

**A comparison of
different work: rest
ratios in a high intensity
interval-training
programme; the effect
on performance and
health parameters.**

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Molly Lloyd Jones

A handwritten signature in black ink, appearing to read 'Molly Lloyd Jones', with a horizontal line underneath the name.

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Abbreviations

ACSM	American College of Sports Medicine
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
A-V O₂ diff	Arterio-venous oxygen difference
EPO	Erythropoietin
EPOC	Excess Post-Exercise Oxygen Consumption
GLUT4	Glucose Transporter type 4
Hex	Hexokinase
HIF-1	Hypoxia-Inducible Factor 1
HIIT	High Intensity Interval Training
HR	Heart rate
MCT1	Monocarboxylate Transporter 1
MPO	Mean Power Output
NIRS	Near Infrared Spectroscopy
OBLA	Onset of Blood Lactate
PAR-Q	Physical activity readiness questionnaire
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
PFK	Phosphofructokinase
PGC-1α	Peroxisome proliferator-activated receptor-co-activator-1 α
PPO	Peak Power Output
Q	Cardiac Output
RSA	Repeated Sprint Ability
SV	Stroke Volume
TSI%	Tissue Oxygen Saturation
TT	Time Trial
VEGF	Vascular Endothelial Growth Factor
VO₂max	Maximal Oxygen Consumption

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Abstract

Background

It is well documented that high intensity interval training (HIIT) is a time efficient and effective method of training to improve exercise performance. However, there are a wide range of intervention protocols used in both research and applied fields, making comparison of data difficult. Previous work from our laboratory (Lloyd-Jones *et al.*, under review) suggested that the work:rest ratio used during this type of training may be important in determining adaptations, and in selecting the most efficient training approach, although this a poorly understood and often neglected consideration. Therefore the aim of this study is to compare different work:rest ratio, all using a 6 second sprint but with either a 1:8, 1:10 or 1:12 work:rest ratio.

Methods

35 active male and females (age: 24 ± 4 years, height: 1.77 ± 0.09 m, body mass: 74.7 ± 12.9 kg, body fat: $16.8 \pm 6.1\%$, height, body mass, body fat) initially completed a VO_2 max step test, followed at least 24hrs later by a thigh occlusion test whilst wearing near-infrared spectroscopy over the vastus lateralis muscle, and a 10km cycling time trial.

Participants then completed 6 sessions of HIIT on a cycle ergometer split over 2 weeks, consisting of 10x6 sec sprints with either 48sec rest (1:8 group), 60sec rest (1:10 group) or 72sec rest (1:12 group), and compared with a control group in a randomised controlled trial. Peak and average power output were calculated for each sprint session and peak power calculated for each individual sprint.

Results

There was no significant change in VO_2 max following 2 weeks of training in any group, or 10km time trial performance (1:8: pre: 780 ± 258 sec post: 751 ± 270 sec, 1:10 pre: 572 ± 138 sec post: 563 ± 134 sec, 1:12: pre: 641 ± 100 sec post: 615 ± 94 sec, Con: pre: 716 ± 207 sec post: 761 ± 229 sec). Peak power output increased significantly in the 1:8 group (1038 ± 330 - 1095 ± 357 watts), 1:10 group (1092 ± 282 - 1148 ± 286 watts) and 1:12 group (1043 ± 201 - 1097 ± 226 watts). The average range of the change in power over the

training session, for all the six sprint sessions for the 1:8 group was 62watts, the 1:10 group was 44watts and the 1:12 group was 45watts. There was no change in muscle oxygen kinetic data in any group following 2 weeks of HIIT as assessed by thigh occlusion.

Conclusion

The performance improvements in 10km time trial performance were largest in the 1:8 and 1:12 groups. It is possible that there was insufficient recovery time in the 1:8 condition, resulting in metabolic stress to the cardiovascular system, and that adaptations in the 1:12 condition may be a consequence of repeatedly achieving peak power output, facilitated by a sufficient recovery period. It is therefore possible that adaptations following HIIT can be elicited from different stimuli, and this should be considered in future research and practice.

Chapter 1: Introduction

It is well established that exercise has cardiovascular, metabolic and psychological benefits, and is a modifiable risk factor for lowering the risk of many chronic diseases (Saaniyoki *et al.* 2015; Warburton *et al.* 2006). More specifically, exercise has been demonstrated to improve body composition, glucose homeostasis and insulin sensitivity and cardiac function, while having the psychological benefits of decreasing stress levels, anxiety and depression (Peluso & Guerra de Andrade, 2005). Despite the well-documented benefits, in 2012 45% of women and 33% of men in England were not completing the recommended amount of exercise (Townsend *et al.* 2015). The American College of Sports Medicine (ACSM) recommends all healthy adults should complete at least 30 minutes of moderate intensity physical activity 5 times a week, or at least 20 minutes of vigorous activity 3 times each week, in addition to completing exercises to maintain or improve muscular strength at least twice a week (American College of Sports Medicine, 2010).

A common barrier that has been reported to prevent participation in exercise is a lack of time due to work or study commitments (Aaltonen *et al.* 2012). High intensity interval training (HIIT) consists of repeated bouts of very brief exercise, performed with an 'all-out' effort, and has been reported to be a time efficient exercise strategy that produces similar adaptations to endurance training (Gibala & McGee, 2008). In runners, high intensity interval training is thought to minimize the risk of overuse and orthopaedic injury, by reducing overall training volume but still improving running performance (Kavaliuskas *et al.* 2015). Bartlett *et al.* (2011) reported that high intensity interval training is more enjoyable than long duration endurance training in healthy recreationally trained participants, and enjoyment is another common barrier that prevents participation in physical activity or exercise (Warburton *et al.* 2006). Additionally, Jung *et al.* (2015) reported greater adherence to HIIT programmes in comparison to moderate intensity continuous exercise programmes one month following the supervised laboratory based interventions were initiated.

Zieman *et al.* (2011) states that high intensity training is an effective training method to improve aerobic capacity and therefore be beneficial for endurance

sports, reporting an increase in maximal aerobic capacity ($VO_2\text{max}$) and a lower blood lactate concentration following 6 weeks of HIIT. Similarly, Burgomaster *et al.* (2008) reported an increase in $VO_2\text{max}$ following 6 weeks of high intensity sprint training, but that there was no difference between the improvement in $VO_2\text{max}$ between the HIIT group and a group that completed continuous endurance training. The oxygenation of the leg muscle group is a primary determinant of maximal exercise performance and may therefore explain the improvements in $VO_2\text{max}$ and time trial performance (Jacobs *et al.* 2013), although results have varied, an increase in muscle oxygen extraction has been reported following HIIT, and consequently may improve exercise performance (Rud *et al.* 2011).

Skelly *et al.* (2014) demonstrated that HIIT, when compared to continuous endurance training, is a time efficient training method that produces similar energy expenditure in the 24 hours following exercise. This suggests that HIIT could improve body composition with less mechanical work and time commitment compared to long duration endurance training. Townsend *et al.* (2013) reported a greater excess post-exercise oxygen consumption (EPOC) following high intensity training compared to endurance training. This elevated EPOC results in increased energy expenditure, and hence an increased caloric expenditure, which explains the decrease in total body mass and percentage body fat, as reported in many studies (Whyte *et al.* 2010; Boutcher, 2011). This decrease in total body mass and percentage body fat that occurs following a HIIT programme, may benefit patients with type 2 diabetes and cardiovascular diseases. There are strong links with hypertension and the development of diseases such as type 2 diabetes (Madsen *et al.* 2015; Swain & Franklin, 2006), however HIIT has been reported to provide a range of health benefits in patients with type 2 diabetes and healthy individuals, including an improved glycaemic control, reduced abdominal fat and reduced hypertension. This improvement in glycaemic control as seen as a result of exercise is due to an increase in the protein glucose transporter 4 (GLUT 4), which facilitates an increased glucose uptake (Little *et al.* 2011). Babraj *et al.* (2009) reported an improvement in glycaemic control and insulin sensitivity following high intensity training in healthy

individuals, demonstrating that HIIT may help reduce the risk of developing type 2 diabetes.

Many protocols have been used in different studies, ranging from a 4 minute high intensity exercise bout to a 6 seconds sprint, with one of the most common being repeated Wingate (30 second) sprints. Lloyd Jones *et al.* (under review) reported no significant difference between performance adaptations including a 10km time trial when comparing a 30 second sprint with 4 minutes recovery (1:8 work:rest) and a 6 second sprint with 48 seconds rest (1:8 work:rest). Additionally, Jakeman *et al.* (2012) reported a significant improvement in time trial performance and peak power output following 10x6 second sprints with 60 seconds rest in between each sprint. Sprints below 10 seconds are much more manageable (Hazell *et al.* 2010), and have been reported to be as effective at improving exercise performance as longer duration sprints, but health benefits of this short duration sprint are currently unknown. Hence more research is needed to find the optimal work to rest ratio for a 6 second sprint training programme, as previous research using the 6 second sprint have used different rest durations but the work to rest ratios have not directly been compared to determine if there is an optimal protocol.

Chapter 2: Literature Review

Moderate intensity exercise training has been reported to improve exercise performance and muscle oxidative capacity and have various health benefits (Combes *et al.* 2016; Burgomaster *et al.* 2005). Although less widely recognized, many studies have reported shorter duration, higher intensity exercise bouts have similar effects to longer duration endurance training (Burgomaster *et al.* 2008; Gibala *et al.* 2006; Helgerud *et al.* 2007). High intensity interval training (HIIT) can be defined as repeated bouts of short duration exercise, completed at an 'all-out' effort, interspersed with brief rest periods (Laursen & Jenkins, 2002). Many studies have directly compared continuous exercise to high intensity interval training and have reported similar metabolic and performance adaptations (Gibala *et al.* 2006; Burgomaster *et al.* 2008).

Cochran *et al.* (2014) also compared continuous training and interval training, reporting an increase in VO_2max with both training methods, but additionally, that both training methods generated similar increases in signalling proteins linked with mitochondrial biogenesis for example the mRNA expression of Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α). Recent research has demonstrated that perceived enjoyment following high intensity interval training was higher compared to continuous running, despite participant's rating of perceived exertion being higher during HIIT (Bartlett *et al.* 2011).

It is well established that continuous low-intensity exercise training causes a variety of both central and peripheral adaptations; however it has also been reported that HIIT produces similar adaptations (Laursen & Jenkins, 2002). Central adaptations occur in the cardiovascular system and help increase the delivery of oxygen to the working muscles (Laursen & Jenkins, 2002). Both Bayati *et al.* (2011) and Helgerud *et al.* (2007) reported an increase in oxygen delivery via an increase in stroke volume (SV). This increase in SV is due to an increase in left ventricular contractile force, and in turn will improve aerobic capacity due to an improved delivery of oxygen to the working muscles. This improvement in stroke volume following training has been linked with training

induced hypervolaemia, the increase in blood plasma volume (Convertino, 1991). This increase in blood volume reduces cardiovascular stress and will improve aerobic capacity and time trial performance (Laursen & Jenkins, 2002). An additional central adaptation commonly reported following HIIT is an increase in mitochondrial biogenesis (Cochran *et al.* 2014; Gibala, 2009; Jacobs *et al.* 2013), the growth and division of existing mitochondria (Jornayvaz & Shulman, 2010). Exercise induced mitochondrial biogenesis is determined by the intensity, volume and duration of exercise (Cochran *et al.* 2014) however; it is unknown how much each of these factors plays a role in causing mitochondrial biogenesis.

Mitochondrial biogenesis is stimulated by the increase in peroxisome proliferator-activated receptor-co-activator-1 α (PGC-1 α) activity, a further adaptation to HIIT (Burgomaster *et al.* 2008; Gibala *et al.* 2009; Cochran *et al.* 2014). Burgomaster *et al.* (2008) reported an increase in PGC-1 α following both low-intensity continuous training and HIIT, showing similar adaptations to mitochondrial enzyme activity following exercise of different intensities. Citrate synthase is another mitochondrial enzyme that has been reported to increase following both high and low intensity exercise (Burgomaster *et al.* 2008; MacDougall *et al.* 1998). Citrate synthase catalyses the initial reaction in the Krebs cycle, which produces ATP to fuel exercise (Barnett *et al.* 2004). Many studies have reported an increase in citrate synthase activity following HIIT programmes (MacDougall *et al.* 1998; Burgomaster *et al.* 2005; Burgomaster *et al.* 2008; Barnett *et al.* 2004), which indicates oxidative metabolism produces the energy for exercise following high intensity exercise training. The increase in oxidative metabolism as the way to produce fuel for exercise is affiliated with the decrease in muscle glycogenolysis following training, as oxidative metabolism is activated earlier during exercise (Perry *et al.* 2008). Many high intensity interval studies have reported a reduction in glycogenolysis during exercise as a result of training (Burgomaster *et al.* 2005; Burgomaster *et al.* 2008), this decrease in glycogenolysis may also explain the prolonged time to reach the onset of blood lactate (OBLA), which is an additional peripheral adaptation to high intensity exercise training (Jakeman *et al.* 2012). An increase in muscle buffering capacity has been previously reported following both HIIT and endurance training (Gibala *et al.*

2006), and results in an improved exercise performance, as Bayati *et al.* (2011) and Burgomaster *et al.* (2006) both reported an improvement in time trial performance. A decrease in lactate accumulation has also been reported to improve the glycolytic yield of ATP (Laursen & Jenkins, 2002), as H⁺ ions have been reported to have an inhibitory effect on glycolytic enzymes for example phosphofructokinase (PFK) and Hexokinase (Hex) (Bayati *et al.* 2011). PFK catalyses the transfer of a phosphate molecule in the process of glycolysis and Hex phosphorylates glucose early in the process of glycolysis (MacDougall *et al.* 1998). The increased glycolytic flux rate as a result of high intensity training may also be responsible for the improved peak power output due to the increased energy supply for muscular contractions (Gollnick *et al.* 1973), which is another common adaptation of HIIT (Burgomaster *et al.* 2005; Jakeman *et al.* 2012; Hazell *et al.* 2010).

GLUT4 is a major transporter of glucose in skeletal muscle; its translocation is stimulated by exercise to increase muscle glucose uptake and utilization (Hawley & Lessard, 2008). It has been reported that exercise causes a rapid increase in GLUT4 protein, which facilitates an increased glucose uptake, which leads to improved glycaemic control (Little *et al.* 2011). Glycaemic control is an important aspect of the treatment for patients with type II diabetes, and it is possible that with training, mitochondrial capacity increases and may contribute to improved glycaemic control. Patients with type II diabetes are recommended to complete low intensity continuous training to help improve glycaemic control. The increase in GLUT4 protein following exercise occurs within 18 hours, as exercise activates the transcriptional signalling pathway (Greiwe *et al.* 2000). This activation of the signalling pathway to increase GLUT4 protein is not stimulated by catecholamines, but by skeletal muscle to ensure the muscle has sufficient levels of fuel. Low intensity exercise training has traditionally been advised to patients with type II diabetes to improve glycaemic control and increase GLUT4 protein. Babraj *et al.* (2009) reported an improvement in insulin sensitivity following high intensity interval training, and although not measured in the study, GLUT4 concentration is an important insulin regulatory factor controlling insulin sensitivity. Kranjou *et al.* (2006) compared two different exercise intensities, 60 minutes at 40%VO₂max and ~30 minutes at 80%VO₂max to assess which

intensity would show the greater increase in GLUT4 protein. Both exercise intensities increased GLUT4 protein to a similar degree, despite different exercise intensities and duration. Likewise, Hood *et al.* (2011) and Little *et al.* (2011) reported an increase in GLUT4 following high intensity exercise and therefore an increase glucose uptake in skeletal muscle and improved glycaemic regulation.

In addition to metabolic adaptations reported following high intensity interval training, exercise studies have reported a upregulation in cytokines, which have a range of effects on the body. Erythropoietin (EPO) is a hypoxic induced cytokine, that stimulates the rapid increase of endothelial cells that express EPO receptors, which stimulates angiogenesis, the development of new blood cells (Beleslin-Cokic *et al.* 2004). EPO is mainly produced in the kidney in adults in hypoxic conditions, which causes hypoxia-inducible factor 1 (HIF-1) to bind to hypoxic response elements which in turn activates the transcription of hypoxic responsive genes and regulates erythropoiesis, angiogenesis and glycolysis (Vogt & Hoppeler, 2010). The hypoxic condition in the cells stimulates the improvement of the oxygen delivery system to improve both the delivery and utilization of oxygen (Dehnert *et al.* 2002). Zafeiridis *et al.* (2015) reported that local hypoxia stimulates mitochondrial biogenesis, and enhances the activity of mitochondrial enzymes and the rapid increase in capillary density, which improves aerobic fitness following training at a range of intensities. Exercise has previously been reported to stimulate erythropoiesis, as in trained athletes both total haemoglobin and total red blood cell volume are elevated (Mairbäurl, 2013). Mairbäurl, (2013) reported that training causes a decrease in renal blood flow, which causes hypoxia of the EPO producing peritubular fibroblasts, a result which may be enhanced as exercise intensities increase. Therefore this may be an explanation for the improved aerobic capacity commonly reported following HIIT. Additionally Mairbäurl, (2013) reported that the elevated total blood volume as a result of erythropoiesis stimulates the vascularization by vascular endothelial growth factor (VEGF) following exercise in hypoxic conditions. VEGF is an angiogenic factor, that increases following exercise, as it responds to myocardial hypoxia, to increase the oxygen availability (Navaravong *et al.* 2014). During exercise the oxygen tension in the muscle decreases causing

local muscle hypoxia, resulting in HIF-1 α and HIF-1 β to cause VEGF gene expression, which in turn stimulates angiogenesis (Gustafsson *et al.* 1999). This increase in VEGF mRNA following exercise may increase capillary proliferation, which will improve muscle blood flow and oxygen kinetics in the form of oxygen extraction and conductance, which may improve endurance exercise performance (Richardson *et al.* 2000).

The delayed blood lactate accumulation can be measured using many different markers for example OBLA and lactate threshold. Lactate threshold is directly linked with ventilatory threshold (Ghosh, 2004) and defines a point at which changes in metabolic acidosis or ventilation are non-linear. Ventilatory threshold occurs when ventilation increases non-linearly to cope with the increase in H⁺ ions and other metabolites when the intensity of work increases (Ghosh, 2004). Ventilatory threshold, or gas exchange threshold has been reported to occur at higher exercise intensities following training (Ghosh, 2004). Previous research states that gas exchange threshold one, the point at which ventilation first increases non-linearly can be used as a predictor of marathon pace (Loftin *et al.* 2007) and therefore is an additional measure of changes in aerobic performance following an exercise training programme due to its link with endurance performance.

Changes in oxygen kinetics, including the reoxygenation of myoglobin and haemoglobin can be measured using the non-invasive and continuous technique of near-infrared spectroscopy (NIRS) (DeLorey *et al.* 2003). Near infrared light easily penetrates the skin and can non-invasively measure the changes in the balance between oxygen utilization and oxygen delivery in skeletal muscle (Bhambhani, 2004). Changes in oxygen kinetics of haemoglobin and myoglobin can be calculated by applying the modified Beer-Lambert law (Hamaoka *et al.* 2011). NIRS measurements are used in localized regions of an individual's muscle to assess the localized muscle metabolic rate. NIRS provides continuous monitoring of oxygen kinetics of the small arterioles, capillaries and venuoles within the muscle (DeLorey *et al.* 2003).

Hamaoka *et al.* (2011) reported that following high intensity maximal voluntary contractions the rate of muscle reoxygenation is linked with oxidative capacity, the higher the oxidative capacity the faster the re-oxygenation rate. The faster

reoxygenation rate has also been linked with a faster PCr recovery (Hamaoka *et al.* 2011; Boushel *et al.* 2001). Similar to Hamaoka and colleagues, Ferrari *et al.* (1997), reported a faster rate of oxygen resaturation following exercise in trained individuals in comparison to sedentary individuals. Many studies have used either venous or arterial limb occlusion to measure muscle oxygen consumption by only allowing oxygen extraction from the capillaries by blocking the oxygen supply (Bae *et al.* 2000). At the point of occlusion, the level of deoxyhaemoglobin rises while the level of oxyhaemoglobin decreases, however during thigh occlusion, previous literature has reported a plateau in both deoxyhaemoglobin and oxyhaemoglobin when saturation levels decreased by about 20% (Ferrari *et al.* 1997). This plateau suggests that not all oxygen stores are depleted during occlusion although it is unclear how training affects oxygen utilisation during occlusion. Oxygen saturation reflects the balance between oxygen delivery and oxygen consumption therefore, during occlusion, when the oxygen delivery is stopped the rate of oxygen desaturation reflects oxygen consumption (Boushel *et al.* 2001). During exercise, the increase in oxygen consumption, decrease in oxyhaemoglobin and the increase in deoxyhaemoglobin in the working skeletal muscle varies depending on the intensity of the exercise, the degree of activation of the muscle group and the level of training of the individual completing the exercise (Boushel *et al.* 2001). Jones *et al.* (2015) reported an increase in desaturation of tissue oxygen saturation (TSI %) and deoxyhaemoglobin and deoxymyoglobin independent of blood volume during post-training sprinting following 2 weeks of high intensity sprint training. These changes indicated an increase in muscle oxygen extraction capacity following training. Jacobs *et al.* (2013) also reported an increase leg deoxygenation during exercise following HIIT, which they suggested shows an increase in oxygen extraction from the working muscle. An improved oxygen extraction rate is a result of an improved diffusive capacity between the capillary and the mitochondria in the muscle. The expansion of the mitochondrial network creates an increased diffusion gradient and therefore an increase in oxygen extraction rate (Jacobs *et al.* 2013)

These peripheral muscle oxygen adaptations following training increases the muscles ability to extract oxygen and may be due to the well documented

increase enzymes including PGC-1 α following HIIT, which results in mitochondrial biogenesis (Burgomaster *et al.* 2008; Little *et al.* 2010). Jones *et al.* (2015) state that the higher the exercise intensity during HIIT the more likely mitochondrial biogenesis is to occur in both type I and type II muscle fibres. Similarly, Zorgati *et al.* (2015) reported that the increase in both the number and size of mitochondria, caused by central adaptations including mitochondrial biogenesis in the skeletal muscle, would affect the relationship between oxygen supply and utilization. Zafeiridis *et al.* (2015) compared different exercise intensities and the effects each has on oxygen delivery and consumption in the working muscle. It was found that all protocols investigated (continuous, long-interval and short interval) increased muscle deoxygenation in the exercising muscle. This suggests that local muscle hypoxia and the 'mismatch' between oxygen delivery and consumption during training improves muscle oxidative capacity. The muscle deoxygenation reflects the balance between oxygen delivery and utilization, the deep muscle for example the vastus intermedius has the potential to maintain a higher oxygen delivery to oxygen utilization ratio than superficial muscle for example the vastus lateralis (Okushima *et al.* 2015). This difference in the oxygen delivery to utilization ratio is a result of the number of oxidative muscle fibres, with deep muscle having more than the superficial muscle (Kalliokoski *et al.* 2000). Okushima *et al.* (2015) previously reported that deoxygenation of the superficial muscle during exercise peaks and plateaus at about 70% of maximum work rate. This may be because the superficial muscle reaches an oxygen extraction capacity at this submaximal exercise intensity, and the deep muscle continues to extract oxygen as it contains more type I muscle fibres. Therefore deep muscle has a greater oxidative capacity compared to the superficial fibres. However, HIIT may affect the fibre type in the superficial muscle fibres, and increase the oxidative capacity of the superficial vastus lateralis muscle.

Previous literature has studied the deoxygenation and re-oxygenation rate during HIIT. Bae *et al.* (2000) reported that during a 30 second sprint muscle oxygenation declines from the 3rd-13th second and then plateaus from the 17th-30th second of the sprint. Additionally Bae *et al.* (2000) reported sufficient re-oxygenation between each 10 second sprint with 20 seconds recovery (1:2

work:rest ratio). This decline in muscle oxygenation during each sprint, suggests that oxidative metabolism significantly contributes to the energy production during sprints of 10 seconds with 20 seconds recovery (Bhambhani, 2004). However, Ross & Leveritt (2001) state that glycolysis is the main contributor of energy (~55-75%) at the onset of high intensity exercise, and when sprints last <10seconds. But when the sprints are repeated with short recovery periods, aerobic metabolism will take over as the main source of fuel. Therefore the duration of the recovery between sprints is likely to play a major part in determining the adaptations caused as a result of HIIT, and when the change in fuel source occurs during the sprint training session.

Although previous research has demonstrated that HIIT produces similar performance adaptations to endurance training, some in as little as just 2 weeks, and is more enjoyable, the influence of different exercise protocols remains unclear. Currently there is very limited information regarding the optimal training programme, including the optimal exercise intensity, duration and number of exercise bouts and recovery duration (Laursen & Jenkins, 2002). Research has generated a variety of ways to adapt the exercise intensity of a training programme including using VO_2 max, or a percentage of VO_2 max, duration of sprint and anaerobic or lactate threshold. Helgerud *et al.* (2007) compared long distance training (70% maximal heart rate) to 3 different high intensity training methods, including one at lactate threshold, one 15sec running with 15sec recovery, and 4x4min of running with 3 min recovery between exercise bouts. Helgerud and colleagues reported significant improvements in the latter two protocols in comparison to the longer duration, lower intensity protocols. Burgomaster *et al.* (2006), Gibala *et al.* 2006 and Creer *et al.* (2004) have reported improvements in cycling time trial performance, muscle buffering capacity, and increased power output following the classic 4-6 x 30 second sprints with 4 minutes recovery. However Jakeman *et al.* (2012), Hazell *et al.* (2010) and Kavaliauskas *et al.* (2015) reported similar adaptations to the longer duration sprint, all with repeated sprints of <10seconds. Performance adaptations observed following shorter duration sprints have also been observed from our own lab (Lloyd Jones *et al.*, *under review*), with repeated 6-second sprints showing similar

improvements to repeated 30 second efforts. This study reported significant improvements in 10km cycling time trial performance and peak power output in two training groups (4x30sec and 20x6sec) which were matched for total sprint duration and work:rest ratio

Shorter duration sprints have been shown to be more manageable to complete as a training protocol in comparison with longer duration sprints (Hazell et al., 2010). Hazell et al. (2010) compared 3 different HIIT protocols, a 30 seconds protocol with 4 minutes active recovery, a 10 second sprint with 4 minutes active recovery and a 10 second sprint with 2 minutes of active recovery. Following 2 weeks of training all training groups improved 5km cycling time trial performance, and the 30 second group and the 10 seconds group with 4 minutes of recovery significantly increased VO_2max , whereas the 10 second group with 2 minutes of recovery did increase VO_2max although it did not reach statistical significance. This suggests that the generation of peak power output at the start of the longer sprint and for the duration of the shorter sprint may be important in stimulating performance adaptations, rather than the total amount of work completed, a finding reported by both Hazell *et al.* (2010) and by Jakeman *et al.* (2012), who have both researched extremely short duration sprint protocols. All the groups significantly increased peak power output but the 10 seconds sprints completed half the overall work per sprint training session.

Jakeman *et al.* (2012) investigated the effect of 2 weeks of HIIT consisting of 6 sessions of 10x6 seconds sprints on aerobic performance in trained triathletes. Training adaptations were assessed using a 10km self-paced cycling time trial and a time to exhaustion test, peak power output was also recorded throughout the sprint sessions. Jakeman *et al.* (2012) reported an increase in peak power output following 2 weeks of HIIT, which may be a result of the increase in glycogen availability due to supercompensation of muscle glycogen stores, a common adaptation found following other longer duration HIIT programmes (Burgomaster *et al.* 2005). Following 2 weeks of this extremely short duration HIIT programme, Jakeman *et al.* (2012) reported a significant improvement in time trial performance (~10%), and although time to exhaustion did not improve significantly, the time to reach the onset of blood lactate accumulation (OBLA) did significantly increase. This delayed

time to reach OBLA may be explained by the increase in glycolytic enzyme activity including the enzyme pyruvate dehydrogenase (PDH), which is responsible for the increase in pyruvate in the process of oxidative metabolism. The delayed onset of blood lactate may also be a result of an increased expression of monocarboxylate transporter 1 (MCT1), which can occur with both endurance and high-intensity training, and is higher in endurance-trained athletes in comparison to sedentary individuals (Thomas *et al.* 2005). MCT1 is found predominantly in oxidative muscles and has been reported to improve the capability to remove lactate and potentially reduce lactate release (Thomas *et al.* 2005).

Kavaliauskas *et al.* (2015) also investigated the effect of a short duration sprint and the training adaptations that occur with different rest durations between each sprint. The training consisted of 6 x 10 seconds sprint with either a 30 second recovery, 80 second recovery or a 120 second recovery. Following 2 weeks of HIIT, Kavaliauskas *et al.* (2015) reported an improvement in 3km running time trial in the 30 second recovery group, but not in either the 80 or 120 second recovery group, this may be due to the short recovery time resulting in a larger cardiovascular demand during training, and therefore a greater performance adaptation. However time to exhaustion did improve in both the 30-second and 80 second recovery group, but not in the 120 second recovery group. It is suggested that a smaller oxygen deficit when starting each sprint would result in an improvement in time to exhaustion, hence the shorter recovery times resulted in an increase in time to exhaustion (Demarle *et al.* 2001). However, time to exhaustion is an exercise capacity test, and it lacks ecological validity in relation to many sports. Peak power output during the sprints was also measured, and increased only in the 6 x 10 second group with 80-second recovery, and not in either the shorter or longer recovery groups. This suggests that the shorter recovery does not allow for either glycolytic enzyme activity to increase.

Dawson *et al.* (1998) reported significant improvements in repeated sprint ability (RSA), VO_2 max and 40m sprint time following 6 weeks of HIIT consisting of <10second running sprints with a work: rest ratio varying between 1:4- 1:6. The significant improvement in VO_2 max is similar to previous studies using a longer sprint duration (Burgomaster *et al.* 2008),

showing that the shorter duration sprint can be as effective as both longer duration sprint HIIT programmes and endurance training. Interestingly, the study by Dawson *et al.* (1998) reported an increase in type II muscle fibres, which explains the improvement in power and the improvement in RSA and 40m sprint time reported. Type II fibres are metabolically suited to sprinting due to the higher myosin ATPase activity (Essen *et al.* 1975 as cited in Dawson *et al.* 1998), however some previous research has reported the opposite, a decrease in type II fibres and an increase in type I fibres (Linossier *et al.* 1993). This difference could be explained by the difference in recovery time used in different protocols, and the different source of fuel used and muscle fibre type recruited.

However, Gaitanos *et al.* (1993) have reported that 30 seconds recovery following 10 x 6 seconds sprints is sufficient to allow the resynthesis of PCr. During the initial sprints this resynthesis is generated from anaerobic glycolysis and PCr degradation, however during the latter sprints the reliance on aerobic metabolism of glycogen for the resynthesis of ATP increases. Similarly, Racinais *et al.* (2007) also reported a decrease in anaerobic metabolism and an increase in aerobic metabolism during the latter of the 10 x 6 second sprints.

The type of recovery between each sprint will contribute to the resynthesis of PCr, Bogdanis *et al.* (1996) reported that heart rate remains higher with active recovery of light cycling at 60rpm compared to a passive recovery, which may enhance the resynthesis of PCr and other muscle metabolites. This is due to the elevated heart rate, which keeps oxygen delivery to the muscle raised and improves the rate of resynthesis of PCr. Bogdanis *et al.* (1996) also reported a higher peak power output in subsequent sprints following active recovery, despite no difference in lactate concentration in the muscles following active and passive recovery. However, Signorile *et al.* (1993) reported an increase in the rate of lactate removal and the utilization of the lactate as a fuel source by neighbouring muscle fibres, following active recovery compared to passive recovery, demonstrating active recovery is beneficial to repeated sprint performance.

However, Spencer *et al.* (2006) reported a larger peak power output decrement following active recovery in comparison to passive recovery

following 6 x 4 second sprints. Dupont *et al.* (2004) demonstrated that active recovery enhances lactate removal, however, also reported a longer time to exhaustion following training with passive recovery between sprints. Dupont *et al.* (2004) reported passive recovery results in higher PCr resynthesis and higher reoxygenation of myoglobin, as there is more oxygen available during passive recovery compared to active recovery.

Therefore, the aim of this current study is to investigate the effects of different work to rest ratios to assess the potential causes of performance adaptations. The sprint duration and repetition number will be identical in each treatment group, but the rest duration between sprints will change. The work to rest ratios will be 1:8, 1:10 and 1:12, the sprint duration will be 6 seconds as previous research has demonstrated this causes performance adaptation (Jakeman *et al.* 2012; Lloyd Jones *et al. under review*). Previous research has suggested that repeatedly reaching peak power output may be the cause of the performance adaptations (Hazell *et al.* 2010) as a 6 second sprint protocol was reported to cause similar performance adaptations to a 30 second sprint protocol (Lloyd Jones *et al. under review*). However, it may be that HIIT repeated sprints cause sufficient stress to the cardiovascular system and hence performance adaptations occur (Wood *et al.* 2016). This study will therefore investigate the effect of different work to rest ratios have on causing performance adaptations in an extremely short duration HIIT programme.

Chapter 3: Methods

Participants

35 healthy, physically active (min 5x45minutes of moderate intensity exercise per week) male and female volunteers (characteristics: age: 24 ± 4 years, height: 1.77 ± 0.09 m, body mass: 74.7 ± 12.9 kg, body fat: $16.8 \pm 6.1\%$) were recruited for the current study via posters sent to local and university sports teams. Inclusion criteria were being aged between 18-35 years, and having no medical condition or injury that would be exacerbated by strenuous exhaustive exercise. Prior to participation all subjects received a copy of the information sheet (appendix 1), explaining experimental procedures and the potential risks. Participants were also required to sign a statement of informed consent (appendix 2) and complete a physical activity readiness questionnaire (PAR-Q) (appendix 3).

The protocol was approved by the Ethical Committee of Oxford Brookes University (Appendix 4).

Study Design

Table 1 shows the study design. Participants were instructed to be well rested and hydrated prior to any exercise testing, and were asked to refrain from exhaustive exercise, smoking and the consumption of caffeine and alcohol for 24hr prior to any exercise test.

Table 1: Study design

Week 1 Baseline testing		Week 2 Training			Week 3 Training			Week 4 Outcome testing	
Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9	Session 10
VO ₂ max test & Occlusion	10km cycling time trial	Sprint training session	Sprint training session	Sprint training session	Sprint training session	Sprint training session	Sprint training session	VO ₂ max test & Occlusion	10km cycling time trial

Baseline and follow-up testing

On the first visit to the lab, participants signed written informed consent, and body mass and composition were measured using bioelectrical impedance analysis (BIA) on Tanita scales (Tanita, BC-418MA, Amsterdam, The Netherlands) to the nearest 0.1kg and 0.1% body fat. Height was measured to the nearest 0.1cm using a stadiometer (Holtain, Crosswell, Wales).

VO₂max

In the same session as the basic anthropometric tests, participants completed an incremental VO₂max test, on a Lode Excaliber cycle ergometer.

The VO₂max test consisted of a 2 minute baseline section, while the participant remained stationary, then a 5-minute warm up, cycling against a 50w load, before resistance being increased by 25 watts every 3 minutes until volitional exhaustion (similar to Dall *et al.* 2002). Cadence was self-selected, but the test was stopped when the participant was unable to maintain a steady cadence above 60rpm. Heart rate (Polar: Finland) and gas exchange (via Cortex Metalyzer 3B, Leipzig, Germany) were measured continuously throughout the test. The VO₂max was determined by calculating the highest recorded VO₂ in the final 30 seconds of the test (any data points \pm 2SD of the average were removed). Rating of perceived exertion (RPE; Borg Scale) data and fingertip blood samples (Lactate pro 2: Australia) were collected at the end of each step. Throughout the VO₂max test participants were given verbal encouragement. From the gas exchange data collected, gas exchange threshold 1 and 2 were both determined using the V- slope method. Two trained individuals separately determined the points at which they believed that threshold occurred, these values were then compared, and any disagreements were decided by the help of a third trained individual.

Time Trial

At least 24 hours after the VO₂max test, participants completed a self-paced 10km time trial, on the Lode Excalibur sport cycle ergometer. A linear factor was chosen to produce a power output corresponding to 70% of the maximum power produced in the VO₂max test at each individuals average pedal cadence during the VO₂max test; however cadence was self-selected during

the time trial. Participants were aware of the distance completed but not the completion time to ensure participants did not utilize a pacing strategy.

Occlusion test

Muscle oxygen kinetics were assessed in the same session as the VO₂max test using an occlusion assessment. A Near-Infrared Spectroscopy (NIRS) device (portaMON, Artinis Medical Systems, the Netherlands) was placed on the right leg over the main body of the vastus lateralis muscle. A small 3x3cm² patch was shaved of the right upper leg; over the vastus lateralis muscle in order to get a clear NIRS reading. The distance from the inguinal fold to the proximal border of the patella was measured and 1/3 of this distance was then measured from the proximal boarder of the patella to find the vastus lateralis muscle; the NIRS holder was placed on the flat side of a contracted muscle to ensure the holder was flush to the skin; the upper leg was wrapped in a bandage to avoid interference of ambient light. The NIRS holder was wrapped tightly in one layer of cling film to ensure the device was not affected by sweat during the tests. The participant lay down on a bed for 5 minutes to collect baseline data with the pneumatic cuff attached around the thigh and connected to an automatic inflation system (Hokanson E20 Rapid Cuff inflator and AG101 Cuff inflator Air Source, PMS Instruments Ltd, Maidenhead, Berkshire, UK). Following the 5 minute relaxation period the cuff was inflated for 3 minutes to a pressure of 300 mmHg (180 mmHg above systolic pressure), causing arterial occlusion (Bae *et al.* 2000). NIRS data were collected throughout occlusion and for a 2-min period following the release of pressure. Figure 1 displays a typical NIRS trace from the occlusion protocol.

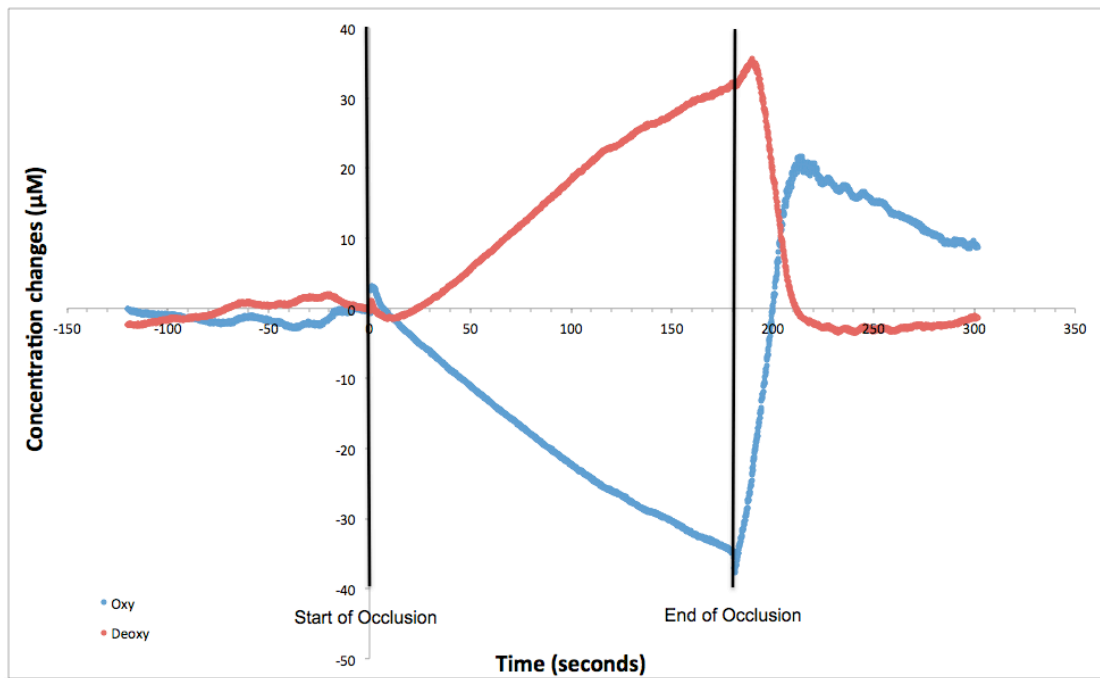


Figure 1: An example of the trace during the occlusion protocol

Training program

Participants were randomly assigned by picking a group out of a hat, to a treatment group or control group ensuring there was an equal split of male and female participants in each group. The groups all completed 10 x 6 second sprints with either a 48 seconds recovery (1:8 work to rest ratio), 60 seconds recovery (1:10 work to rest ratio) or 72 seconds recovery (1:12 work to rest ratio), to complete 1 minute total work (table 2), similar protocol to Gaitanos *et al.* (1993). Three sessions were completed each week for two consecutive weeks. All sprinting took place on a Lode Excalibur cycle ergometer in the lab with a resistance set equal to 7.5% body mass (kg) for each subject, following a 5 minute self-paced warm up. Participants received verbal encouragement throughout each sprint to ensure it was an 'all-out' effort. Heart rate (Polar: Finland) was continuously monitored throughout each sprint session and the participant's power output (Watts) for each sprint was recorded using Lode Ergometry Manager software. Peak power output was calculated as the highest recorded power output per sprint session, the average power per sprint was calculated; this was then averaged to get the mean power output per sprint session.

Table 2: Sprint training protocol

Group/ Work:rest ratio	Rest duration	Repetitions	Total work duration (s)
1:8	48 seconds	10	60
1:10	60 seconds	10	60
1:12	72 seconds	10	60

Data and statistical analysis

A repeated measures ANOVA test was used to analyse VO₂max, time trial, power output and the Near-infrared Spectroscopy occlusion data and a one-way ANOVA was used to analyse participant characteristics. The Mauchly sphericity test was used to check for homogeneity of variance, and if this was violated, the Greenhouse-Geisser correction was used. The statistical significance level was set at $p < 0.05$, and the Scheffé post hoc test was used where appropriate. All data is presented as mean \pm standard deviation unless stated otherwise. Cohens D effect sizes (d) were calculated, with $d < 0.2$ a trivial effect, $d = 0.2 - 0.5$ a small effect, $d = 0.6 - 1.1$ medium and $d = 1.2 - 1.9$ large effect size respectively (Cohen, 1988). Confidence intervals were calculated and set at 95%.

Chapter 4: Results

Performance adaptations

There was no significant difference between participant's characteristics for age, height, body mass and percentage body fat between the 4 groups at baseline ($p > 0.05$, table 3).

Table 3: Participants characteristics at baseline

	1:8 (n= 9)	1:10 (n= 8)	1:12 (n= 9)	Con (n= 9)
Age (years)	23 ± 4	26 ± 5	24 ± 4	24 ± 4
Height (m)	1.77 ± 0.12	1.80 ± 0.09	1.77 ± 0.09	1.75 ± 0.09
Body mass (kg)	74.3 ± 15.8	77.3 ± 14.3	74.6 ± 11.0	72.8 ± 10.7
Percentage body fat (%)	14.9 ± 4.6	19.0 ± 8.2	16.4 ± 6.9	17.1 ± 4.9

VO₂max

There was no significant time, group or interaction effect for VO₂max ($p > 0.05$), VO₂max data are shown in table 4. The VO₂max of the 1:8 training group decreased by 1% from pre to post training, with both the 1:10 and control group demonstrating a decrease in VO₂max from pre training to post training of 3%, and the 1:12 groups' VO₂max decreased by 4%. The 1:10, 1:12 and Con group all had a small effect size ($d = 0.25$, $d = 0.31$, $d = 0.20$, respectively).

Table 4: VO₂max data

Variable	Group		Pre	Post	<i>d</i>
VO ₂ max (ml.kg.min ⁻¹)	1:8 (n=9)	<i>Mean ± SD</i>	51 ± 9	51 ± 9	0.00
		<i>95% CI</i>	33 - 69	33 - 69	
	1:10 (n=8)	<i>Mean ± SD</i>	55 ± 8	53 ± 8	0.25
		<i>95% CI</i>	39 - 71	37 - 69	
	1:12 (n=9)	<i>Mean ± SD</i>	53 ± 6	51 ± 7	0.31
		<i>95% CI</i>	41 - 65	37 - 65	
	Con (n=9)	<i>Mean ± SD</i>	54 ± 11	52 ± 9	0.20
		<i>95% CI</i>	32 - 76	34 - 70	

Time Trial

There was no time or group effect for 10km time trial performance ($p>0.05$), however there was a significant interaction effect following training. The 1:8 group improved by 5%, the 1:10 group by 1% and 1:12 group improved by 4%, while the control groups time trial performance worsened by 6% (Figure 2). There was no significant interaction effect following a scheffe post hoc test in any group. All groups had a small effect size (Table 5).

Table 5: 10km time trial performance data

Variable	Group		Pre	Post	<i>d</i>
TT (seconds)	1:8 (n=9)	<i>Mean ± SD</i>	780 ± 258	751 ± 270	0.11
		<i>95% CI</i>	274 - 1286	222 - 1280	
	1:10 (n=8)	<i>Mean ± SD</i>	572 ± 138	563 ± 134	0.07
		<i>95% CI</i>	302 - 843	300 - 826	
	1:12 (n=9)	<i>Mean ± SD</i>	641 ± 100	615 ± 94	0.27
		<i>95% CI</i>	445 - 837	431 - 799	
	Con (n=9)	<i>Mean ± SD</i>	716 ± 207	761 ± 229	0.21
		<i>95% CI</i>	310 - 1122	312 - 1210	

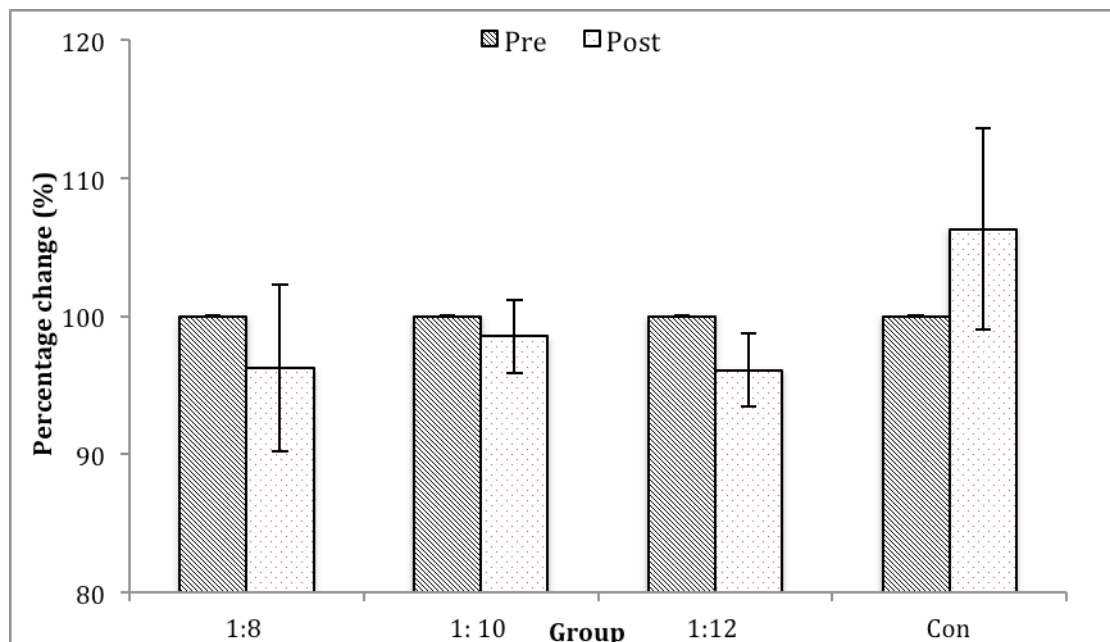


Figure 2: Percentage change in 10km time trial performance

Power Output

Peak power output significantly increased over time ($p < 0.05$) however there was no group effect or interaction effect following training ($p > 0.05$; Figure 3). Peak power output increased by 5.4% ($1038 \pm 330 - 1095 \pm 357$ watts), 5.4% ($1092 \pm 282 - 1148 \pm 286$ watts) and 5.0% ($1043 \pm 201 - 1097 \pm 226$ watts) from sprint session 1-6, in the 1:8, 1:10 and 1:12 group respectively.

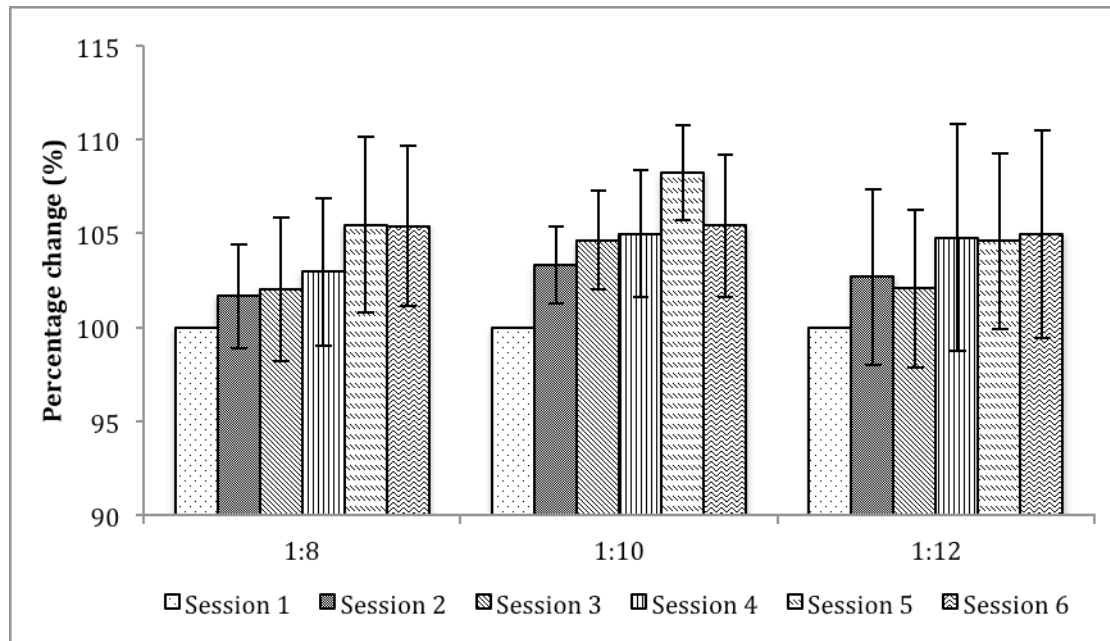


Figure 3: Percentage change in peak power output from session 1

Sprint peak power output for each sprint during each training session decreased in the 1:8 group (figure 4), this contrasts with the other two training groups where individual sprint peak power output tended to increase (figure 5 and 6). Additionally, there was a significant main effect for time on the range of sprint peak power output (highest vs. lowest) which tended to decrease in the 1:10 and 1:12 groups, but which increased in the 1:8 group ($p = 0.056$). The average range for all the sprint sessions for the 1:8 group was 62watts, the 1:10 group was 44watts and the 1:12 group was 45watts.

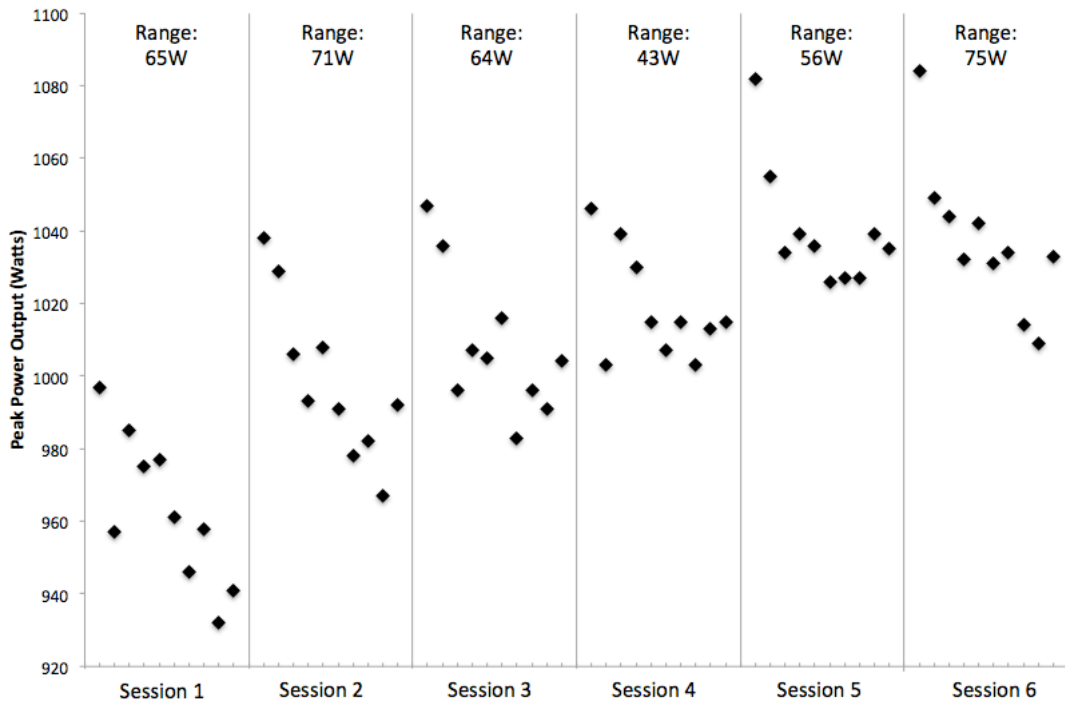


Figure 4: Peak sprint power output for training sessions from the 1:8 group, also showing range of power outputs.

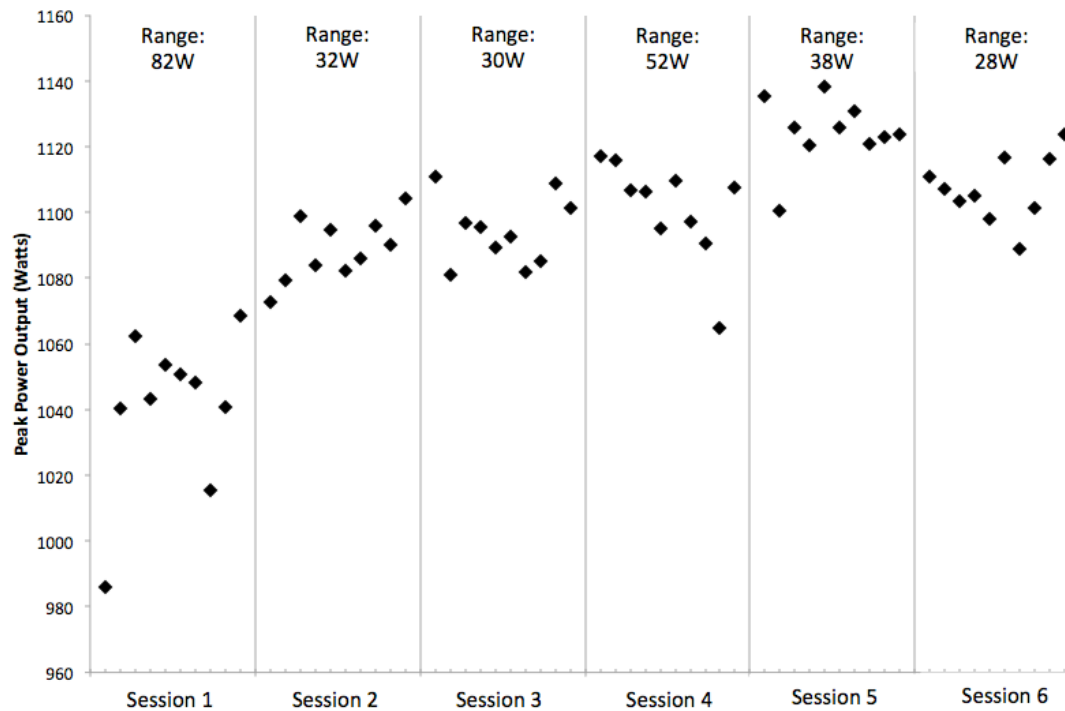


Figure 5: Peak sprint power output for training sessions from the 1:10 group, also showing range of power outputs.

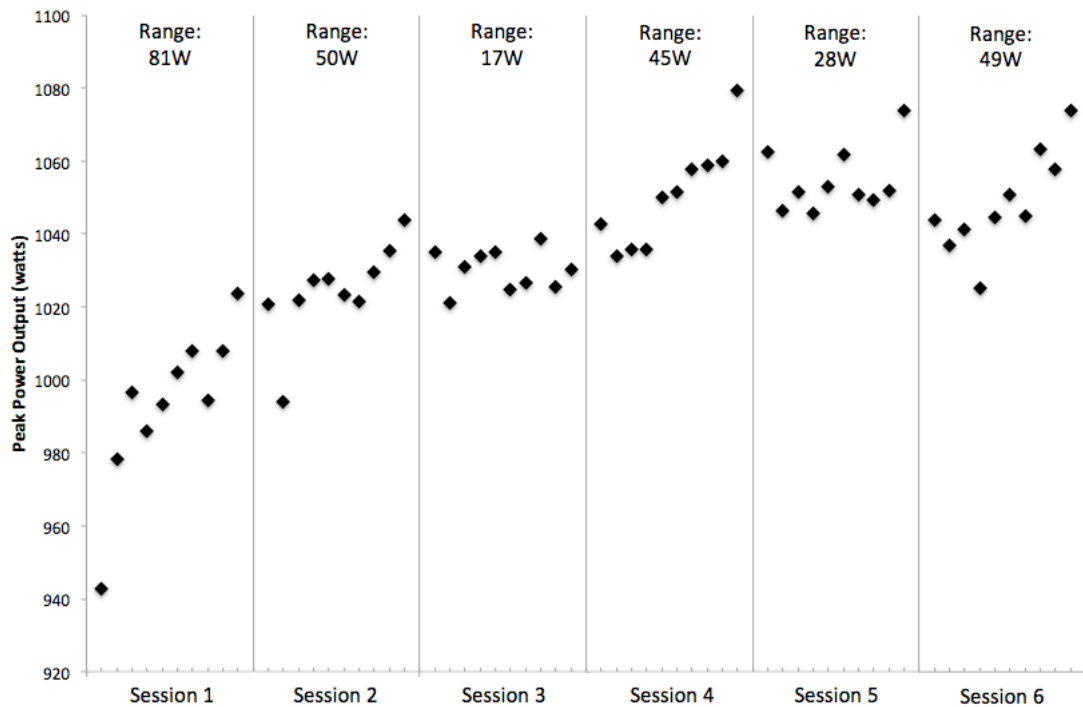


Figure 6: Peak sprint power output for training sessions from the 1:12 group, also showing range of power outputs.

There was a main effect for time ($p < 0.05$) for average power output per sprint session, however there was no significant group or interaction effect ($p > 0.05$). The 1:8 group improved mean power output by 4.5% ($887 \pm 272 - 927 \pm 283$ watts), the 1:10 group by 4.6% ($951 \pm 230 - 992 \pm 229$ watts) and the 1:12 group by 5.5% ($924 \pm 182 - 951 \pm 192$ watts) (Figure 7).

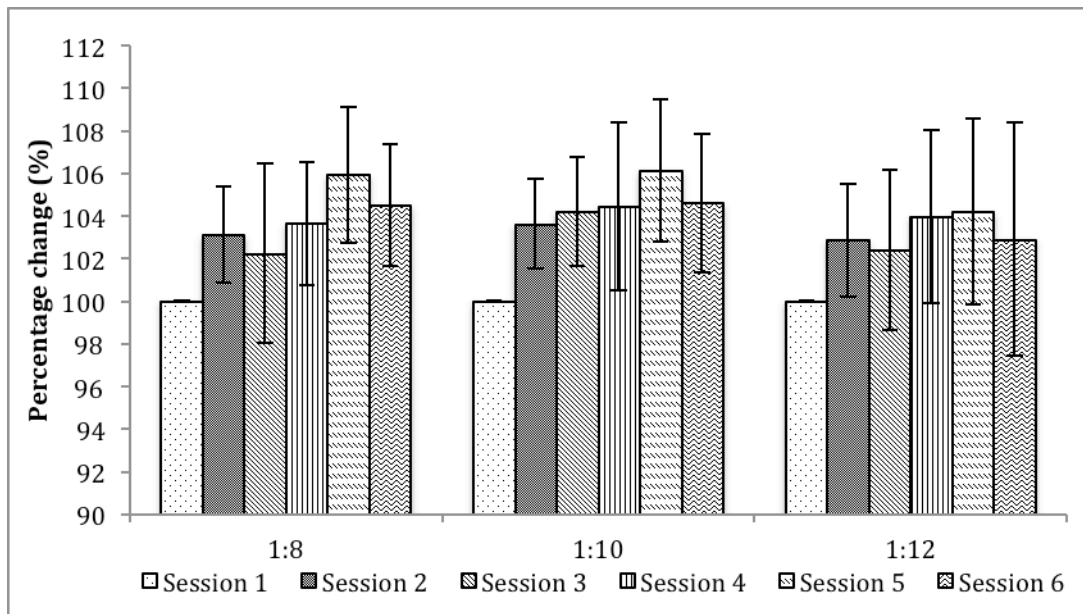


Figure 7: Percentage change in average power output

Gas exchange threshold

There was no significant time, group or interaction effect for gas exchange threshold one (GET1) from pre to post training ($p>0.05$; table 6). The 1:10 group neared a medium effect size ($d=0.39$). Similarly there was no time, group or interaction effect for gas exchange threshold two (GET2) following 2 weeks of training ($p>0.05$; table 6).

Table 6: Gas exchange threshold data

	Group		Pre	Post	<i>d</i>
GET1 (Watts)	1: 8 (n=9)	<i>Mean ± SD</i>	169 ± 61	181 ± 65	0.19
		<i>95% CI</i>	49 - 289	53 - 308	
	1: 10 (n=7)	<i>Mean ± SD</i>	181 ± 32	166 ± 44	0.39
		<i>95% CI</i>	118 - 244	80 - 252	
	1:12 (n=9)	<i>Mean ± SD</i>	156 ± 50	153 ± 65	0.05
		<i>95% CI</i>	58 - 254	26 - 280	
Con (n=9)	<i>Mean ± SD</i>	181 ± 53	175 ± 54	0.11	
	<i>95% CI</i>	77 - 285	69 - 281		
GET 2 (Watts)	1: 8 (n=9)	<i>Mean ± SD</i>	219 ± 60	206 ± 75	0.19
		<i>95% CI</i>	101 - 337	59 - 353	
	1: 10 (n=7)	<i>Mean ± SD</i>	231 ± 37	236 ± 38	0.13
		<i>95% CI</i>	158 - 304	162 - 310	
	1:12 (n=9)	<i>Mean ± SD</i>	197 ± 52	214 ± 74	0.27
		<i>95% CI</i>	95 - 299	69- 359	
	Con (n=9)	<i>Mean ± SD</i>	214 ± 59	211 ± 55	0.05
		<i>95% CI</i>	98 - 330	103 - 318	

A post-hoc power calculation with time trial performance as the main outcome measure indicated that the current study had power of 71%. A total of 42 participants would be needed to achieve a statistical power of 80% (Faul *et al.* 2007).

Near Infrared Spectroscopy

Oxyhaemoglobin

There was no time, interaction or group effect for the change in slope gradient of the oxyhaemoglobin during the 3-minute occlusion from pre-testing to post-testing ($p>0.05$) (Figure 8).

Similarly there was no time, group or interaction effect for the slope of the 2-minute recovery following the occlusion for oxyhaemoglobin ($p>0.05$).

There was no time, group or interaction effect for the minimum recorded value for oxyhaemoglobin during the occlusion ($p>0.05$) between the pre to post training (Table 7), however both the 1:10 and 1:12 group had a medium effect size (table 7).

Table 7: Minimum values for oxyhaemoglobin during occlusion

Group	Pre	Post	<i>d</i>
1:8	-23.9 ± 10.2	-20.6 ± 11.0	0.31
1:10	-17.4 ± 10.3	-22.9 ± 9.5	0.55
1:12	-24.1 ± 7.9	-20.2 ± 6.7	0.53
Control	-21.2 ± 8.2	-21.1 ± 7.9	0.01

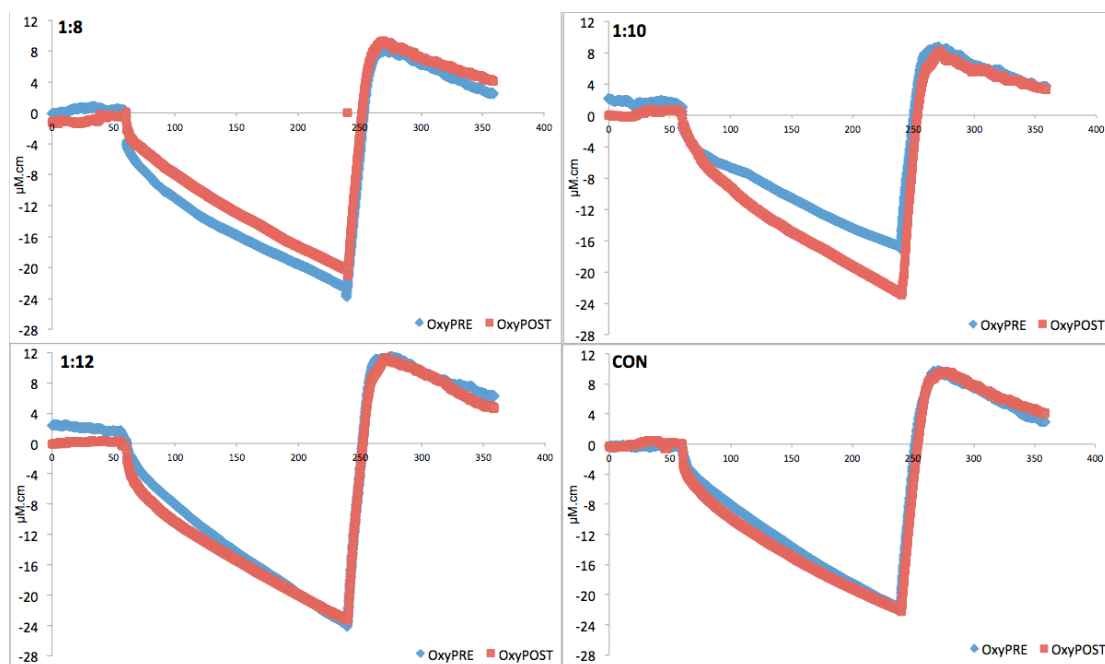


Figure 8: Oxyhaemoglobin during 3 minutes occlusion and 2 minutes recovery from both pre-training and post-training.

Deoxyhaemoglobin

There was no time, interaction or group effect following training for the change in slope gradient during the 3-minute occlusion, for the deoxyhaemoglobin ($p>0.05$). Additionally there was no time, group or interaction effect for the slope of the 2-minute recovery following the occlusion for deoxyhaemoglobin ($p>0.05$) (Figure 9).

There was no time, group or interaction effect ($p>0.05$) for the maximum-recorded point during the 3-minute occlusion for deoxyhaemoglobin (Table 8) with the 1:12 group approaching a medium effect size.

Table 8: Maximum value for deoxyhaemoglobin during occlusion

Group	Pre	Post	<i>d</i>
1:8	10.0 ± 7.3	13.4 ± 10.7	0.37
1:10	8.4 ± 7.3	11.1 ± 11.7	0.27
1:12	15.5 ± 11.7	11.2 ± 6.9	0.45
Control	12.5 ± 11.3	9.3 ± 6.8	0.15

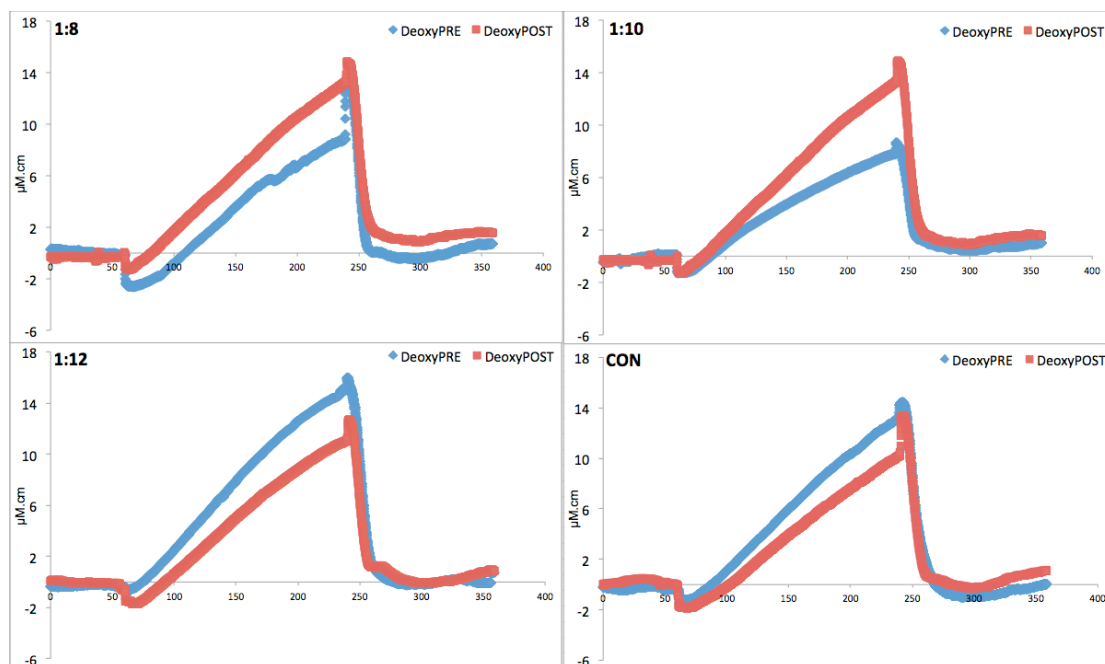


Figure 9: Deoxyhaemoglobin during 3 minutes occlusion and the 2 minutes recovery from pre-training and post-training

Total

There was no change in total haemoglobin during occlusion from pre to post training in any group ($p>0.05$; table 9), the 1:10 group showing a meaningful change through a large effect size ($d= 0.91$).

Table 9: Total Haemoglobin during Occlusion

Group	Pre	Post	<i>d</i>
1:8	1.23 ± 4.58	0.75 ± 1.19	0.14
1:10	2.94 ± 4.30	0.15 ± 0.43	0.91
1:12	0.22 ± 3.43	-0.68 ± 2.85	0.29
Control	-0.02 ± 2.73	0.18 ± 0.66	0.10

Chapter 5: Discussion

Performance Adaptations

The current study shows that 6 sessions of high intensity interval training (HIIT) over 2 weeks, consisting of 10 x 6 second sprints with varying rest durations improved 10km time trial performance, however none reached statistical significance. There was also no change in maximal aerobic capacity or gas exchange thresholds in any group as a result of extremely short duration HIIT. All treatment groups also improved both peak and average power output following 2 weeks of training, however there was no difference between groups.

VO₂max

There was no significant change in maximal aerobic capacity (VO₂max) following 2 weeks of training in any of the groups (1:8, 1:10, 1:12 or control groups). These data are similar to that previously reported by Burgomaster *et al.* (2008) and Gibala *et al.* (2006) who reported no change in VO₂max with just two weeks of HIIT. Previous research that has reported improvements in VO₂max following HIIT programmes have been over 6 weeks in duration and consisted of 4-6 x 30 second sprints rather than shorter (<10sec) sprints (Burgomaster *et al.* 2008). This suggests that 2 weeks is not long enough to induce changes in maximal aerobic capacity. Changes in VO₂max following training according to Helgerud *et al.* (2007) are dependent on the intensity, frequency and duration of training. As other studies of longer duration have reported improvements in VO₂max when exercising at similar exercise intensities 3 times a week, it suggests it is the duration of training that has resulted in a lack of change in VO₂max. VO₂max is limited by cardiac output, as this determines the oxygen delivery to the muscles, any change in cardiac output and therefore an improvement in VO₂max will be due to changes in stroke volume (Bayati *et al.* 2011), as maximal heart rate typically does not change with exercise. However changes in stroke volume occur as left ventricular contractile force and/or cardiac filling pressure increases (Laursen

& Jenkins, 2002) and this may take 12-38 days to occur, although changes in stroke volume are very difficult to assess other than through an increase in $VO_2\text{max}$. This increase in stroke volume is associated with hypervolaemia, an adaptation that occurs to reduce cardiac stress by preventing the decrease in cardiac filling, mean arterial pressure and central venous pressure (Sawka *et al.* 2000). However, similar to the increase in stroke volume itself, hypervolaemia has been reported to take weeks to occur, as although plasma volume occurs almost immediately following exercise, erythrocyte volume takes weeks, and both contribute to improvements in aerobic power, and hence will not have an effect on $VO_2\text{max}$ in the current study (Sawka *et al.* 2000).

Mitochondrial biogenesis is an adaptation reported following both endurance (Bishop *et al.* 2013) and HIIT (Little *et al.* 2010); this increases mitochondrial density and hence improves the delivery of oxygen to the muscles, and improves $VO_2\text{max}$. At the start of muscle contractile activity, signalling proteins for example kinases are activated, this initiates exercise-induced mitochondrial biogenesis by changing content and activity of sensor enzymes such as regulators, transcription factors and co-activators. This causes an increase in mRNA of these enzymes and proteins and as a result activates the transcription process (Bishop *et al.* 2013). However, although mitochondrial biogenesis has been reported following training, Laursen and Jenkins (2002) state that several weeks of training are required to improve $VO_2\text{max}$ and for changes to occur to mitochondrial density. Mitochondrial biogenesis is stimulated by local cell hypoxia, which in turn increases capillary density, mitochondrial enzyme activity and therefore improves maximal aerobic capacity (Zafeiridis *et al.* 2015). HIIT causes local muscle cell hypoxia (Zafeiridis *et al.* 2015), which would suggest that mitochondrial biogenesis would occur following this type of training, however this indicates that the current study duration was not long enough to elicit these changes.

There are many mitochondrial enzymes for example peroxisome proliferator-activated receptor-co-activator-1 α (PGC-1 α), which is the major regulator of mitochondrial biogenesis, that have previously been reported to improve as a result of HIIT. Cochran *et al.* (2014) and Burgomaster *et al.* (2008) reported an increase in PGC-1 α activity as a result of a HIIT programme, although the

improvement was reported following 6 weeks of training, and therefore may explain why no adaptations to maximal aerobic capacity were reported in the current study. Gibala *et al.* (2009) reported an increase in PGC-1 α mRNA after just a single HIIT session, however no increase in PGC-1 α protein content. This suggests that a study of several weeks in duration would be needed to increase PGC-1 α activity and to stimulate mitochondrial biogenesis.

Time Trial

10km time trial performance improved in all treatment groups, the 1:8 group improved by 5%, the 1:10 group by 1% and the 1:12 group by 4%, and the control group worsened by 6%, however no group reached statistical significance. Many previous studies have reported improvements in time trial performance similar to the current study; Jakeman *et al.* (2012) reported a 10% decrease in 10km time trial time, following 2 weeks of HIIT consisting of 10x6 second sprint with 60 second recovery in between each sprint, the same protocol as the 1:10 group in the current study. The larger increase in the Jakeman *et al.* (2012) study may be due to the method used, the resistance and cadence both being self-selected, whereas in the current study the cadence was self-selected but the resistance was set using a linear factor. This meant that the only way the participants could produce a faster time trial time in the current study would be to consistently hold a higher cadence throughout the second time trial. Hazell *et al.* (2010) reported that both the short sprint duration groups consisting of either 10 sec work, 4 min recovery or 10 sec work, 2 min recovery, improved 5km time trial performance by 3.5% and 3.0% respectively. These improvements in performance are similar also to those reported by Burgomaster *et al.* (2006), Babraj *et al.* (2009) and Jacobs *et al.* (2013) who reported significant improvements in time trial performance of varying lengths (250kJ, 250kJ and 40km, respectively) all following HIIT programmes consisting of repeated 30 second sprints. All these studies reported significant improvements, which is different to the current study, where the improvements did not reach statistical significance.

Although the improvements in the current study did not reach statistical significance, Paton and Hopkins (2006) reported a 0.6 % improvement in time

trial performance is a meaningful change, and that in a real sporting environment, anything above that would be seen as an improvement. According to this definition, all treatment groups demonstrated a meaningful change in time trial performance that would cause an improvement in performance in a race situation. Additionally, although not significant, using a time trial to assess changes in performance is superior in comparison to a VO_2max test or time to exhaustion test, as it is more exercise and race specific (Lansley *et al.* 2011).

The faster time to complete 10km cycling as reported in many previous studies and the current study, have been reportedly due to the increased ability to sustain a higher average power output for a fixed work bout (i.e. 10km) that is dominated by aerobic metabolism (Burgomaster *et al.* 2006). Changes in time trial performance and the improvement in exercise capacity are likely to have occurred as a result of peripheral adaptations (Gibala & McGee, 2008). Laursen *et al.* (2005) state that the improvement in time trial performance is likely to be due to an increase in oxygen uptake and an increase in H^+ buffering capacity. An increase in muscle buffering capacity has previously been reported in studies consisting of 30 sec sprints (Burgomaster *et al.* 2006; Gibala *et al.* 2006). Additionally short duration sprints studies (6 sec), including a study by Jakeman *et al.* (2012), have reported an increased time to reach OBLA. The reduction in lactate in the muscle as a result of training could be a result of decreased glycogenolysis, another commonly reported adaptation of HIIT (Burgomaster *et al.* 2008; Harmer *et al.* 2000), or an increase in the enzyme pyruvate dehydrogenase (PDH) activity. PDH will increase the use of pyruvate as a source of fuel in the process of oxidative metabolism, and may trigger the up-regulation of mitochondrial enzymes, which will improve aerobic performance (MacDougall *et al.* 1998) and hence time trial time.

The increase in muscle buffering capacity has been linked with an improvement in time trial performance following exercise training, may be related to the increase in enzyme activity for example phosphofructokinase (PFK). Elevated concentrations of H^+ ions has an inhibitory effect on enzyme activity and hence an improved muscle buffering capacity, will therefore indirectly contribute to an improved glycolytic yield of ATP and enhance an

individuals ability to work at a higher exercise intensity during the set work bout (Laursen & Jenkins, 2002).

Additionally whole body K^+ regulation during exercise is altered following HIIT, with a reduction in the rise of plasma K^+ and an increased Na^+-K^+ pump concentration also reported (McKenna *et al.* 1997). The expression of Na^+-K^+ -ATPase and sarcoplasmic reticulum Ca^{2+} -ATPase are also altered following HIIT, and this in turn will regulate the activity of pumps involved in cation transport, which will maintain muscle membrane potential (Laursen & Jenkins, 2002). This change to enzyme activity may be another possible mechanism that causes an improvement in endurance exercise performance, and hence may contribute to the meaningful improvement in time trial performance.

The large difference between the improvement in time trial performance in the treatment groups (1:8 = 5%, 1:10 = 1% and 1:12 = 4%) suggests the adaptations may occur from different stimuli. Previous literature has speculated that the adaptations from HIIT come from metabolic stress to the cardiovascular system which then causes central and peripheral adaptations (Wood *et al.* 2016). This could be the case in the 1:8 group. As shown in figure 4, the power output during the sprint session decreases, showing an incomplete recovery, which suggests the adaptation occurs from stress to the system. Additionally it demonstrates that 48 seconds rest may not be sufficient to allow full recovery between sprints, due to a decrease in PCr stores (Hazell *et al.* 2010). The PCr stores are limited by oxygen availability (Bogdanis *et al.* 1998), suggesting that the metabolic system will be sufficiently stressed, and that this may be the driver of the performance adaptations. It also indicates a switch over to the aerobic system to fuel exercise occurs early within the training session as the muscle is forced to regenerate ATP quickly, with a decreasing contribution from anaerobic sources (Barnett *et al.* 2004). Some literature has suggested that the adaptation from sprint training comes from continuously achieving peak power output and hence shorter duration sprints (<10secs) show similar adaptations to longer duration sprints (Hazell *et al.* 2010). Figure 5 shows that peak power output tended to increase throughout each sprint session and over the two week training intervention in the 1:12 group, this suggests that 72 seconds is sufficient to cause a complete recovery of ATP as peak power output can be

generated every sprint and that it actually improves somewhat over the individual session. As the 1:10 group only improved by 1%, which although according to Paton & Hopkins (2006) is still a meaningful change, it is still a much smaller improvement than the 1:8 or 1:12 group. The 1:10 group improved by a smaller amount than either of the other two treatment groups although this could still be considered a meaningful change (Paton & Hopkins, 2006).

Power Output

Peak power output improved in all treatment groups (1:8, 1:10 & 1:12) with no statistical significant difference between the changes observed in each group. The increase in peak power output is similar to previous HIIT studies for example Jakeman *et al.* (2012), who reported an improvement in peak power output of 6% following 2 weeks of 10 x 6 second sprints. The current study reported an increase of 5.4% with the same work to rest ratio as the Jakeman *et al.* (2012) study (1:10 group), and a 5.4% and 5.0% increase in peak power output in both the 1:8 and 1:12 groups, respectively. Similar performance adaptations have been reported following different HIIT programmes with long sprint durations (30sec), for example Burgomaster *et al.* (2007) reported a 5% improvement in peak power output as a result of 6 weeks of training and MacDougall *et al.* (1998) reported an improvement of 9%, following 7 weeks of training. The increase in peak power output following HIIT of differing durations suggests that it may be that repeatedly achieving peak power output causes the adaptations (Hazell *et al.* 2010). The increase in peak power output has been linked with an increase in glycolytic enzymes for example phosphofructokinase (PFK), which is commonly reported to be elevated as a result of HIIT (Rodas *et al.* 2000; MacDougall *et al.* 1998). PFK catalyses the transfer of phosphate molecules in the process of glycolysis (MacDougall *et al.* 1998), and consequently increases the rate of glycolysis, and hence increases the peak power output as there is a higher ATP yield. During a 30 second sprint the first 5-10 seconds of the sprint are reliant on glycolysis for fuel (Beneke *et al.* 2002), therefore glycolysis is likely to initially fuel sprints in the current study, however it is likely that oxidative phosphorylation will take over during the latter sprints (Hazell *et al.* 2010).

Linossier *et al.* (1993) reported a 25% increase in peak power output of a Wingate test following 7 weeks of training, utilising a similar protocol to Jakeman *et al.* (2012), both studies consisted of repeated <10 seconds sprints comparable to the current study. Previous studies have reported that each high intensity training session uses similar levels of muscle glycogen (Gaitanos *et al.* 1993), with a higher turnover reported as a result of training. However with training skeletal muscle can store more glycogen (Burgomaster *et al.* 2005), therefore it is likely that the increase in muscle glycogen stores are linked with the increase in peak power output (Jakeman *et al.* 2012). Hexokinase is an additional glycolytic enzyme that has been reported to increase as a result of HIIT, which will also increase peak power output (Laursen & Jenkins, 2002). Hexokinase (Hex) phosphorylates glucose early in the process of glycolysis, and hence increases the glycolytic flux rate. MacDougall *et al.* (1998) reported a 56% increase in Hex from baseline testing following 7 weeks of training consisting of longer duration sprints. This increase in Hex activity is likely to increase the rate of glucose utilization during both the sprints and the rest between intervals (MacDougall *et al.* 1998). Despite neither the increase in Hex or PFK being measured in the current study, the increase in peak power output may be attributable to the increase in both the glycolytic enzymes.

Mean power output significantly improved in all groups (1:8 group= 4.5% from 887 ± 272 - 927 ± 283 watts, 1:10 group= 4.6% from 951 ± 230 - 992 ± 229 watts and 1:12 group= 5.5% from 924 ± 182 - 951 ± 192 watts), and there was no significant difference between the improvements in any groups.

The increase in mean power output is most likely due to the increased ability to sustain a high power output as a result of training. Similarly, Hazell *et al.* (2010) reported an increased ability to sustain a higher power output during a 10 second sprint. This increased ability to sustain power may be due to an increase in glycolytic flux rate due to the increase in glycolytic enzyme activity as previously discussed. Additionally, the range of the peak power output per sprint in each session significantly decreased from session 1 to session 6 in all treatment groups, and the group effect approached significance ($p=0.056$). As indicated in figures 4-6 there is a significant change in the range of power outputs within each session. The average range of sprint peak power outputs

was 62 watts in the 1:8 group, 44 watts in the 1:10 group and 45 watts in the 1:12 group. The difference in sprint peak power output range may communicate some valuable information regarding the mechanisms of adaptation involved in sprinting with these work:rest ratios.

Gas exchange threshold

The current study reported no change in gas exchange threshold one (GET1) or gas exchange threshold two (GET2) in any of the training groups or control group. Gas exchange threshold one, is also known as ventilatory threshold and represents the first point when an individual's ventilation shows a non-linear increase (Ghosh *et al.* 2004), it is commonly used as a marker of aerobic fitness for example as a predictor of marathon pace (Loftin *et al.* 2007). Burke *et al.* (1994) reported an increase in ventilatory threshold following interval training consisting of either 2min work: 2 min rest or 30 sec work: 30 sec rest, and stated that the duration of the work interval is not important in determining the change in ventilatory threshold. However, the study by Burke *et al.* (1994) was 7 weeks in duration and therefore may explain why no change in ventilatory thresholds were reported in the current study when the duration was only 2 weeks. Similarly, Poole *et al.* (1985) reported increases in ventilatory threshold following both continuous and interval training, however, this study was 8 weeks in duration. Poole *et al.* (1985) states that there are many possible mechanisms for increasing ventilatory threshold, including the increased oxygen delivery to skeletal muscle fibres, increased rate of lactate and pyruvate clearance from the blood and a reduction in lactate formation. It has commonly been reported that HIIT has increased glycolytic rate (MacDougall *et al.* 1998), which will increase pyruvate clearance and hence the reduction in lactate build up in the muscles. However, these adaptations may take over 2 weeks to occur, and hence may explain why there was no significant change in gas exchange thresholds in the current study.

Near Infrared Spectroscopy

There was no difference in the slope or rate of change of oxyhaemoglobin or deoxyhaemoglobin during 3 minutes occlusion in any group as a result of training. Additionally there was no difference in the minimum recorded value of oxyhaemoglobin and the maximum recorded value for deoxyhaemoglobin during occlusion following 2 weeks of HIIT in any group. As oxygen delivery is halted during occlusion, the rate of oxygen desaturation reflects the rate of oxygen consumption within the Vastus Lateralis muscle (Boushel *et al.* 2001). The results from the current study suggest that there is no change in the rate at which oxygen was being extracted from the Vastus Lateralis muscle during occlusion following HIIT. Calbet *et al.* (2005) state that there are several factors that may change oxygen extraction in the muscle; these include the kinetics of the off-loading of oxygen from haemoglobin, maximal muscle oxidative capacity and blood flow and mean transit time. Bassett & Howley (2000) state that an increase in capillary density as a result of training will lengthen mean transit time, however this is referring to endurance training, rather than HIIT. Yet any change in capillary density as a result of extremely short duration sprints (<10sec) high intensity interval training similar to the current study, is yet to be reported. During exercise, local muscle hypoxia is caused as the oxygen tension in the muscle decreases, this in turn causes HIF-1 α and HIF-1 β to cause VEGF gene expression, which results in angiogenesis (Gustafsson *et al.* 1999). VEGF mRNA has been reported to increase following HIIT (Mairbäurl, 2013); this may increase capillary proliferation, which improves oxygen extraction and muscle blood flow (Richardson *et al.* 2000). Exercise training at higher intensities causes an increase in recruitment of type II muscle fibres, which may cause an increase in the number of capillaries supplying type II fibres (Jensen *et al.* 2004). Jensen *et al.* (2004) reported that following high intensity leg extensor training, capillary growth occurs in weeks 2-4 of training, and no further capillarization occurs after 7 weeks of training. This may explain why there was no reported change in oxygen extraction in the Vastus lateralis muscle, as changes in capillarization take over two weeks to be observed. The off-

loading of oxygen from haemoglobin during exercise is increased by acidification and the rise in temperature of the blood (Calbet *et al.* 2005), it may therefore be that in the current study, the duration of the sprint and the total sprint session did not create an acidic enough environment to cause any changes in the off-loading of oxygen from the haemoglobin molecule.

Current literature also states that an increase in mitochondrial biogenesis with training will also lead to an increase in muscle oxygen extraction (Jones *et al.* 2015), however it is unlikely that 6 sessions of HIIT is sufficient to stimulate mitochondrial biogenesis (Laursen & Jenkins, 2002), and hence no improvement in muscle oxygen extraction was reported in the current study. Roca *et al.* (1992) reported that following 9 weeks of interval and endurance training, leg muscle oxygen extraction increased by approximately 10%, along with an improvement in maximal aerobic capacity, which most likely explains the change. Increases in mitochondrial enzyme activity is known to occur with exercise training, and this increase in mitochondrial enzyme activity may allow for a slightly improved oxygen extraction from the blood (Bassett & Howley, 2000). As there is no change in the oxygen extraction rate during occlusion in the post-testing compared to pre-testing, it is unlikely that there is any change in maximal oxidative capacity as a result of the current study. However, Burgomaster *et al.* (2005) did report an increase in muscle oxidative capacity following just 6 sessions split over 2 weeks of HIIT consisting of 4-6 x 30 second sprints. This suggests that either the current HIIT protocol consisting of just 6 second sprints with either a 48sec, 60sec or 72sec recovery is not sufficient to elicit changes in muscle oxidative capacity, or that the NIRS technique is not sensitive enough to detect the change. Additionally, Burgomaster *et al.* (2005) reported changes in muscle oxidative capacity by using the invasive technique of a muscle biopsy, it may be that changes can be found using biopsies after just 2 weeks, but these adaptations are too small to be seen using NIRS following 2 weeks of training.

Although not statistically significant, figure 8 does show that there is a slightly faster oxygen extraction rate in the 1:10 group as a result of training, additionally the 1:10 group has the largest effect size ($d=0.55$) showing the most meaningful change occurred in this group. This is interesting as the 1:10 group saw the smallest change (1%) in time trial performance, but the largest

change in the oxygen extraction rate. This suggests that the 60 second recovery may stimulate mitochondrial biogenesis or and increase in capillary density, which may have an effect on oxygen extraction rate but that potentially the study was too short to elicit significant changes in either time trial performance or oxygen extraction rate. Therefore, more research should be completed to see if over a longer training duration, is the 60-second recovery the most suitable for altering oxygen kinetics, and causing enough metabolic stress to increase the aerobic enzymes for example PGC-1 α to stimulate mitochondrial biogenesis and consequently improve oxygen extraction rate in the muscle.

There was no difference in the rate of recovery of oxyhaemoglobin and deoxyhaemoglobin for the 2 minutes following occlusion in any of the groups, suggesting that oxygen is not flushed back into the muscle any quicker following 2 weeks of extremely short duration high intensity interval training. The reoxygenation rate following maximal voluntary contractions has been reported to be faster in trained rowers when compared to sedentary individuals (Chance *et al.* 1992). Similarly, the rate of muscle reoxygenation following high intensity voluntary muscle contractions has been suggested as a method to estimate muscle oxidative capacity (Hamaoka *et al.* 2011). An increase in the rate of reoxygenation has been reported following training, this may be due to an increase in capillary density, which will lengthen mean transit time, which enhances oxygen delivery to the muscle (Bassett & Howley, 2000).

Arterio-venous oxygen difference (A-V O₂ diff) depends on the muscles oxidative capacity and the exchange area between the capillaries and the muscle cells. Daussin *et al.* (2007) reported an increase in both A-V O₂ diff and cardiac output following interval training, suggesting that 8 weeks of interval training can increase the muscles ability to extract oxygen from the blood. This increase in A-V O₂ diff may be a result of a rightward shift in the O₂Hb disassociation curve, which enhances oxygen delivery and extraction of the working muscle (Bassett & Howley, 2000). However, changes in A-V O₂ diff as a result of training studies have occurred following longitudinal studies rather than over just 2 weeks like the current study, therefore this may be

another contributing factor to the reason there was no change in oxygen delivery and extraction.

Additionally, a fast reoxygenation rate has been linked with fast PCr recovery rate (Hamaoka *et al.* 2011; Boushel *et al.* 2001). The resynthesis of PCr is reliant on oxidative metabolism, and is closely linked with pulmonary oxygen uptake kinetics and therefore related to an individual's oxidative capacity (Forbes *et al.* 2008). Training has an effect on the recovery rate of PCr, as Johansen & Quistorff (2003) reported PCr recovery rate to be approximately 2 times faster in distance trained runners, compared to untrained individuals. Forbes *et al.* (2008) reported that following 2 weeks of HIIT consisting of 4-6x 30 second sprints, PCr resynthesis was faster which is due to an increase in oxidative enzymes for example citrate synthase and an increase in PGC-1 α protein, which in turn improve oxidative capacity of the individual. However in the current study PCr recovery rate and changes in enzyme activity were not measured, so it isn't possible to state if this occurred. An increase in capillary density has previously been reported as a result of training, which will lengthen mean transit time, enhancing oxygen delivery to the muscle (Bassett & Howley, 2000). However, as there was no change in VO₂max as reported in the previous chapter, and no change in the reoxygenation rate in any group, it is likely that either a longer duration study is needed, or the duration of the 6 second sprint is not long enough to cause these adaptations over just 2 weeks.

Total

There was no significant difference between total haemoglobin (tHb) content from pre to post testing in any group, which is a measure that has previously been reported to reflect changes in blood volume (Jones *et al.* 2015). Hypervolaemia is an adaptation previously reported with continuous exercise training programmes (Laursen & Jenkins, 2002), however Menz *et al.* (2015) reported no change in blood volume following HIIT consisting of four-minute exercise bouts. Jones *et al.* (2015) reported similar findings to the current study, no significant change in muscle blood volume (tHb) as a result of training. Schmidt & Prommer (2008) state that the regulation of tHb mass is controlled by the hormone erythropoietin (EPO) which stimulates

angiogenesis. However, as tHb did not change in the current study either 2 weeks is not long enough to stimulate and increase in EPO, or the repeated 6 second sprints are not creating a hypoxic enough environment in the muscle to stimulate the change. As Mairbäurl, (2013) reported an increase in reticulocytes and tHb 1-2 days after both endurance and strength training sessions indicating the exercise stimulates erythropoiesis. It is therefore more likely that the latter is correct in the current study, and that the 6 second sprints are not long enough to cause a sufficiently hypoxic environment, and all the different recovery durations are too long to cause muscle cell hypoxia.

Limitations

One major limitation of using near infrared spectroscopy is that an overlying fat thickness of 5mm can hinder the intensity of the signal getting into the muscle by 20% (Hamaoka *et al.* 2011). Although there was particular inclusion criteria participants were required to complete in order to participate, there was no cut off for fat thickness, this was also not measured at the site where the NIRS holder was placed, and therefore was not taken into account in the current study. Additionally, due to the technology in the laboratory malfunctioning, the wavelength depth used in the current study was 757nm rather than the desired 843nm. Furthermore, in the current study the occlusion was completed on a separate day to the sprints, if the occlusion was done immediately following sprints, it may be that the results would be more sensitive to changes. Therefore, this is an area for further research, to assess the changes in oxygen kinetics as a result of a training programme, but completing the occlusion immediately following a sprint session.

An additional limitation is the duration of the study, the 2 weeks of training is not sufficient to elicit central adaptations to training, so it is difficult to assess what the drivers are for changes in performance following this extremely short duration high intensity interval training, and to assess if different rest durations have an effect on changes to maximal aerobic capacity.

Chapter 6: Conclusion

The aim of this study was to investigate the effects of difference work: rest ratios to assess the potential causes for performance adaptations.

The current study found that two weeks of repeated 6 second sprints with varying recovery duration does not elicit changes in VO_2 max. Meaningful improvements in time trial performance were observed in all treatment groups but not the control group, although this did not reach statistical significance.

Although improvements in time trial performance did not reach statistical significance, there was a meaningful improvement in all the treatment groups but not the control group. The 1:8 and 1:12 groups showed the largest improvements in time trial performance, were observed in the 1:8 and 1:12 groups, which may occur as a result of incomplete recovery causing metabolic stress and in the 1:8 group and sufficient enough recovery in the 1:12 group to allow peak power to be repeatedly reached, causing performance adaptations. The smaller change in the 1:10 group may reflect the fact that recovery was sufficient enough to not result in adaptations associated with the 1:8 group but not complete enough to elicit the performance adaptations observed in the 1:12 group.

Consistent with previous literature, high intensity interval training resulted in increases in peak power output across all treatment conditions.

There was no significant change in the rate of oxygen extraction following 2 weeks of training, which may be a result of the training duration being insufficient to elicit changes that cause adaptations in oxygen kinetics, or that the short sprint duration does not cause enough of a stimulus to elicit a response, further research is needed to investigate this. Future studies may consider the impact of a variety of work:rest ratios collecting more complex data such as those available from muscle biopsy, blood borne protein response and longer duration sprint exercise training protocols could also be investigated. Differences in work:rest ratio may be of interest to practitioners, and should be considered when planning future research studies.

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Appendices

Appendix 1



1st November 2015

Information to Research Participants

Title of the study: A comparison of different work:rest in a high intensity interval training programme; the effect on performance and health parameters.

Contact: Molly Lloyd Jones- 15039685@brookes.ac.uk

Place of the experiment: Human Performance Laboratory, School of Health Science, Headington Campus, Gypsy Ln, Oxford OX3 0BP

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

The purpose of this study is to compare different extremely short high intensity interval protocols for performance adaptations and health related markers. Each protocol will use a different work to rest ratio, all using a 6 seconds sprint with either 48 seconds, 60 seconds or 72 seconds rest between each sprint respectively.

Previous research has found that high intensity training can improve exercise performance, and has similar effects to endurance training. However little research has been done looking at the optimal work:rest ratio. This study will assess changes in performance by participants completing a VO₂max test, a 10km time trial on cycle ergometers, and the reoxygenation of muscle following arterial occlusion, and changes in blood protein levels, before and after a 2 week training period, consisting of 3 training sessions a week.

Why have I been invited to participate?

We are recruiting men and women, aged 18-35 who complete a minimum of 5x45minutes of exercise per week.

Exclusion criteria includes personal history of cardiovascular or pulmonary disease, anaemia, diabetes, any musculoskeletal injury, which may be exacerbated by strenuous exercise or currently taking any medication/drugs for any reason as this may confound interpretation of the results.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason, and no information or data about you will be held by the researchers or university. Your decision to participate or not will not have any impact on your academic progress or regard to which you are held by the university.

What will happen to me if I take part?

You will be asked to make 10 visits to the Human Performance Laboratory, Oxford Brookes University, over a 4-week period during February- June to complete a series of exercise tests. You can read below what you will be asked to do on each visit.

What is involved?

All testing will take place in the human performance laboratory on Gipsy Lane campus. Participants are required to report to the laboratory in training kit (short, t-shirt and trainers) well rested and hydrated. Participants are requested to refrain from exhaustive exercise, smoking and the consumption of caffeine and alcohol for 24h prior to any exercise test and caffeine consumption for two hours following exercise.

There are a total of 10 sessions, 4 of which are base line and follow up testing and 6 of which are training. The outline is given in the table below, and a detailed description of what is involved in each session is described below.

	Week 1 Baseline testing		Week 2 Training			Week 3 Training			Week 4 Outcome testing	
	Session 1	Session 2	Session 3	Session 4	Session 5	Session 6	Session 7	Session 8	Session 9	Session 10
			Overnight fast					Overnight fast		
	VO ₂ max test & Occlusion	10km cycling time trial	Venous blood sampling, & Sprint training session	Sprint training session	Sprint training session	Sprint training session	Sprint training session	Venous blood sampling, & Sprint training session	VO ₂ max test & Occlusion	10km cycling time trial
Total duration	1.5 hours	30 minutes	2.5 hours	30 minutes	30 minutes	30 minutes	30 minutes	2.5 hours	1.5hours	30 minutes

Session 1: Participants are required to complete a pre-exercise health questionnaire, to identify any participants that may be at risk by completing the exercise. A series of anthropometric tests will then be completed, including height, weight, skinfold measures, and blood pressure (using an automated cuff).

Then you will have the small electronic box placed on your leg to measure the oxygen in your blood. It is totally non-invasive and leaves no lasting marks, as it is taped to your skin. You should let us know if you are allergic to glue (as found on sticky tape), or nickel as we may ask you to shave a small portion of your leg to achieve a good signal. If you have an allergy, or do not wish to have part of your leg shaved, please let us know, and we will omit this part of the testing. You will be asked to lay down and a pneumatic cuff will be placed around the thigh. The cuff will be inflated which will cause blood flow to stop, it remains inflated for 3 minutes while data is collected, you will then be asked to remain still for the following 2 minutes, to collect recovery data.

Then you will be asked to complete a VO₂max test on a cycle ergometer. This will involve cycling against a progressively increasing resistance, until you cannot cycle any further. During this, we will collect expired gas using a face-mask, blood lactate, using a fingertip blood sample, and heart rate. You should be aware that, although exceptionally rare, this procedure does carry the risk of irregular heart beat and collapse. At all times, a member of staff trained in the use of an automated external defibrillator and first aid will be on site, and available for assistance. We will also ask

you, during this test, to wear the small electronic box on your leg to measure the amount of oxygen in your blood, and can give us some valuable information about how your body is working.

Session 2: 24-48 hours after session 1, a 10km time trial will be completed on a cycle ergometer, where we will ask you to complete the 10km as fast as possible. Following these tests, volunteers will be randomly assigned into either a 1:8, 1:10 or 1:12 work to rest ratio sprint training groups.

Sessions 3-8: During the training sessions, you will complete a high intensity training (HIT) programme on a cycle ergometer to complete a total of 1 minutes work. Depending on the group you are in, you will perform a 6 second sprint with either a 48 seconds recovery (1:8 work to rest ratio), 60 seconds recovery (1:10 work to rest ratio) or 72 seconds recovery (1:12 work to rest ratio), repeated 10 times to complete 1 minutes total work.

Before session 3 and session 8 only, we will insert a cannula into your arm at the elbow, and we will use this to take blood before the sprint session, immediately after, and 30, 60, 90 and 120 minutes after the exercise. Therefore prior to session 3 & 8 only we ask that you come to the lab having fasted for 12 hours, and do not eat or drink anything other than water until all blood samples have been taken, water will be supplied in the lab, and you will be free to leave the lab between samples if you wish.

Session 9 & 10: Repeat of sessions 1-2, to record follow up data.

What will happen to the results of the research study?

The data collected during this study will be used to complete a dissertation for a sport science research masters degree under the supervision of Dr. Martyn Morris (Senior Lecturer in Sport Science) and Dr. John Jakeman (Lecturer in Sports Science and Coaching). No personal information or identification will be mentioned and will remain strictly confidential and anonymous throughout the creation of the dissertation and possible publication. All data will be de-identified by giving a unique code to anonymise the data (a reversible process in order for us to be able to provide you feedback on your own performance). All data will be kept anonymously and stored electronically on a password protected computer. The participant may be sent a summary of the final research paper if they wish.

This study has been approved by the Department Research Ethics Committee, Oxford Brookes University. If you have any concerns about the way in which the study has been conducted, please contact the Chair of the Department Research Ethics Committee on dido.green@brookes.ac.uk.

This project is supervised by Dr Martyn Morris (mgmorris@brookes.ac.uk) & Dr John Jakeman (jjakeman@brookes.ac.uk).

Should you wish to take part in this study, or for more information please contact Molly Lloyd Jones by email (15039685@brookes.ac.uk).

Thank you for taking time to read this information sheet.

Appendix 2

DREC Reference 1215_25

CONSENT FORM



Title of the study: A comparison of different work: rest in a high intensity interval training programme; the effect on performance and health parameters.

Molly Lloyd Jones

Supervisors

Dr. Martyn Morris (mgmorris@brookes.ac.uk) &

Dr. John Jakeman (jjakeman@brookes.ac.uk)

Please initial box

I confirm that I have read and understand the information sheet for the above research project and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.

I am aware that a risk assessment has been administered for the activities I am about to partake and have the right to view the risk assessment if I choose.

I understand that where the sample size is very small, it may be impossible to guarantee anonymity/confidentiality of participant identity.

I agree to take part in the above research project.

Please tick

I agree to anonymised data from this study being stored securely for use in future publication or presentation

Yes

No

Name of Participant

Date

Signature

Name of Supervisor

Date

Signature

Appendix 3



Please read the following questions carefully and answer as accurately as possible.

Medical history	Yes	No
1. Has a doctor ever said you have heart trouble?	_____	_____
2. Do you suffer frequently from chest pains?	_____	_____
3. Do you often feel faint or have spells of dizziness?	_____	_____
4. Has a doctor ever said you have epilepsy?	_____	_____
5. Has a doctor ever said you have high blood pressure?	_____	_____
6. Has a doctor ever said you have diabetes?	_____	_____
7. Has a doctor ever said you have asthma?	_____	_____
8. Do you have a bone, joint or muscular problem which may be aggravated by exercise?	_____	_____
9. Do you have any form of injury?	_____	_____
10. Are you currently taking any prescription medications?	_____	_____
11. Have you suffered from a viral illness in the last two weeks	_____	_____
12. Are you lactose intolerant?	_____	_____
13. Are you anaemic?	_____	_____

Adapted from Chisholm, D. M., Collins, M. I., Davenport, W., Gruber, N. and Kulak, L. L. (1975) PAR-Q validation report *British Columbia Medical Journal* 17.

If you have answered YES to any of the above questions please inform a member of the teaching staff/research team.

If any of the information you have provided changes in any way, you must inform a member of the teaching staff BEFORE you participate in any physical assessment



Exercise and Sports Science Research Group
School of Biological and Molecular Sciences
Oxford Brookes University
Oxford
OX3 0BP

If, prior to participation in a physical assessment, the answer to any of the following questions is 'yes', I will inform a member of the research team

Pre-exercise activity	Yes/No
1. Have you eaten within the last 2 hours?	
2. Have you drunk coffee or tea within the last 2 hours?	
3. Have you smoked a pipe or cigarette within the last 12 hours?	
4. Have you consumed alcohol within the last 24 hours?	
5. Have you performed exhaustive exercise within the last 48 hours?	

I understand that the information provided above is important, and I will inform a member of the research team if I believe there is a medical reason I should not participate in this research.

The procedure for the current study has been outlined and explained to me, and I have had the opportunity to address any points on which I need increased clarity. I therefore consent to volunteer for the current study.

Name

Signature

Date

Appendix 4

E3/FH&LS

Oxford Brookes University
Faculty of Health and Life Sciences
Decision on application for ethics approval

The Departmental Research Ethics Officer (DREO) / Faculty Research Ethics Committee (FREC) has considered the application for ethics approval for the following project:

Project Title: A comparison of different work: rest in a high intensity interval training programme; the effect on performance and health parameters

REC number: 1215_25

Name of Applicant/s: Molly Lloyd-Jones

Name of Supervisor/s: Dr John Jakeman, Dr Martyn Morris

Please tick one box

1. The Departmental Research Ethics Officer / Faculty Research Ethics Committee gives ethical approval for the research project.

Please note that the research protocol as laid down in the application and hereby approved must not be changed without the approval of the DREO / FREC

2. The Departmental Research Ethics Officer / Faculty Research Ethics Committee gives ethical approval for the research project, subject to the following::

3. The Departmental Research Officer / Faculty Research Ethics Committee cannot give ethical approval for the research project. The reasons for this and the action required are as follows:

Signed:  Approval Date: 29.1.2016

Designation: Departmental Research Ethics Officer

(Signed on behalf of the Faculty Research Ethics Committee)

Date when application reviewed (*office use only*): 15.12.2015