater decrement in performance that that seen in type II fibres which have a greater anaerobic capacity (Westerblad *et al.,* 2010; Slot et al., 2014).

VE in hypoxia was greater than in normoxia, however all groups followed a similar incremental pattern across each exercise intensity. Sprint trained individuals displayed the greatest values for VE throughout all exercise intensities following rest. In normoxic conditions all groups increased in a similar pattern with the greatest differences seen at 50%

Wmax with a significant difference between sprint trained and untrained individuals ($p = 0.001$). Due to the reduction in F_iO_2% during hypoxia, VE increases in order to maintain VO_2 and expire the excess VCO_2 that accumulates during hypoxia (Kennedy *et al.,* 2008). Deeper breathing (hyperpnoea) is one mechanism behind coping with hypoxia (Chu *et al.,* 2005) and is a likely factor contributing to the difference of VE during hypoxia in the current study.

Lactate levels in normoxia followed a similar pattern amongst all training status groups, however, there was some variation in the magnitude of lactate between groups, with sprinters having the greatest magnitude of lactate accumulation followed by endurance trained individuals with the untrained individuals having the lowest mean lactate through all exercise intensities. Lactate in hypoxia however increased at a greater magnitude than in normoxia particularly in sprint trained individuals. The increased lactate accumulation is due to the reduced ability to buffer H^+ and remove bi-products from the working muscle resulting in an excessive build-up during hypoxia, which leads to acidosis (Ghosh, 2004; Woorons *et al.,* 2010; Thomas *et al.,* 2011). During hypoxia increased blood lactate concentration in the muscle leads to a greater desaturation of O_2 in the muscle and a decrease in pH (Heubert *et al.,* 2005). Lower pH, due to an increase in lactic acid is associated with a reduction in arterial O_2 saturation for a given F_iO_2, although, haemoglobin disassociation increases therefore facilitating the delivery of O_2 in the capillaries (Heubert *et al.,* 2005).

Lactate has previously been referred to as a key indicator of RER during high intensity exercise (Goedecke *et al.,* 2000). Exercising during hypoxia, causes a great challenge on the body physiologically, with even low exercise intensities causing a severe challenge on the metabolic system. The increased intensity caused by exercising during hypoxia, leads to greater concentrations of blood lactate to accumulate (Fig. 7) which is related to greater CHO metabolism, and anaerobic metabolism of phosphocreatine and ADP (Scott, 2005). According to Goedecke *et al.* (2000) RER would be expected to be greater during hypoxia as it increases at a similar rate to blood lactate.

A great enough increase in exercise intensity, leads to ventilatory and blood lactate thresholds to be crossed; this is the point where VE and blood lactate concentrations increase rapidly and non-linearly, which is known as the anaerobic threshold (Ghosh, 2004). The anaerobic threshold of the endurance trained group would be expected to be greatest, closely followed by the sprint trained group and the untrained group would have the least tolerance to increased VE and blood lactate (Ghosh, 2004). Although sprint trained individuals have a greater H^+ buffering capacity and can continue to exercise with although their lactate threshold has been exceeded (Thomas *et al.,* 2011). These adaptations occur

because of the training that is performed which is often around or over the anaerobic threshold (Kenney *et al.,* 2011; Thomas *et al.,* 2011). Therefore it may be suggested that sprint trained individuals will respond differently during hypoxia, as they can continue to exercise with higher concentrations of blood lactate. In the present study mean blood lactate was greatest in the sprint trained group at 40% and 50% Wmax during normoxia and hypoxia, this suggests that at a given intensity sprint trained individuals have a greater accumulation of lactate concentration, which could be linked to the greater use of CHO and anaerobic substrates during exercise (Scott, 2005; Westerblad *et al.,* 2010). Although the lactate threshold increases with training, VE has been found to remain unaltered with exercise training (Ghosh, 2004).

During hypoxia there is an increased concentration of blood lactate and H^+ accumulation, which leads to an increased VE (Ghosh, 2004; Woorons *et al.,* 2010) which could cause an overestimation of RER due to the increased expiration of CO_2 (Katayama *et al.,* 2009). Furthermore hyperventilation can also impact RER, with increased CO_2 production, greater values of RER are produced, which may not represent a true estimation of substrate metabolism (Péronnet *et al.,* 2006). Because of this, indirect calorimetry has been criticised for its accuracy, particularly when used for exercise during hypoxia (Janyacharoen *et al.,* 2009).

In the present study mean heart rate significantly increased with exercise intensity in all training status groups during both hypoxia and normoxia. In normoxia mean heart rate steadily increased in the sprint trained group with a significant difference between sprint trained individuals and untrained individuals at 50% ($p = 0.03$). In the endurance and untrained groups there was a plateau between 30% Wmax and 40% Wmax, with a slight increase at 50% Wmax in endurance trained individuals. In hypoxia all the groups mean heart rate followed a similar pattern, increasing rapidly from rest to 30% Wmax, followed by a steady increase from 30% onwards. Untrained and sprint trained individuals showed similar mean heart rates throughout, whereas endurance athletes had slightly lower values at all intensities. The increase in mean heart rate in hypoxia is related to the reduction in O_2 content in the blood, therefore, for the working muscles to receive enough O_2, the heart has to pump more blood than it would at the same intensity during normoxia (Benoit *et al.,* 2003; Kennedy *et al.,* 2008). However in contrast to findings in the current study where heart rate increased in all conditions during hypoxia, it has previously been found that cardiac output decreases during acute hypoxia from a reduction in stroke volume and heart rate due to lower end diastolic volume and pre-load (Noakes, 2004; Kennedy *et al.,* 2008). Furthermore

peak heart rate has been found to be reduced at altitude alongside VO_2max, particularly in endurance trained individuals (Benoit *et al.,* 2003). This was not found in the current study as participants did not reach maximal heart rate, because steady state exercise at a moderate intensity was used.

The majority of physiological differences between training status groups and the greatest magnitude of difference between conditions were seen at 50% Wmax. The findings from the current study show that the characteristics derived from training status are displayed most clearly during hypoxia at 50% Wmax. This is where the differences in RER, blood lactate, VE and heart rate between each group can be appreciated, showing how the characteristics of their training influences their physiological response to hypoxic exercise. This suggests that a greater exercise stimulus is required to elicit different responses between training statuses and that the variables measured are intensity dependent (Van Loon *et al.,* 2001). This indicates that it is only at higher intensities or at greater altitude that the adaptations to training have an effect.

Limitations

RER is an appropriate method for indicating substrate metabolism during steady state exercise of an intensity up to ~60% VO_2max and is only measurable up to a value of 1 (Janyacharoen *et al.,* 2009). RER scores greater than 1 are questionable to their validity and accuracy, as this hasn't been accounted for in the equation for measuring substrate metabolism from RER, and may not be a representable value relative to aerobic metabolism (Westerblad *et al.,* 2010). In the present study, during hypoxia RER was greater than 1 throughout the entire protocol, the exact mechanisms behind this are currently unknown, however, it may be a consequence of increased VCO_2 (Kennedy *et al.,* 2008; Katayama *et al.,* 2009), greater exercise intensity and physiological challenge (Goedecke *et al.,* 2000; Van Loon *et al.,* 2001), greater anaerobic metabolism contribution (Scott, 2005; Westerblad *et al.,* 2010), or the inability to remove waste products (Ghosh, 2004; Woorons *et al.,* 2010). RER values greater than 1 suggest that anaerobic metabolism is taking place, and therefore different substrates such as phosphocreatine and ADP through glycolytic resynthesis of ATP would be used for energy production (Scott, 2005; Westerblad *et al.,* 2010).

Although there were differences between means of the sprint trained group and endurance and untrained groups, due to a low sample size of sprint trained individuals this group lacked statistical power and therefore few significant differences were found and this data should be viewed with caution, as the sample is not fully representative of the sprint trained population.

The exercise intensity used in the current study was not high enough to elicit enough stress to cause responses between groups, particularly during normoxia, 40% Wmax, 50% Wmax and 60% Wmax may have been better at showing the different responses of training status groups. Also the use of the supine tilt bike, although appropriate for the cardiac measurements that were used in a separate area of the study, the use of a treadmill would have been more appropriate for the athletes (all runners) and measures that were used in the current study.

Future research

In future research the use of a greater intensity, may produce more distinct responses between groups, an investigation into the effect of high intensity bouts of exercise during hypoxia using endurance, sprint and untrained groups, this would provide an interesting insight into metabolism during hypoxia. Further research into sprint trained individuals exercising during hypoxia and its effect on metabolism is required as currently the literature in this area is particularly limited, and there is clearly interesting differences between sprint trained and endurance trained individuals. Furthermore an investigation into the substrate metabolism of hypoxia tolerant individuals in comparison to substrate metabolism of endurance and sprint trained sea level residents exercising at different intensities during normoxia and hypoxia.

Practical implications

In respect to the greater utilisation of anaerobic metabolism during hypoxia, it may be beneficial for athletes to incorporate some high intensity anaerobic training prior to performing at altitude, if implemented early enough then the development of type II fibres may help to improve performance due to the characteristics that type II fibres possess, most importantly resistance to fatigue when O_2 availability is low. It is also important to be aware that maximal performance will be reduced, lower peak heart rate and greater accumulation of blood lactate will restrict maximal exercise capacity. To help energy production a high CHO diet may help to maintain the higher intensities experienced at high altitude and reduce cardiovascular strain.

CHAPTER 6

CONCLUSION

The aim of the study was to investigate the effect of training status on substrate metabolism during acute hypoxia. The key findings were that RER was greater during hypoxia at rest and during exercise, VE in endurance trained and untrained individuals, was significantly greater at 50% Wmax, blood lactate was significantly greater at 50% Wmax during hypoxia in all training status groups. RER shifts towards greater CHO utilisation during hypoxia at rest, and during exercise regardless of training status. Therefore the hypothesis that RER would increase irrespective of training status during hypoxia was accepted. The increase in RER is likely due to an increased intensity of exercise and therefore the ability to metabolise O_2 aerobically is reduced, increasing the reliance on anaerobic metabolism. Other mechanisms may also contribute towards greater CHO utilisation, such an increased glucose disposal (Heinonen *et al.,* 2011), reduced cardiovascular strain (Heinonen *et al.,* 2011) and inhibition of FFA oxidation at higher exercise intensities (Van Loon *et al.,* 2001). The implications of the study suggest that a diet high in CHO should be used to help energy production and reduce cardiovascular strain. In conclusion RER increases regardless of training status with exercise intensity and is significantly greater in hypoxia, at rest and during exercise in untrained and endurance trained individuals.

REFERENCES

Alkhatib, A., Seijo, M., Larumbe, E. and Naclerio, F. (2015). Acute effectiveness of a “fat-loss” product on substrate utilization, perception of hunger, mood state and rate of perceived exertion at rest and during exercise. *Journal of the International Society of Sports Nutrition*, **12(1)**, pp. 44-52.

Baker, J. S., McCormick, M. C. and Robergs, R. A. (2010). Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of Nutrition and Metabolism*, **2010**, pp. 1–13.

Beidleman, B. A., Muza, S. R., Fulco, C. S., Cymerman, A., Sawka, M. N., Lewis, S. F. and Skrinar, G. S. (2008). Seven intermittent exposures to altitude improves exercise performance at 4300 m. *Medicine & Science in Sports & Exercise*, **40(1)**, pp. 141–148.

Benoit, H., Busso, T., Castells, J., Geyssant, A. and Denis, C. (2003). Decrease in peak heart rate with acute hypoxia in relation to sea level VO2max. *European Journal of Applied Physiology*, **90(5-6)**, pp. 514–519.

Calbet, J. A. L. (2003). Chronic hypoxia increases blood pressure and noradrenaline spillover in healthy humans. *Journal of Physiology*, **551(1)**, pp. 379-386.

Carter, J.M., Jones, D.A. and Jeukendrup, A.E. (2002). Validity and reliability of three commercially available breath-by-breath respiratory systems. *Medicine & Science in Sports & Exercise*, **34(5)**, p. S4.

Carter, S., Rennie, C. and Tarnopolsky, M. (2001). Substrate utilization during endurance exercise in men and women after endurance training. *AJP: Endocrinology and Metabolism*, **280(6)**, pp.E898-E907.

Chu, A.L., Jay, O. and White, M.D. (2006). The effects of hyperthermia and hypoxia on ventilation during low-intensity steady-state exercise. *AJP: Regulatory, Integrative and Comparative Physiology*, **292(1)**, pp. R195–R203.

Cleuziou, C., Perrey, S., Lecoq, A. M., Candau, R., Courteix, D. and Obert, P. (2005). Oxygen uptake Kinetics during moderate and heavy intensity exercise in humans: The influence of Hypoxia and training status. *International Journal of Sports Medicine*, **26(5)**, pp. 356–362.

Emery, P. (2015). Basic metabolism: protein. *Surgery (Oxford)*, **33(4)**, pp.143-147.

Flat, J. P. (1995). Body composition, respiratory quotient, and weight maintenance. *American journal of clinical nutrition*, **62(5)**, pp. 1107S-1117S.

Friedmann, B., Bauer, T., Menold, E. and Bartsch, P. (2004). Exercise with the Intensity of the Individual Anaerobic Threshold in Acute Hypoxia. *Medicine & Science in Sports & Exercise*, **36(10)**, pp.1737-1742.

Gallagher, C.A., Willems, M.E.T., Lewis, M.P. and Myers, S.D. (2014). Effect of acute normobaric hypoxia on the ventilatory threshold. *European Journal of Applied Physiology*, **114(8)**, pp. 1555–1562.

Ghosh, A.K. (2004). Anaerobic Threshold: Its Concept and Role in Endurance Sport. *Malaysian Journal of Medical Science*, **11(1)**, pp. 24–36.

Goedecke, J. H., Gibson, A. S., Grobler, L., Collins, M., Noakes, T. D., Lambert, E. V. (2000). Determinants of the variability in the respiratory exchange ratio at rest and during exercise in trained athletes. *AJP: Endocrinology and Metabolism*, **279**, pp. E1325-E1334.

Gollnick, P.D. (1985). Metabolism of substrates: energy substrate metabolism during exercise and as modified by training. *Federation Proceedings*, **44(2)**, pp. 353-353.

Gore, C. J. (2014). The challenge of assessing athlete performance after altitude training, *Invited Editorial*, **116(6)**, pp. 593–594.

Gormley, S. E., Swain, D. P., High, R., Spina, R. J., Dowling, E. A., Kotipalli, U. S. and Gandrakota, R. (2008). Effect of intensity of aerobic training on VO2max. *Medicine & Science in Sports & Exercise*, **40(7)**, pp. 1336–1343.

Heinonen, I., Kemppainen, J., Kaskinoro, K., Peltonen, J., Sipila, H., Nuutila, P., Knuuti, J., Boushel, R. and Kalliokoski, K. (2011). Effects of adenosine, exercise, and moderate acute hypoxia on energy substrate utilization of human skeletal muscle. *AJP: Regulatory, Integrative and Comparative Physiology*, **302(3)**, pp.R385-R390.

Henderson, J., Withford-Cave, J. M., Duffy, D. L., Cole, S. J., Sawyer, N. A., Gulbin, J. P., Hahn, A., Trent, R. J. and Yu, B. (2005). The EPAS1 gene influences the aerobic–anaerobic contribution in elite endurance athletes. *Human Genetics*, **118(3)**, pp. 416–423.

Hughson, R.L., Green, H.J. and Sharratt, M.T. (1995). Gas exchange, blood lactate, and plasma catecholamines during incremental exercise in hypoxia and normoxia. *Journal of Applied Physiology*, **79(4)**, pp. 1134–1141.

Huso, M., Hampl, J., Johnston, C. and Swan, P. (2002). Creatine supplementation influences substrate utilization at rest. *Journal of Applied Physiology*, **93(6)**, pp.2018-2022.

Infante-Vázquez, O., Cadena-Méndez, M., Escalante-Ramírez, B. and Azpiroz-Leehan, J. (2013). VO2 and VCO2 variabilities through indirect calorimetry instrumentation. *SpringerPlus*, **2(1)**, pp. 688-700.

Jacobs, R. A., Rasmussen, P., Siebenmann, C., Diaz, V., Gassmann, M., Pesta, D., Gnaiger, E., Nordsborg, N. B., Robach, P. and Lundby, C. (2011). Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *Journal of Applied Physiology*, **111(5)**, pp. 1422–1430.

Janyacharoen, T., Auvichayapat, P., Tsintzas, K., MacDonald, I. A. and Leelayuwat, N. (2009). Effect of gender on fuel utilization during exercise at different intensities in untrained Thai individuals. *European journal of applied physiology,* **107(6)**, pp. 645-651.

Katayama, K., Goto, K., Ishida, K. and Ogita, F. (2010). Substrate utilization during exercise and recovery at moderate altitude. *Metabolism*, **59(7)**, pp. 959–966.

Kennedy, M.D., Warburton, D.E., Boliek, C.A., Esch, B.T., Scott, J.M. and Haykowsky, M.J. (2008). The oxygen delivery response to acute hypoxia during incremental knee extension exercise differs in active and trained males. *Dynamic Medicine*, **7(1)**, pp. 11–23.

Kenney, L. W., Wilmore, J. H. and Costill, D. L. (2011). *Physiology of sport and exercise*. 5th edition. Champaign, IL: Human Kinetics.

Kubukeli, Z. N., Noakes, T. D. and Dennis, S. C. (2002). Training techniques to improve endurance exercise performances. *Sports Medicine*, **32(8)**, pp. 489–509.

Liversey, G. and Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition*, **47(4)**, pp. 608–628.

Lundby, C. and Van Hall, G. (2002). Substrate utilization in sea level residents during exercise in acute hypoxia and after 4 weeks of acclimatization to 4100 m. *Acta Physiologica Scandinavica*, **176(3)**, pp. 195–201.

Mamede, A. C., Abrantes, A. M., Pedrosa, L., Casalta-Lopes, J. E., Pires, A. S., Teixo, R. J., Gonçalves, A. C., Sarmento-Ribeiro, A. B., Maia, C. J. and Botelho, M. F. (2013). Beyond

the limits of oxygen: Effects of Hypoxia in a hormone-independent prostate cancer cell line. *ISRN Oncology*, **2013**, pp. 1–8.

Mezzani, A., Corrà, U., Bosimini, E., Giordano, A. and Giannuzzi, P. (2003). Contribution of peak respiratory exchange ratio to peak VO2 prognostic reliability in patients with chronic heart failure and severely reduced exercise capacity. *American Heart Journal*, **145(6)**, pp. 1102–1107.

Morishima, T. and Goto, K. (2014). Successive exposure to moderate hypoxia does not affect glucose metabolism and substrate oxidation in young healthy men. *SpringerPlus*, **3(1)**, pp. 370-378.

Noakes, T.D. (2004). Central regulation of skeletal muscle recruitment explains the reduced maximal cardiac output during exercise in hypoxia, *AJP: Regulatory, Integrative and Comparative Physiology*, **287(4)**, pp. R996–R1002.

Péronnet, F., Massicotte, D., Folch, N., Melin, B., Koulmann, N., Jimenez, C., Bourdon, L., Launay, J.C. and Savourey, G. (2006). Substrate utilization during prolonged exercise with ingestion of ^{13}C-glucose in acute hypobaric hypoxia (4,300 m). *European Journal of Applied Physiology,* **97**, pp. 527-534.

Pogatschnik, C. and Steiger, E. (2015). Review of Preoperative carbohydrate loading. *Nutrition in Clinical Practice*, **30(5)**, pp. 660–664.

Prabhakar, A., Quach, A., Zhang, H., Terrera, M., Jackemeyer, D., Xian, X., Tsow, F., Tao, N. and Forzani, E. S. (2015). Acetone as biomarker for ketosis buildup capability - a study in healthy individuals under combined high fat and starvation diets. *Nutrition Journal*, **14(1)**, pp. 41-52.

Ramos-Jimenez, A., Hernandez-Torres, R. P., Torres-Duran, P. V., Romero-Gonzalez, J., Mascher, D., Posadas-Romero, C. and Juarez-Oropeza, M. A. (2008). The Respiratory Exchange Ratio is Associated with Fitness Indicators Both in Trained and Untrained Men: A Possible Application for People with Reduced Exercise Tolerance. *Clinical Medicine: Circulatory, Respiratory and Pulmonary*, **2**, pp. 1–9.

Saraslanidis, P. J., Manetzis, C. G., Tsalis, G. A., Zafeiridis, A. S., Mougios, V. G. and Kellis, S. E. (2009). Biochemical evaluation of running workouts used in training for the 400-m sprint. *Journal of Strength and Conditioning Research*, **23(8)**, pp. 2266–2271.

Slot, I.G.M., Schols, A.M.W.J., Vosse, B.A.H., Kelders, M.C.J.M. and Gosker, H.R. (2014). Hypoxia differentially regulates muscle oxidative fiber type and metabolism in a HIF-1α-dependent manner. *Cellular Signalling*, **26(9)**, pp. 1837–1845.

Subudhi, A. W., Lorenz, M. C., Fulco, C. S. and Roach, R. C. (2007). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: Effect of oxygenation on maximal performance. *AJP: Heart and Circulatory Physiology*, **294(1)**, pp. H164–H171.

Subudhi, A. W., Miramon, B. R., Granger, M. E. and Roach, R. C. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *Journal of Applied Physiology*, **106(4)**, pp. 1153–1158.

Tarnopolsky, M. A. (2008). Sex differences in exercise metabolism and the role of 17-beta Estradiol. *Medicine & Science in Sports & Exercise*, **40(4)**, pp. 648-654.

Thomas, C., Bernard, O., Enea, C., Jalab, C. and Hanon, C. (2011). Metabolic and respiratory adaptations during intense exercise following long-sprint training of short duration. *European Journal of Applied Physiology*, **112(2)**, pp. 667–675.

Van Loon, L. J. C., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. M. and Wagenmakers, A. J. M. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, **536(1)**, pp. 295–304.

Westerblad, H., Bruton, J.D. and Katz, A. (2010). Skeletal muscle: Energy metabolism, fiber types, fatigue and adaptability. *Experimental Cell Research*, **316(18)**, pp. 3093–3099.

Vogt, M., and Hoppeler, H. (2010). Is hypoxia training good for muscles and exercise performance? *Progress in Cardiovascular Diseases*, **52(2010)**, pp. 525-533.

Wadley, G. D. (2005). Effect of exercise intensity and hypoxia on skeletal muscle AMPK signaling and substrate metabolism in humans. *AJP: Endocrinology and Metabolism*, **290(4)**, pp. E694–E702.

Wismann, J. and Willoughby, D. (2006). Gender differences in carbohydrate metabolism and carbohydrate loading. *Journal of the International Society of Sports Nutrition*, **3(1)**, pp. 28-34.

Woorons, X., Bourdillon, N., Vandewalle, H., Lamberto, C., Mollard, P., Richalet, J.-P. and Pichon, A. (2010). Exercise with hypoventilation induces lower muscle oxygenation and higher blood lactate concentration: Role of hypoxia and hypercapnia, *European Journal of Applied Physiology*, **110(2)**, pp. 367–377.

Workman, C. and Basset, F. A. (2012). Post-metabolic response to passive normobaric hypoxic exposure in sedentary overweight males: A pilot study. *Nutrition & Metabolism*, **9(1)**, p. 103.

APPENDICES

APPENDIX A

Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

RESEARCH PROJECT

"How does blood pressure influence the untwisting of the Heart muscle?"

Name of principal investigator: **Mr Jake Samuel**

Name of co-investigators: **Dr. Eric Stöhr**

This document provides information on:

1) The background and aim of the research project
2) The role of the researchers
3) Your role as a participant
4) Benefits of taking part
5) How data will be collected
6) Risks
7) How the results will be used
8) Your rights

IMPORTANT: The purpose of this document is to assist you in making an informed decision about whether you wish to volunteer for this research project by promoting transparency in the research process.

1) Background and aims of the research

- Untwisting of the heart is an important mechanism of filling of the heart with blood.
- The rate of untwisting is reduced in aerobically fit individuals at rest and during submaximal exercise. At present, scientists think this reflects a greater efficiency of the trained heart to draw blood in to the heart.

- During this phase the heart muscle also supplies itself with oxygen by increasing the blood flow to this area.
- However, it is not known whether the blood pressure influences how much blood is distributed to the heart.
- It is possible that the lower untwisting rate in fit individuals during rest and submaximal exercise is caused by their increased ability to supply the heart itself with oxygen.
- **The aim of this study** is to investigate the influence of blood pressure on cardiac untwisting rate in individuals with low and high aerobic fitness at rest and during exercise, with and without altered oxygen consumption. The response to acute exercise may help to understand whether untwisting of the heart is related to oxygen supply of the heart and whether this is altered when oxygen availability is reduced.

2) The role of the researchers:

The researchers (Jake Samuel and Dr Eric Stöhr) will be responsible for conducting and overseeing all stages of the research project. In addition to carrying out the scientific part of the project, we are there to **provide you with personal guidance and assistance** in relation to any questions or issues you may have. During your first visit, we will **explain all the project details** so that you feel comfortable with knowing exactly what will be required. We will also assist you with the completion of the health questionnaire and answer **any questions you may have** in relation to the project. While our research interest is to collect novel and valuable information on your heart's response to breathing different oxygen concentrations, your health and safety is of primary importance to us and has precedence over our research interests. In relation to this, you will not only be able to ask questions and inform us about issues while in the laboratory but you will also be able to contact Dr Eric Stöhr at any time outside laboratory contact time. We encourage you to talk to us at any time should you feel uncomfortable or have questions related to the project.

3) Your role as a participant:

We are very grateful should you choose to take part in this research project and we will treat you with respect at all times and try our best to explain everything to you so that you have an enjoyable experience. While we need to ensure the scientific quality of our work, we also aim to create a friendly and fun environment in the laboratory during your visits.

Your role is to **visit the laboratory on two occasions** and enable us to assess your health through completion of a brief questionnaire, assess your current aerobic fitness through a standardized fitness test on a supine bike and measure the function of your heart whilst exercising and breathing two different levels of oxygen: **normal (21%)** and **low oxygen (12%)**.

4) Direct and indirect benefits of taking part:

Your participation is potentially of direct and indirect benefit to you:

Direct: By participating you will learn how your heart responds to different levels of inspired oxygen. You will also get to know about your general fitness as assessed in the fitness test and you will experience how state-of-the-art research is conducted in an exercise laboratory.
Indirect: Your participation will contribute to our general knowledge on the human heart. Your participation will inform researchers and clinicians and may help to develop further research into the influence of exercise intensity or oxygen consumption on heart function during exercise.
We will be happy to share your personal results from this study with you.

5) How data will be collected:

You will need to attend the laboratory on two occasions for two time points of assessment.

The purpose of each assessment and what you will be required to do is outlined below.

Assessment 1 (estimated duration ~30 minutes)

During your first assessment, you can ask questions about the research and, when all your questions are answered, you will:

- Fill out a consent form and a health questionnaire (see both attached). According to the guidelines of the American College of Sports Medicine (ACSM), you will be considered healthy and free to enroll in this study if you do not have more than one cardiovascular risk factors. We will then measure your height and weight. Should the completion of the questionnaire reveal that you do have more than one risk factors, you will, unfortunately, not be able to partake in this research project.
- Perform a standard maximal exercise test according to ACSM guidelines. The purpose of this test is to assess your fitness. To do so, you will be asked to sit on a supine bicycle and wear a face mask through which you will breathe normal room air. We will collect your expired air in a computer attached to the face mask at rest

  and during exercise. Exercise intensity will start at a very easy level and become progressively harder until you are unable to continue to cycle. At this point, we will lower the exercise intensity but ask you to keep cycling for another three minutes. During the last 30 seconds of each exercise, at the end of the last stage and 2 minutes after exercise a small blood sample will be taken from your finger tip for post-test lactate analysis. This cool down is for your own safety to avoid dizziness following your exercise effort. Following your recovery, this assessment is completed.

Assessment 2 (estimated duration ~2 hours 30 minutes)

During assessment two, upon arrival we will:

- Measure your height and weight again and you will lie down on a medical couch. You will be required to remove the clothing on your upper body for the purpose of the heart scans and **you will wear sports shorts**.

- We will attach a normal blood pressure cuff on your upper right arm and a small cuff on your middle finger for continuous measurement of your blood pressure. We will also place a clip onto your right ear lobe in order to measure the level of oxygen in your blood. Your normal oxygen level is expected to be ~98-100% when breathing room air. Oxygen levels during hypoxia are expected to drop to approximately 6080%. Previous studies have seen safe reductions at 60%. We will continuously monitor your oxygen level and should it fall below 60% for more than 30 seconds we will stop the research and you will breathe room air immediately.
- We will also stick three ECG pads on your chest that will continuously monitor your heart rate.
- In addition, a wrist tonometer will be placed over your right wrist in order to measure the blood pressure in your wrist.
- Also, three electrodes and an infra-red box will be attached to your right upper leg to measure the electrical activity and oxygen consumption of your leg muscle, respectively.
- Once all the equipment is attached, we will measure the function of your heart **at rest** using ultrasound. We will take pictures of your heart from two locations on your chest: the upper left side of your chest and from the middle left side.
- You will then exercise at low intensities which will progressively increase until you reach 30% of the maximal effort you achieved in the first visit. Then, you will

  exercise for 5 minutes at 30%, 40% and 50%, respectively, while either breathing normal room air (21%) or low oxygen (12%). Following this bout of exercise you will rest for 45 minutes.
- Following your rest period, you will be weighed again before performing a second exercise bout, with the same intensities as the previous effort but in the other breathing condition (either 21% or 12% oxygen).
- To avoid biased results, we will not tell you the order of conditions (21% or 12%) until you have completed the whole test.

6) Risks

For healthy individuals, there are two risks associated with this project.

1) You may feel light-headed or dizzy following the maximal exercise effort during visit one. This can be avoided by continuing to cycle at low exercise intensities after the maximal effort. We will ensure that you get 3 minutes of recovery at low intensity (25 Watts), thereby minimizing the risk of dizziness or light-headedness.

2) The inspiration of hypoxic gas may cause dizziness or light headaches or chest pain (unexpected in healthy individuals). Previous studies have used lower oxygen concentrations than this study and have not reported complications. However, should you feel dizzy, light-headed or report a headache or chest pain during exercise, we will immediately remove the mouth piece and let you breathe normal room air. The

symptoms should disappear within 30 seconds and we will not continue the trial. We strongly encourage you to notify us should you feel uncomfortable or unwell.

7) How the data / research will be used:

In agreeing to become a voluntary participant, you will be allowing us to use your results to statistically analyse, interpret and publish the findings according to the principles of scientific conduct. We will not refer to your personal data in any way during conference presentations or in peer-review publication.

8) Your rights

IMPORTANT: Your right as a voluntary participant is that you are **free to enter or withdraw from the study at any time**. This simply means that you are in full control of the part you play in informing the research and what anonymous information is used in its final reporting.

Protection to your privacy

Your identity will not be disclosed in any written transcripts, notes or associated documentation that informs the research and its findings. Furthermore, any personal information about you will remain confidential according to the guidelines of the Data Protection Act (1998).

Contact details

If you require further information or have any outstanding queries, feel free to contact the principal investigator or a co-investigator.

Jake Samuel

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APPENDIX B

Cardiff Metropolitan University | Prifysgol Metropolitan Caerdydd

CONSENT FORM

Title of Project:

"How does blood pressure influence the untwisting of the heart muscle?"

Principal Investigators:
Dr Eric Stöhr estohr@cardiffmet.ac.uk
Mr Jake Samuel st20008009@outlook.cardiffmet.ac.uk Participant Identification Number:

Please tick the appropriate box for each question / statement.

1. I confirm that I have read and understand the information sheet related to this study. I have had the opportunity to consider this information, ask questions and have had my questions answered satisfactorily.	**YES** ☐	**NO** ☐
2. I understand that my participation is voluntary and that I am free to withdraw from this study at any time, without giving any reason, without my medical care or legal rights being affected.	**YES** ☐	**NO** ☐
3. I understand that the data collected in this project will not be used for commercial purposes.	**YES** ☐	**NO** ☐
4. I agree that if I become unable to continue giving my consent during the project, I will be withdrawn from the project and data or tissue already collected under consent will be anonymised and used for research purposes.	**YES** ☐	**NO** ☐
5. While this is a research study, we may observe abnormalities in the function of your heart or arteries. Do you wish to be informed about these should we think something warranted further medical consultations?	**YES** ☐	**NO** ☐

Please turn over

CONSENT FORM
(continued)

Please tick the appropriate box for each question / statement.

6. I agree that the principal and co-investigators of this study may use my data anonymously in other research projects with the purpose of answering new research questions. **YES** ☐ **NO** ☐

7. I agree to take part in the above study. **YES** ☐ **NO** ☐

Name of participant:	**Date:**	**Signature:**
_____	_____	_____

Name of person taking consent:	**Date:**	**Signature:**
_____	_____	_____

APPENDIX C

AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire

Assess your health needs by marking all *true* statements.

History

You have had:

___ A heart attack

___ Heart surgery

___ Cardiac catheterization

___ Coronary angioplasty (PTCA)

___ Pacemaker/implantable cardiac defibrillator/rhythm disturbance

___ Heart valve disease

___ Heart failure

___ Heart transplantation

___ Congenital heart disease

*If you marked any of the statements in this section, consult your physician or other appropriate healthcare provider before engaging in exercise. You may need to use a facility with a **medically qualified staff**.*

Other health issues

___ You have diabetes

___ You have or asthma other lung disease

___ You have burning or cramping in your lower legs when walking short distances

___ You have musculoskeletal problems that limit your physical activity

___ You have concerns about the safety of exercise

___ You take prescription medication(s)

___ You are pregnant

Symptoms

___ You experience chest discomfort with exertion

___ You experience unreasonable breathlessness

___ You experience dizziness, fainting, blackouts

___ You take heart medications

_____ _____

Cardiovascular risk factors

___ You are a man older than 45 years

___ You are a woman older than 55 years, you have had a hysterectomy, or you are postmenopausal

___ You smoke, or quite within the previous 6 months

___ Your BP is greater than 140/90

___ You don't know your BP ___ You take BP medication

*If you marked two or more of the statements in this section, you should consult your physician or other appropriate healthcare provider before engaging in exercise. You might benefit by using a facility with a **professionally qualified exercise staff** to guide your exercise program.*

___ Your blood cholesterol level is >200 mg/dL ___ You don't know your cholesterol level

___ You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister)

___ You are physically inactive (i.e., you get less than 30 min. of physical activity on at least 3 days per week) ___ You are more than 20 pounds overweight

___ None of the above is true

You should be able to exercise safely without consulting your physician or other healthcare provider in a selfguided program or almost any facility that meets your exercise program needs.

Balady et al. (1998). AHA/ACSM Joint Statement: Recommendations for Cardiovascular Screening, Staffing, and Emergency Policies at Health/Fitness Facilities. *Medicine & Science in Sports & Exercise*, *30*(6). (Also in: *ACSM's Guidelines for Exercise Testing and Prescription*, 8th Edition, 2009. Lippincott Williams and Wilkins http://www.lww.com)

www.acsm-msse.org/pt/pt-core/template-journal/msse/media/0698c.htm