The Ergogenic Effects of Oxygen Supplementation on Cycling Performance

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Quote

"We all have dreams. But in order to make dreams come into reality, it takes an awful lot of determination, dedication, self-discipline, and effort."

Jesse Owens

Dedication

This thesis is dedicated to all the people that motivated me along the way and especially those that had a big impact in my sporting endeavours from a young age till now. Finally, I would like to dedicate this thesis to those that were lost through the PhD process. I made it!

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Thesis Abstract

Over the past 20 years oxygen supplementation has been proposed as an ergogenic aid in a range of sports and endorsed by various governing bodies (including the NFL and the NBA). Originally the use of oxygen supplementation in research was related time trial and time to exhaustion performances, i.e. predominately aerobic activities. However, more recently, oxygen supplementation has also been shown to benefit high intensity short duration activity.

This thesis aimed to further examine how oxygen supplementation may offer performance enhancing benefits, particularly during repeated sprint exercise. The first aim, examined in study 1, was designed to investigate the effects of oxygen supplementation on repeat sprint cycle performance. Studies 2 and 3 followed on with aims to investigate mechanisms that underlie these performance enhancements. Next, we aimed to determine how the exact timing of oxygen supplementation could potentially influence repeated sprint performance (study 4). The final aim, examined in study 5 was to identify whether the observed short-term performance benefits of oxygen supplementation, would transfer to performance improvements as a result of a supplemented training intervention. These aims allowed us to investigate some of the gaps in the existing literature, whilst developing a progressive narrative within oxygen supplementation research.

Investigating the mechanisms behind the change in acute and chronic performance involved the use of both near infrared spectroscopy (NIRS) and twitch potentiation. The use of these devices alongside oxygen supplementation resulted in novel studies. Similarly, our training study was conducted on competitive level cyclists, making our results of great interest to the cycling community.

Throughout this thesis, oxygen supplementation was shown to be a beneficial ergogenic aid during repeat sprint cycling which can be applied to training on a session by session

basis. Further research is needed to evaluate its chronic effectiveness when used during high intensity exercise and explore more of the underlying mechanisms.

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Abbreviations

ANOVA	Analysis of Variance
ATM	Atmosphere
ATP	Adenosine Triphosphate
BLa	Blood Lactate
COPD	Chronic Obstructive Pulmonary Disease
DPF	Differential Pathlengths Factor
EPO	Erythropoietin
FI	Fatigue Index
FiO_2	Fraction of Inspired Oxygen
H	Oxygen Supplementation (FiO_2 1.00)
H^+	Hydrogen Ion
HbO_2	Oxyhaemoglobin
HHb	Deoxyhaemoglobin
HIIT	High Intensity Interval Training
Oxygen Supplementation	Manipulation of Oxygen Inspired
O_2Supp	Oxygen Supplementation
Pi	Inorganic Phosphate
IT	Interpolated Doublet Twitch
LT	Lactate Threshold
N	Normoxia (FiO_2 0.21)
NIRS	Near Infrared Spectroscopy
mA	Millliampere
Mb	Myoglobin
MPO	Mean Power Output

ms	Millisecond
MVC	Maximal Voluntary Contraction
P_aO_2	Partial Pressure of Oxygen in the Alveoli
PCr	Phosphocreatine
PPO	Peak Power Output
PTF	Potentiated Interpolated Twitch
RBC's	Red Blood Cells
ROS	Reactive Oxygen Species
RPE	Rating of Perceived Exertion
RPM	Revolutions Per minute
RST	Repeat Sprint Training
S_aO_2	Saturation of Arterial Blood with Oxygen
SIT	Sprint Interval Training
SRS	Spatially Resolved Spectroscopy
tHb	Total Haemoglobin Concentration
TI	Twitch Interpolation
TSI	Tissue Saturation Index
TT	Time Trial
TTE	Time Trial to Exhaustion
VA	Voluntary Activation
$\dot{V}O_2max$	Maximum Rate of Oxygen Consumption During Exercise
W	Watts
WADA	World Anti-Doping Agency

1 - Introduction

Introduction to the Thesis Chapters

1.1 Introduction

Oxygen supplementation (O_2Supp) is the administration of medical grade oxygen to athletes during or between periods of work [1]. To date O_2Supp has been applied to a variety of different training modalities (running, cycling and rowing) at varying degrees of concentration (percentage of oxygen consumed) [1–6].

Many performance and recovery aids are already used by a large proportion of the sporting population (such as muscle stimulation, foam rolling, bicarbonate, and stretching), and athletes are continuously searching for the more performance enhancers - oxygen supplementation (O_2Supp) is one of the newer options.

A common focus within O_2Supp research the effect on performance during high intensity interval training (HIIT). HIIT is short duration, intense periods of work interspersed with periods of active or passive recovery. It is during these periods of recovery where O_2Supp may be most effective.

Researchers have already begun to apply O_2Supp to a variety of different training modalities (rowing, cycling, swimming, running) with varying degrees of effectiveness. As the concentration of oxygen varies across studies the findings are not easy to compare. In order to reduce the dose response component, the research in this thesis will look at the effectiveness of O_2Supp during high intensity repeated sprint cycling using only 100% medical grade oxygen concentration (fraction of inspired oxygen - FiO_2 1.00). The experimental chapters within this thesis will incorporate consistent methodology to identify the effectiveness of O_2Supp as a performance aid.

1.2 Aims of Research

Several studies [5,7,8] have begun to identify the effectiveness of O_2Supp in a variety of different sporting modalities, but not specifically repeat sprint cycling.

This research aims to assess the effectiveness of O_2Supp on repeat sprint cycling and will aim to answer the following;

- During which component of a repeat sprint cycling training session is O_2Supp most effective at increasing performance?
- Which physiological components does O_2Supp effect to allow for improvements in performance capacity?
- Can a short-term training intervention influence performance or metabolic markers of performance?

2 – Literature Review:

The Use of Oxygen Supplementation and its Role Within an Exercise Programme

2.1 Overview

This literature review provides an in-depth description of O_2Supp, and its purported effects on exercise performance with a particular focus on high intensity interval training (HIIT).

HIIT training and its main limiting factor (peripheral fatigue) will be addressed first, followed by a description of oxygen supplementation, and how it has been used within sports performance research. Published studies exploring both acute and chronic supplementation and the resultant effects on both performance and exercise metabolism will be examined.

This review will also explore many of the mechanisms that are believed to underlie the effects of O_2Supp (such as increased muscle & whole-body oxygenation, reduced blood lactate accumulation, enhanced phosphocreatine resynthesis and reduced peripheral muscle fatigue,). Within this section, the two of methods used to determine the extent to which oxygen supplementation affects physiology are examined in depth – these methods are near infrared spectroscopy (NIRS) and twitch interpolation (TI). These methodologies will play a vital role in the forthcoming experimental studies.

The review will finish with a brief outline of gaps in the literature related to supplementation and performance, followed by the aims and hypotheses of the current thesis.

2.2 HIIT / SIT training

High-intensity interval training (HIIT) is a common, time efficient training method for improving cardiorespiratory and metabolic function and in turn, physical performance in athletes [9]. HIIT involves recurring bouts of short high-intensity exercise (45 s – 4 min) interspersed with short periods of recovery [10]. HIIT has been shown to be an optimal

stimulus for eliciting maximum cardiovascular and peripheral adaptations in relatively short training sessions [11,12]. This is achieved by subjecting the athlete to an increased amount of time in the 'red zone', which is characterised by working at greater than 90% of their $\dot{V}O_2max$ [10,12]. Most HIIT session only last for several minutes with the majority of that in the 'red zone', eliciting both acute (lactate accumulation, peripheral fatigue) and chronic (myocardial enlargement and oxidative muscle adaptation) changes.

HIIT consists of the variation of nine variables, including the work interval intensity and duration, the recovery interval recovery and duration, exercise modality, number of repetitions, number of sets, as well as the between set recovery and duration [13]. The manipulation of these variables affects the acute physiological responses to HIIT. The most common variables to manipulate are the work and recovery durations. HIIT has been split into four main areas that incorporates the many different work durations; short intervals, long intervals, repeated sprint training (RST) and sprint interval training (SIT) [9,13]. Short interval HIIT has a work duration of less than 60 s and long interval HIIT has a work duration of 60 s or longer. RST and SIT are types of short interval HIIT as they both have work durations of less than 60 s. RST commonly has work durations of 3-10 s in length and whereas SIT has work periods of 30-45 s in duration [13].

It is suggested that with a change in work duration, comes a reduction in critical maintainable velocity. Taken from Buchheit *et al.*, 2013 [13], velocity for SIT ranges from 85 -100% of maximum sprint speed or 160-200% of velocity of $\dot{V}O_2max$ ($v\dot{V}O_2max$), whereas, RST can be conducted at a relatively slower intensity of 120 – 160% $v\dot{V}O_2max$. The main reason for this drop in maximal velocity is down to the recovery durations following the work. RST has short high intensity intervals followed by short (30 s) recovery periods; therefore, the velocity must be reduced to maintain multiple repetitions. SIT has

slightly longer intervals with long recoveries (2-4 min), allowing for complete recovery and increased work velocity.

It is evident that HIIT training can be applied to an array of different sports (Hockey, cycling, running and footboall [14–17]). HIIT training can take many forms, some lending themselves to be more effective types of training than others. The most common protocol for building peak power is both SIT and RST. SIT allows an individual to work at a very high-power output and repeat it until chronic adaptations occur (common in 100 m training), whereas RST allows the athlete to test their recovery capabilities when producing large power outputs (common in track cycling)[18–20]. The adaptations that occur during SIT and RST are both metabolic and musculoskeletal. The metabolic adaptations that occur are the increased efficiency of converting phosphagens and ADP into adenosine triphosphate (ATP), restoration of phosphocreatine (PCr), and the anaerobic breakdown of carbohydrates, whereas the cardio and musculoskeletal adaptations that occur include increase stroke volume, increase microvascular size of the peripheral muscles vessels, and muscle fibre recruitment changes [21–23]. Short and long duration HIIT work the aerobic system that aids endurance performance by increasing $\dot{V}O_2max$ and the velocity at which it is achieved. These are achieved by increasing the efficiency of muscle glycogen utilisation and the cardiopulmonary circuit.

Performance during both SIT and RST training are limited by several physiological factors; resynthesis of anaerobic sources during recovery, muscular fatigue, and the metabolic homeostasis within the cells, all of which can improve an individual's performance [15,16,24].

2.3 Fatigue

Fatigue comprises many different pathways and symptoms and therefore, researchers define fatigue as largely different entities. In this thesis the combination of Edwards [25], and

Bigland- Ritchie *et al.*, [26], definitions will be used *"The inability to maintain the required level of strength or power output, leading to exercise cessation"* & "*Any exercise induced reduction in maximal force or power regardless of whether the task can be sustained or not*". These are two definitions that describe what occurs to the physiological performance parameters during sport.

Fatigue is often quantified as either peripheral and central fatigue [27,28]. Central fatigue represents the decrease in the ability of the recruitment of muscle motor units at the start of muscle force generation [29]. There are several hypothesises as to why this occurs; increased brain serotonin levels, an altered ratio between tryptophan and branch chain amino acids [30] and the irritation of nerve endings. Central fatigue is, in essence, those chemical imbalances or fluctuations within the brain during daily life and especially during exercise. Central fatigue can be measured in many ways; intrusively by taking brain blood samples to identify the fluctuations within the biochemistry of the brain, the perception of fatigue (ratings of perceived exertion), peripheral twitch interpolation, transcranial magnetic stimulation and electromyography.

Peripheral fatigue on the other hand refers to physiological changes that occur as a result of alterations in nerve impulses at a muscular level. Peripheral fatigue thus, is a decrease in the contractile strength of the muscle fibres due to changes in the frequency and strength of the muscle action potentials [28]. Peripheral fatigue can be measured in a plethora of ways, such as; a decline in performance, reduced force produced during maximal voluntary contraction (MVC) and twitch interpolation (TI) [31–33]. TI is the most common way of measuring peripheral fatigue and is the measurement of force being produced by a single stimulus. The amplitude of the twitch then identifies the contraction intensity and level of excitation of the motor neurons [34]. A large twitch indicates a larger level of peripheral fatigue at a muscular level.

2.3.1 Central Fatigue

Central fatigue is the reduction in the supraspinal recruitment of muscle motor units that generate muscle force. Central fatigue governs the supraspinal and spinal changes in physiology capable of inducing an excitation in the motor neuron (reduction in neurotransmitters and conductive capability at the neuron junction).

Central fatigue is generated from the cerebellum and the spinal cord that make up the central nervous system. These organs are extremely sensitive to alterations in their homeostatic regulation, as well as invasive instruments to measure it. Resultantly, due to the nature of central fatigue it is problematic to recognise the 'true' underpinning mechanisms and fatiguing components during exercise. Ethically it would be impermissible to attach probes to an individual's brain to directly monitor the chemical composition and oxygenation. Therefore, direct measures are not frequently used. Many indirect measures occur as a result; cerebral NIRS, transcranial magnetic stimulation, and invasive measures such as cranial cannulas [28,31].

Central fatigue is one factor that results in performance decline throughout a race. Gandevia [28] identified that a 25% drop-in force of the motor unit identified using transcranial magnetic stimulation was attributed solely to central fatigue. Further, he found that during sub maximal low intensity exercise the significant reduction in force was again largely (25%) attributed to central fatigue, due to the reduced excitation stimulated by the motor cortex. It has also been shown that the amount of central fatigue experienced is directly linked to the amount of oxygen available. An inadequate supply of oxygen to the brain and low mitochondrial oxygen tension has been shown to influence the function of neurons, and thereby their ability to maintain motor activation [35]. During hypoxic conditions the 'central governor' reduces the muscle contractile force to deal with the ischaemic

conditions the body's organs are being subjected too. The opposite is hypothesised to happen for hyperoxic conditions. The central governor model states that the brain dynamically regulates the physical exertion by different inputs to the body, allowing task completion in a safe manner [36]. The central governor is able to divert greater oxygen supply to the working muscles because of an increased availability, supply and volume [37].

2.3.2 Peripheral Fatigue

Peripheral fatigue occurs when the motor drive within the central nervous system is unchanged, but the full electrical excitation produced within the motor units is no longer adequate to support maximal voluntary contractions [26]. Due to the nature of the neuromuscular junction and the mechanism behind muscle contraction many would suggest that peripheral fatigue is also multidimensional in nature.

The neuromuscular junction is where neural signals are transferred from a neuron to its target. It comprises a synaptic terminal and a synaptic cleft. The terminal between the synaptic terminal, otherwise known as the end of the axon terminal, and the motor end plate is regulated by multiple biochemical reactions. Inorganic phosphate (Pi) induces a desensitisation in the terminal leading to a reduction in force production [38,39]. Subsequently, acetylcholine is released and is the primary chemical used to regulate the terminal space to ensure full sensitivity and polarisation [40].

Huxley's sliding filament theory [41] states that the H bands (myosin) and the I bands (Actin) get smaller during a contraction, the zones then overlap and the Z lines move closer together (actinin attached with titin). The contraction weakens when the I band disappear, and the H bands and the Z line connect. If calcium is not released from the sarcoplasmic reticulum efficiently, the muscle fibres cannot contract. The control of calcium production is governed by both neural control and build-up of other compounds such as phospholamban.

Biochemical changes within the muscles are associated with imbalances within the sarcolemma and its electrical potential [42]. A change in the electrical signal is a direct result of biochemical imbalances, which in turn reduces the force generating capacity of local muscles resulting in muscular fatigue [43]. A small change in the cellular homeostatic balance within the muscle fibres lead to large reductions in the force generating capacity. Factors that have the capacity to alter this homeostasis are; the depletion of ATP, depletion of PCr, accumulation of inorganic phosphate, and decrease in pH, to name a few.

ATP availability has been closely correlated to the fatiguability of local muscles. As ATP depletion increases fatigue within the skeletal muscles. However it is still up for debate whether this phenomenon is coincidental or causative [43]. It has been shown that intramuscular supply of ATP and PCr are never completely depleted, as a protective mechanism with the body, so levels are regulated to maintain integrity within the cells. Bergstrom *et al.,* [44] found that ATP and PCr were between 10-70% of their rested values during exhaustive exercise. Although, they state that ATP is stored within multiple compartments (such as mitochondria and cytoplasm), the local store of ATP maybe depleted but the overall cellular level remains intact.

Lactate and hydrogen ions (H^+) play a large role in the materialisation of peripheral fatigue during exercise lasting between 15 s and 15 min. During high intensity exercise the build-up of lactate and H^+ is due to the inefficiency of the Cori cycle and production subsequently exceeds removal. The effects of lactate in peripheral fatigue are 85% attributed to the increase in H^+, leading to an exercise induced acidosis [45]. Intramuscular acidosis has been shown to cause an inhibition of the energy systems (glycolysis), and consequently a reduction in power output follows [46].

Accumulation of inorganic phosphate may also result in peripheral fatigue as it has been linked with limiting force generation via inhibiting calcium secretion within the

sarcoplasmic reticulum [47]. Further, inorganic phosphate desensitises the cross bridges at the neuromuscular junction [38,48].

2.4 Recovering from fatigue

Fatigue is an outcome that is a consequence of numerous processes that are a result of exercise. Physiological fatigue (referred to as performance decline in the following chapters) is the result of the acute physiological stress that a single fatiguing training session evokes, which is also associated with; minor muscle damage, inflammation, nervous system fatigue, and oxidative fatigue [49]. To minimise the effects of these stresses, multiple recovery strategies have been proposed over the years; adequate nutrition, cool down, massage, compression, cold-water immersion and 'gas therapy' [49–51]. These strategies have been proposed to aid recovery post a repeat sprint session. As well as post exercise recovery, minimising the amount of fatigue experienced during repeat sprint training is also vital for performance. Acute fatigue that is as a result of one sprint is associated with increase pH, reduced fuel availability (PCr) and accumulation of lactate. Acute fatigue requires different recovery strategies than that of chronic fatigue, these strategies may include bicarbonate supplementation, creatine loading and O_2Supp [52]. This next section of this review will be on the recovery from acute fatigue with the impetus on O_2Supp.

2.4.1 Recovery Techniques for Acute Fatigue

Gas therapy, or oxygen therapy, is the use of various gas mixtures to aid in recovery depending on type of exercise [53]. O_2Supp during recovery has been shown to decrease lactate production and increase both peak power and mean power [7,54]. There are two distinct form of O_2Supp: normobaric and hyperbaric.

Hyperbaric O_2Supp is the administration of oxygen at a barometric pressure higher than that of the pressure at sea level [53]. Hyperbaric O_2Supp increases the blood plasma concentration of oxygen from 0.3 ml per decilitre to 1.5 ml per decilitre [55], simply by increasing the pressure gradient at a cellular level. Due to the large pressures at which the gas must be administered, hyperbaric O_2Supp typically occurs in an airtight chamber. This enables the correct manipulation of pressures and composition of the air. There are important health implications to be considered when using hyperbaric O_2Supp. Oxygen is needed to sustain life, however too much oxygen is toxic to the central nervous system [56,57]. Oxygen toxicity is not easily identified until it is in its advanced stages, as it is masked by the hyperventilation response that comes from O_2Supp.

Potential prolonged use of O_2Supp (greater than 6-weeks) may cause health problems as a result of cell damage [1]. Long term exposure to high concentrations of oxygen ($FiO_2 > 0.60$) even at normobaric pressure, may cause oxygen toxicity through the formation of excessive reactive oxygen species (ROS) [56,58–60]. However, there is no difference in haematological and urinary markers of oxidative stress during high intensity prolonged training periods with FiO_2 of < 0.60 [61]. ROS formation within the mitochondria has been reported to increase linearly with inhaled oxygen concentration. However, to date no negative effects of short term inhalation of oxygen enriched air during exercise at sea level has been reported [7,62], although it has been hypothesised that the risk exponentially grows with FiO_2 concentration [1]. Therefore, during high intensity exercise it cannot be identified whether it is the exercise or the O_2Supp toxicity that causes a hyperventilation response. This is one of the many reasons, hyperbaric O_2Supp is not widely used within the sporting community. Normobaric O_2Supp is the administration of oxygen at a barometric pressure equivalent to pressure at sea level (760 mmHg or 1 Atmosphere [ATM]). Normobaric O_2Supp is the

most widely used tool for O_2Supp because it can be administered using a plethora of techniques; Douglas bags, air tight tent, or regulated oxygen cylinder [2]. The most crucial benefit of normobaric O_2Supp above hyperbaria is the pressure of administration is at normal air pressure, which is less likely to cause significant adverse effects.

2.5 Near Infrared Spectroscopy

NIRS is an imaging tool that was designed for clinical and emergency medicine. Additional uses have become evident. NIRS is now used in human physiology research to quantify and non- invasively measure the oxygenation status of human tissues [63]. The oxygenation status of a human tissue can be identified by measuring the oxygen saturation of haemoglobin molecules, based on the absorbance of near infrared light (Figure 2-1). NIRS historically used expensive large spectrometers using fibre optic cables, however with the advancements in technology these are now no bigger that a cellular phone. The increase in the resolution of near infrared light and the changes in the theoretical models (Beer Lambert Law to the Modified Beer Lambert law) has led to NIRS being widely used to measure tissue haemodynamics (Figure 2-2).

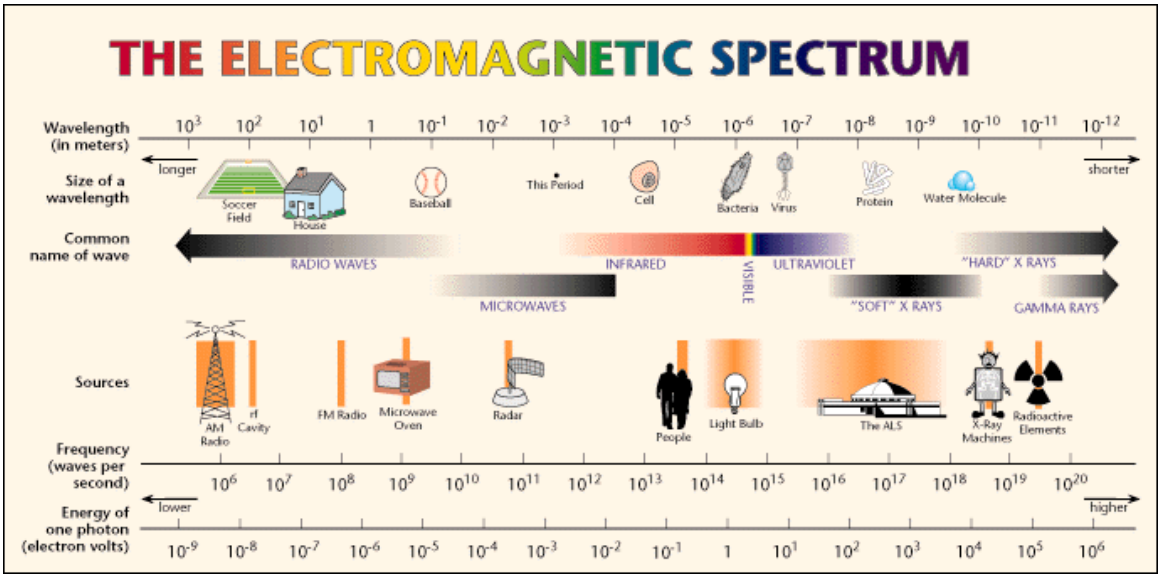

Figure 2-1. Electromagnet Spectrum – Liberated from a Lawrence Berkeley Laboratory web page https://www2.lbl.gov/MicroWorlds/ALSTool/EMSpec/EMSpec2.html.

The light absorbing compounds in human tissue within the near infrared range are called chromophores [64]. The primary chromophores detected by NIRS are oxyhaemoglobin (HbO_2) and deoxyhaemoglobin (HHb), their concentration varies with time and oxygenation status [65]. HHb and HbO_2 are responsible for the transport, delivery and removal of oxygen and carbon dioxide through-out the human body [63]. NIRS works by detecting the amount of light absorbed by the tissue and identifying the amount that is refracted back and received by the device. This is used to identify the rate of oxygenation and deoxygenation during exercise and, along with measures of cardiac output (heart rate and blood flow), helps to identify the central and peripheral components of the exercise. This is assessed by detecting the changes in oxygenation and blood flow at both a cerebral and peripheral muscle level.

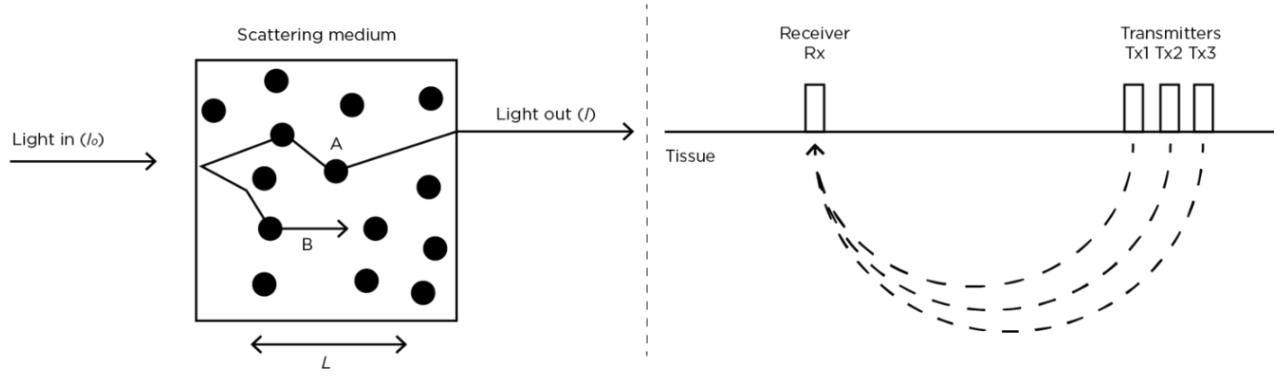

Figure 2-2. Visual diagram of infrared light pathway through muscle tissue. Liberated from Artinis Medical Systems (https://www.artinis.com/theory-of-nirs).

NIRS has been found to be a useful tool to measure blood flow and volume, which is required to determine regional oxygen delivery and uptake [66]. Although NIRS is not without its downfalls; the proxy of the total NIRS reading (tissue saturation index for example) has been shown to comprise 5% skin haemodynamics and the remainder muscle [67]. This is to be considered when looking at desaturation rates. Desaturation and resaturation rates of the skeletal muscle cells during exercise have been considered the main limiting factor during repeated sprint efforts [68]. This was measured using pulse oximetry which validates NIRS devices to identify arterial O_2 saturation. Muscle oxygen desaturation occurs when the working muscle is using more oxygen than is being supplied and beginning to work anaerobically. Resaturation is the recovery of the working muscles oxygen levels back to a pre exercise state, and this typically occurs during periods of rest. NIRS has been shown to be affected by the thickness of adipose tissue. A recent study found that thicker adipose tissue the decreased the sensitivity of desaturation and overestimation of actual muscle oxygenation levels [69]. Other measures (such as muscle biopsies, arterial blood gases) are more accurate for measuring skeletal muscle

oxygenation, although all are invasive. NIRS is non-invasive and does not affect concurrent human locomotion, so despite its limitations, it is a valuable tool within sport science research.

The development of portable NIRS technology has allowed research into tissue oxygenation and localised haemodynamic in an applied exercise environment. The portable nature of NIRS lends itself to research to occur during human locomotion, to identify biochemical aspects during exercise performance. The invention of portable NIRS devices allows a coach or athlete to monitor their muscle oxygenation directly and alter training modality, and training/ recovery duration to elicit the greatest response from training they can. A coach using NIRS has the ability to be able to identify when an athlete has fully resaturated their peripheral muscles with oxygen. This information can direct athletes to start training once they resaturate fully.

HIIT and SIT based research have employed NIRS extensively [70,71]. The rate of muscle oxygen resaturation has strong links with the recovery rate of PCr, a metabolite that is a vital fuel for high intensity exercise [72]. Distinct changes in muscle oxygenation (HbO_2 and HHb) profiles have been observed, characterised by large desaturation / resaturation profiles (Figure 2-3). It has been documented that these profiles (desaturation/ resaturation) are strongly correlated ($r^2 = 0.51$) with the rate of resynthesis of PCr [72]. The rate of muscle oxygen recovery reflects the utilisation of oxygen after exercise to resynthesise PCr. Increases in HbO_2 between conditions would demonstrate this increase PCr resynthesis. It is also suggested that the recovery of HbO_2 resaturation could also be used as an index of muscle oxidative capacity [72,73]. The faster the resaturation of HbO_2 in the working muscles following exercise, indicates an increase in oxidative capacity of the muscles. A proxy of muscle oxidative capacity can be assessed directly using arterial occlusions and NIRS [74,75].

This measure looks at the speed at which HbO_2 recovers following arterial occlusions and the rate at which HHb declines during said occlusions.

Therefore, the use of these muscle oxygen profiles can provide insight (albeit indirectly) into which training programmes and aids are more effective at increasing the resynthesis of PCr. PCr resynthesis and muscle oxygenation profile are vital for most exercise, particularly HIIT and SIT based sessions. HIIT & SIT are commonly used to enhance performance by eliciting improvements in the oxidative capacity of working muscles and maximal oxygen uptake [20]. Interestingly yet unsurprisingly HIIT/SIT based training programs have also demonstrated performance benefits via peripheral adaptations (mitochondrial density/ increased microvascular size and amount/ increased enzyme activity) [18,70]- that lead to enhanced oxidative capacity of working muscles and maximal oxygen uptake.

It has been found that the consumption of hyperoxic gas causes significant changes in NIRS signals. Vanhatalo *et al.*, [76] mentioned that local muscle delivery increased by 29.5% during O_2Supp, compared to Normoxia. They went on to show that the increased availability of oxygen did not change the overall kinetics; as O_2 availabilty became higher, O_2 extraction reduced to maintain equilibrium. The baseline muscle saturation level was at an elevated level but recorded similar levels of desaturation as Normoxia. Vanhatalo *et al.*, also highlighted that even though there was an increase in muscle oxygen delivery, muscle fractional O_2 extraction was reduced by 36% -estimated using HHb. HHb can be used to assess the relationship between muscle resaturation and muscle desaturation.

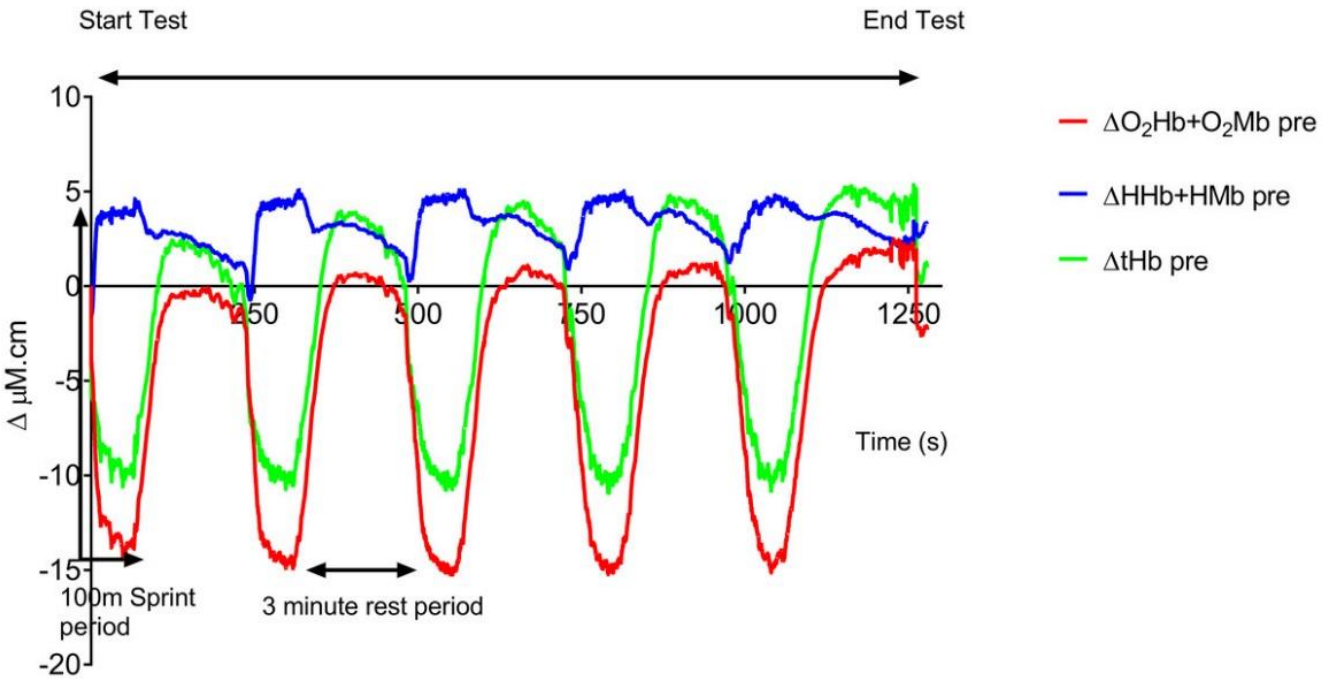

Figure 2-3. Example of NIRS muscle hemodynamic responses during a swim test. Taken from Jones *et al.*, 2018 [77].

Legend: ΔO_2Hb+O_2Mb, change in Oxyhaemoglobin and oxymyoglobin levels; ΔHHb+HMb, change in deoxyhaemoglobin and deoxy myoglobin levels; ΔtHb change in total haemoglobin oxygenation.

Supplementing high intensity exercise with hyperoxia has been shown to enhance the clearing of BLa and prevent the rate of muscle desaturation [54,78]. It has also been noted that the prevention of less than 10% drop in arterial O_2 saturation, significantly lessens the amount of exercised induced force reduction of the quadriceps muscles, following cycling tasks [79]. Although, not directly measured using NIRS, changes in arterial saturation will affect the periphery further down the oxygen cascade where NIRS measures are taken (peripheral muscle oxygen levels) [80]. Preventing a decrease in arterial oxygenation will aid the maintenance of the homeostasis in the periphery. It has been shown that a decrease in arterial oxygen saturation significantly effects peripheral muscle fatiguability [81,82]. A

reduction in the muscle oxygen level aligns with local muscle fatigue at a peripheral level, and this is visibly evident by large reductions in muscle resaturation.

The recovery of muscle oxygen (resaturation rate) strongly correlates with the resynthesis of PCr during recovery periods [72]. Resynthesis of PCr is a key component which affects performance during repeated sprint efforts. Additionally, Balsom *et al.,* [83] found that post exercise multiple subcutaneous injections of erythropoietin (EPO) during hypoxia and O_2Supp, enhanced anaerobic metabolism, and an increased O_2 availabilty (due to 10% increase in haemoglobin concentration) which led to a significant increase in PCr resynthesis during 15 x 6 s cycling sprints. Similar increases in PCr resynthesis are proposed to occur during O_2Supp due to the increased oxygen availability, comparable to EPO injections.

2.6 Oxygen Supplementation

Hyperoxia occurs when cells, tissues and organs are exposed to an excess supply of oxygen or higher than normal partial pressure of oxygen [84]. O_2Supp causes a hyperoxic response within the body. High oxygen availability is used to treat patients with chronic obstructive pulmonary diseases (COPD). Oxygen can be used to supplement their air supply, so they have sufficient oxygen delivery to their cells in light of their disease. Comparable to this, altitude training has similar physiological restraints as COPD [85].

One point of note is the correct use of the term hyperoxia. Hyperoxia occurs when cells, tissues and organs are exposed to an excess supply of oxygen or higher than normal partial pressure of oxygen [59]. Hyperoxia is the internal change due to the consumption of high oxygen content gas, not the physical gas that is being administered.

2.6.1 Normal Respiratory Physiology

During breathing, normal atmospheric air (~21% oxygen, 0.04% carbon dioxide, 78% nitrogen and ~1% other gases) travels down the pulmonary ventilation system into the lungs where it diffuses into the capillaries at a speed of up to 3 $L \cdot min^{-1}$ of oxygen (during exercise). The average oxygen pressure gradient between alveoli (100 mmHg) and capillaries (40 mmHg) is 60 mmHg. A healthy lung at rest will reach an equilibrium in pressure (atmospheric pressure and alveoli pressure difference) within 0.25 s which is about ⅓ of the bloods transit time around the lung. During vigorous exercise blood transit time is increased by as much as 3-fold, and this increase in velocity does not usually restrict the loading of oxygen in the blood [86,87]. This increase in velocity of the blood coincides with an increase in the ventilatory equivalent (ratio of the volume of gas expired per minute to the volume of oxygen consumed per minute), enabling the maintenance of oxygen loading to the blood.

Once oxygen has passed into the blood it combines with the metallic compound haemoglobin (iron containing globular protein). There are over 280 million haemoglobin molecules per red blood cell (and 25 trillion RBCs in the body). The nature of RBCs means that greater than 70 times more oxygen is combined within haemoglobin than dissolved in the plasma There is on average 15 g of haemoglobin per 100 ml of blood with an oxygen carrying capacity of 1.34 ml $O_2 \cdot g^{-1}$. Therefore, per 100 ml of blood around 20 ml of oxygen is carried at any one time.

The saturation of haemoglobin with oxygen is altered by internal fluctuations in oxygen pressure, core temperature, cellular pH and carbon dioxide, as indicated by an oxyhaemoglobin dissociation curve. [88].

Myoglobin, the iron globular protein that is found in muscle microvascular, on the other hand is less affected by these internal changes. Oxygen is transported from the alveoli to

the muscles by haemoglobin and is then passed onto myoglobin to be used at muscular level. Additionally, myoglobin facilitates the transfer of oxygen to the mitochondria at the beginning of exercise and during intense exercise in an attempt to attenuate a large drops in cellular partial pressure of oxygen [89]. Unlike haemoglobin, myoglobin retains a large percentage (95%) of its bound oxygen even when pressures are very low (5 mmHg).

Mole *et al.,* [90] indicated that myoglobin may provide a source of readily available O_2 at the onset of exercise and increase the PO_2 gradient from capillary to muscle cell even at low levels of activity, suggesting that myoglobin has a role that is intermediate between two other functions, O_2 storage and facilitated O_2 diffusion [91].

Once oxygen has been transported from atmosphere to mitochondria it can be used during cellular respiration [92]. Cellular respiration is the combination of processes and reactions where biochemical energy from nutrition is 'converted' into ATP. Cellular respiration can be split into two distinct categories: aerobic and anaerobic. Aerobic respiration brings about the biggest energy yield, using oxygen to resynthesise ATP. Oxidative respiration occurs during the citric acid cycle and oxidative phosphorylation [92] and more than 90% of the total ATP synthesis is achieved during these processes. Oxygen is during these processes to bind with hydrogen to form water and remove the acidity from the mitochondria and buffer it elsewhere.

PCr resynthesis during HIIT and SIT with a recovery duration of 45 s is insufficient in duration to fully replenish PCr stores (only ~75% of resynthesis occurs in this time). Therefore, after several repeated sprints (~5 x 15 s sprints), PCr resynthesis would be diminished, with most PCr stores depleted. Significant performance reductions would be evident with such a reduction in PCr resynthesis capacity. A continued depletion of ATP-PC stores and an inadequate resynthesis of PCr will lead to a reduction in performance until other energy systems are able to produce larger amounts ATP and aid in the resynthesis

process. Mendez- Villanueva *et al.,* [93] found that an 8% increase in PCr resynthesis during 30 s recovery bouts from 10 x 6 s cycling sprints significantly enhanced peak and mean power. Similarly Glaister, [52] identified that an increase in peak and mean power has been attributed to an enhanced PCr resynthesis during repeated high intensity exercise.

There is a small (< 10%) aerobic contribution to energy production during a short (up to 30 s duration) maximal effort sprint and is suggested that this contribution increases as sprints are repeated, perhaps due to the alterations in oxygen kinetics [94–96]. This is part due to increased oxygen demand post sprints in order to re-establish the saturation of myoglobin with oxygen and PCr. This may result in subsequent sprints starting at progressively higher $\dot{V}O_2$. Additionally, Bogdanis *et al.,*[97] found that aerobic metabolism during the second sprint (30 s) provided ~49% of the energy yield. These values were estimated due to an increase in oxygen uptake by ~0.5 $L \cdot min^{-1}$ (increase of 20%). These findings can be explained by Wagner [98] who concluded that an increased FiO_2 raises the partial pressure of oxygen in the blood and resultantly increases $\dot{V}O_2max$ (sparing anaerobic energy at higher workloads). Importantly, oxygen is also used for the resynthesis of PCr, the resynthesis of PCr occurs using products from both the Krebs cycle and aerobic glycolysis, which have been shown to be enhanced following the use of O_2Supp [99].

2.6.2 Hyperoxia

O_2Supp principally works on the basis of increasing the availability of oxygen, thus more is ready to be utilised by the body. O_2Supp can be administered two ways; hyperbaric and normobaric.

Hyperbaric hyperoxia is when additional oxygen is pressurised greater than 1 ATM (760 mmHg) and administered to an individual, whereas normobaric O_2Supp is administered at 1 ATM or lower. Hyperbaric O_2Supp is provided to patients in hospital either through nasal

cannulas or hyperbaric chambers. This additional pressure overcomes the physiological restraints the patients experience due to the increased pressure gradient at an alveoli/ capillary level.

Hyperbaric O_2Supp works on the premise that oxygen is pressured into the body cells thus increasing delivery. Increasing the intrapulmonary pressure within the lungs due to pressurised air (3 ATM) increases the pressure gradient between alveolus walls and the capillary wall by 10-fold [100]. Such a dramatic increase in pressure results in additional oxygen 'leaking' and dissolving into the blood plasma. The increase in dissolved oxygen within the plasma is sufficient to meet resting cellular requirements without any contribution from haemoglobin. Additionally, this dissolved oxygen can be provided to the areas into which red blood cells are unable to pass, such as the interstitial fluid [101,102].

O_2Supp has also been shown to cause vasoconstriction within the microvascular, thus reducing heart rate and blood flow, which is compensated by the increased plasma oxygen content [103,104]. This is typically used to treat crush injuries or burns but it is speculated that it can be applied to a variety of sporting population, shooting, archery and biathlon. This is due to vasoconstriction having a calming effect on the body and reduces heart rate, preventing the shakes during precision sports.

Normobaric O_2Supp refers to additional oxygen greater than a FiO_2 of 0.21 at 1 ATM of pressure, so supplementing the cells with additional oxygen, rather than higher pressurise. Normobaric O_2Supp does not differ from hyperbaric oxygen in terms of its effect on oxygen delivery and consumption, other than the difference in pressure that it is administered. Normobaric O_2Supp is believed to be safer than hyperbaric O_2Supp as the increase in pressure may cause oxygen toxicity through the formation of excessive reactive

oxygen species (ROS) [56,58–60]. The formation of ROS is still possible during normobaric O_2Supp but much less likely than when the oxygen is pressurised before administration.

2.6.3 Cellular Response to Oxygen Supplementation

O_2Supp causes large changes within the cellular equilibrium of the body. Firstly, it increases the partial pressure of oxygen (P_aO_2) within the alveoli, and consequently the entire oxygen cascade. Increasing the FiO_2 to 1.00 (O_2Supp), leads to a subsequent six fold increase within the arterial P_aO_2, to approximately 600 mmHg [105]. Hypothetically this gradient could increase further with hyperbaric hyperoxia, even though the determining factor for oxygen diffusion is the gradient between plasma and cells.

The body has various mechanisms to deal with the change in equilibrium that occurs during the administration of O_2Supp to prevent an accumulation of ROS. Most of the mechanisms associated with O_2Supp have a direct influence on cardiac output; changes within rate of blood flow, and heart rate, impacting cardiac output. One of the most pronounced responses to O_2Supp is the change in the microvascular blood flow [78,106] as the immediate response to O_2Supp is vasoconstriction. Chemoreceptors detect the change in P_aO_2, which is relayed to the brain and triggers the medulla oblongata to respond with peripheral vasoconstriction of the blood vessels, reducing the diameter of the vessels restricting the amount of blood that can pass through them. Thus, less blood is flowing through the microvascular, lowing the oxygen concentration of the tissue [78,107,108].

Another response to O_2Supp is a reduction in heart rate. Again, changes in P_aO_2 are detected and the medulla oblongata to responds via the parasympathetic nervous system, reducing heart rate at any given workload [78,109,110]. The final response is the change in energy

pathway, when more oxygen is available at a cellular level there is an increased efficiency within the mitochondria and an elevated rate of cellular respiration [111]. Additionally, an attenuation of metabolic by-products resulting from a reduced need for glycogenolysis, glycolysis and pyruvate production is evident during O_2Supp [112–114]. Reducing the need for these processing spares fuel and the attenuates the accumulation of by-products till later in exercise. Current research also suggests that O_2Supp during exercise would be enough of a stimuli to counteract the decline in the saturation of arterial haemoglobin (S_aO_2) during exhaustive exercise [115,116].

These responses are likely to be at least partially responsible for the effects sporting performances. Research in the field has increased over the last 6-8 years, with the ban of inhalation of oxygen enriched air being lifted by WADA in 2005. This has opened a door for many researchers to explore the effects of O_2Supp on recovery, as a training aid, and its use longitudinally. Currently due to the size of the equipment needed for safe administration, it is not widely accessible nor practical, although beneficial effects during exercise by decreasing lactate accumulation and increasing cycling power output are evident and worth the challenges O_2Supp poses at this moment in time [1]. Training with concurrent supplementary oxygen in an environmental chamber allows the user to be free from masks and tubing, which is more natural for the user. Environments (hyperbaric) chambers allow the user to change the oxygen content of the air safely and quickly.

2.6.4 Mechanisms

During exercise BLa levels increase with increasing demand on the metabolic systems. Lactic acid is a by-product of anaerobic glycolysis, and quickly converted to

lactate and hydrogen ions. Lactate is produced within the cytoplasm and distributed within the blood and is cleared by active muscles, liver and kidneys.

Blood lactate has been shown to increase in an exponential manner when accumulation exceeds removal, this is known as the as the lactate threshold (LT). LT is typically numerically referred to as 4 $mmol \cdot L^{-1}$. LT is exceeded very early on in a repeat sprint protocol. The response of the BLa during repeated sprints gives practitioners an idea that the participant is responding to the training correctly. For example, during 10 repetitions of an intense cycling activity, lactate increases until it reaches a physiological ceiling and then plateaus. Ergogenic aids are any aid that leads to a direct increase in performance capacity, some aids have been shown to increase this ceiling or reduce the BLa concentration for any given workload [117].

The performance improvements with O_2Supp are shown as increased physical work and changes in metabolism too. O_2Supp effects the accumulation of BLa by increasing the efficiency of the aerobic metabolic processes [113]. Increasing the efficiency of these pathways results in the sparing of fuel and attenuates accumulation of by-products. BLa accumulation and increase of hydrogen ions (H^+) leads to the desensitisation of the cross bridges which subsequently causes peripheral fatigue and the termination of exercise (See section 2.3.2 Peripheral Fatigue). Acutely, decreased lactate accumulation has been credited in part to an increase in performance [7]. Additionally, decreased lactate accumulation has also been shown to attenuate the build-up of peripheral fatigue [46].

However, BLa is not always attenuated by O_2Supp, as some studies have shown BLa to be similar during Normoxia. This has been proposed to be due to the individual participants reaching the ‘ceiling’ for the amount of O_2 being utilised by the working muscles [113,118]. Participants are unable to utilise the additional oxygen available, so it is simply exhaled.

The increase in full body oxygenation associated with O_2Supp [3] has been shown to improve the efficient of multiple metabolic pathways, leading to an increase in performance. S_aO_2 increases a result of O_2Supp, and some researchers have hypothesised that a small increase (1-3%) in S_aO_2 is enough to improve performance by as much as 3% [108,119,120]. Contrary to this, others speculate that due to the already high S_aO_2 at rest (99%), it would be pointless to give O_2 during recovery bouts of less than 1 min because it will have no effect on S_aO_2, which will naturally restore during this bout [121].

One noteworthy reason for O_2Supp being an effective recovery tool between bouts of exercise is its ability to increase the rate of PCr resynthesis. PCr resynthesis during HIIT and SIT with a recovery duration of 45 s is insufficient in duration to fully replenish PCr stores (only ~75% of resynthesis occurs in this time). Therefore, after several repeated sprints (~5 x 15 s sprints), PCr resynthesis would be diminished, with most PCr stores depleted. Significant performance reductions would be evident with such a reduction in PCr resynthesis capacity. A continued depletion of ATP-PC stores and an inadequate resynthesis of PCr will lead to a reduction in performance until other energy systems are able to produce larger amounts ATP and aid in the resynthesis process. Mendez- Villanueva *et al.*, [93] found that an 8% increase in PCr resynthesis during 30 s recovery bouts from 10 x 6 s cycling sprints significantly enhanced peak and mean power. Similarly Glaister, [52] identified that an increase in peak and mean power has been attributed to an enhanced PCr resynthesis during repeated high intensity exercise.

As shown, there are important acute effects of O_2Supp which subsequently aid performance though the sustained improvements in performance through chronic use is unknown.

Whether the acute responses to O_2Supp are an adequate stimulus to lead to long term physiological adaptation is yet to be established, and it is likely that multiple sessions with O_2Supp are needed to lead to chronic adaptations.

2.7 Effects of Oxygen Supplementation

O_2Supp has many potential applications in the sporting world (e.g. increased power output & decreased BLa) as well as in the pharmaceutical world – (temporarily reversing the effects of COPD). Much is known about the therapeutic use of O_2Supp and its effects, though little is known about the effects of O_2Supp following repeated intersession use on performance. The acute use (lasting seconds to hours but less than one day) have been shown to improve performance. Little is known about the effects of long term (chronic - days) use of O_2Supp and whether it is detrimental to health and performance [122]. This section will explore the effects of acute and chronic use of O_2Supp on sports performance.

2.7.1 Effects of Acute Oxygen Supplementation

The consumption of O_2 has been shown to improve a variety of physiological and performance parameters within the short term (effects diminish within 30 min of removal from O_2Supp). The acute performance effects of O_2Supp are what most research has been focused on. The parameters O_2Supp has been shown to improve are; increase time to exhaustion (TTE), decreased accumulation of lactate, increase power output and increase S_aO_2.

O_2Supp can be applied to any part of a session, the pre-loading phase before exercise, during the exercise bout, during the recovery between repetitions (inter sprint recovery) and during recovery between sets of exercise (inter exercise recovery).

Due to the natural high oxygen saturation of the blood at rest, research has moved away from administering O_2Supp prior to exercise, in turn giving it as a recovery aid between bouts of exercise [121]. Sperlich *et al.,* [121] found that pre-loading an athlete with 25 breaths of O_2Supp (FiO_2 0.6) before 15 s maximal cycling sprint was not sufficient at eliciting a response in mean power output (MPO), peak power output (PPO), BLa, pH or H^+.

O_2Supp during either of the two recovery periods, has two distinct main effects; its effect on high intensity exercise and its effect on endurance performance following inter exercise recovery bouts. O_2Supp during high intensity exercise has been shown to increase power and S_aO_2, with minimal changes in BLa and pH [1,6,123]. The effects of O_2Supp on subsequent endurance performance is yet to be documented conclusively, although some include improvements in $\dot{V}O_2$peak, $\dot{V}O_2$max, and oxygen kinetics [6,124].

2.7.2 Acute Supplementation during Exercise

O_2Supp has been shown to be effective during the recovery periods for maintaining/ increasing power output during sprint-based exercise, and it has also been shown to be beneficial for improving longer duration efforts. This could be due to endurance performance being largely limited by oxygen uptake.

The work by Bonetti *et al.,* [125] found that participants who received O_2 (FiO_2 0.6) during 'work' periods experienced positive effects on oxygen efficiency (lower oxygen demand for same work output) and $\dot{V}O_2$peak. Participants consumed less oxygen for any given workload during O_2Supp. Furthermore, work by Ulrich *et al.,* [6] examined the effects of a medium O_2Supp dosage (FiO_2 0.5) on endurance performance, and found that maximal workload (progressive exercise) and endurance time (constant load) increased

meaningfully during O_2Supp. Most importantly they found significant systemic increases in O_2 delivery (measured by NIRS), S_pO_2 and $\dot{V}O_2$. They also found significant increases in tissue oxygenation in both cerebral and local muscle tissue. Bartholomew *et al.,* [126] also found that the higher the concentration of O_2, given during a test, the greater the improvements in Wingate cycling performance compared to Normoxia. O_2Supp has also been shown to be beneficial in increasing TTE, by up to a 131% [81,112,114,127–130]. Similarly, Amann *et al.,* [81] found that PPO and MPO were higher during a 5 km time trial with increased oxygen content (FiO_2 1.00).

Opposing these findings, Church *et al.,* [131] Robbins *et al.,* [132] and Snell *et al.,* [133] all found that O_2Supp of FiO_2 0.55-1.00 is insufficient to effect performance or oxygen kinetics when used during performance or as a recovery tool. Given that O_2Supp increases the delivery of O_2 therefore, it is likely that the methodology (long submaximal durations) of these studies was not sufficient to elicit the responses others found during O_2Supp.

2.7.3 Acute Supplementation during Recovery Periods

O_2Supp has also been shown to be a useful tool when given during acute exercise as well as when given in the recovery periods between successive exercise bouts. Findings from research by both Holmberg *et al.,* [7] and Kay *et al.,* [126] has found that the delivery of FiO_2 0.6-1.00 during recovery of 4-6 min in duration following repetitions of 30 s – 1 min was sufficient to significantly improve both PPO in swimmers and cyclists alike. Maeda *et al.,* [54] also replicated these findings. Maeda *et al.,* found that during exercise at 70% of $\dot{V}O_2$max for 5 x 5 min with O_2Supp during 6 min recovery periods was enough to elicit significant reductions in BLa. They also found that recovery was significantly faster during the higher concentration (FiO_2 0.8) than the four lower concentrations.

O_2Supp as a useful recovery tool has also been demonstrated by Hamalainen *et al.,* [134] who found that O_2Supp attenuates the decrease in S_aO_2 during subsequent exercise. Similarly, Andersson *et al.,* [135] also found a similar increase in S_aO_2 following a hyperoxic response when used during the recovery periods between (2 min) a 6 x 3 min repeated sprint protocol. Neither studies were able to find any significant decrease in pH level or BLa because of O_2Supp.

The success of O_2 as a performance enhancing tool is equivocal. Sperlich *et al.,* [3] found that a duration of O_2Supp (FiO_2 1.00) for 6 min during recovery periods between 30 s sprints was not sufficient to elicit any change in PPO, MPO or even BLa, despite each athlete having a lower rating of perceived exertion. Additionally, Hauser *et al.,* [136] found that O_2Supp (FiO_2 1.00) during recovery from repeated sprint skiing was not a significant stimuli to increase MPO, even though there was a large positive difference in endurance performance variables. It is speculated that O_2Supp may in fact cause reductions in performance if the duration is to short and delivery is intermittent due to rapid changes in internal oxygen equilibrium.

Additional, details of these studies can be seen in Table 2-2 and Table 2-3. The tables also highlight many of the limitations of using O_2Supp during exercise or subsequent recovery periods. There is currently a lack of consistency in the FiO_2 used, with it varying from 0.4 to 1.00. A consistent use of one concentration should be proposed so a consistent narrative can be formed. Additionally, a consistent FiO_2 will enable direct comparisons between studies - something that is currently lacking in O_2Supp research. Another inconsistency regarding when to use O_2Supp - the most effective ‘time’ to administer O_2Supp needs to

be established before training modalities are manipulated effectively. Future research should therefore focus on answering these research questions: 1) When exactly is O_2Supp most effective during a training session? 2) what is the common consensus in O_2Supp research when using a consistent FiO_2 of 1.00?

2.7.4 Effects of Chronic Supplementation

Few studies have examined the long-term use of O_2Supp so little is known about the chronic physiological adaptations that O_2Supp may stimulate. Those that have tried to identify the long-term benefits of O_2Supp have reported mixed results (Table 2-4). Most of the O_2Supp research applies a HIIT protocol to a multi week training intervention. One commonly referenced study in HIIT is that of Burgomaster *et al.,* [70], who found that similar adaptations are established during HIIT as those experienced through endurance training (without additional aids). The major benefit of HIIT is it is significantly less training duration to elicit the same adaptations.

Perry *et al.,* [137], Morris *et al.,* [138], and Murray *et al.,* [8] found that 3-5 hyperoxic sessions per week for 3-6 weeks was enough to stimulate an improvement in time to exhaustion, PPO and power at lactate threshold and power at maximum lactate steady state during cycling (FiO_2 0.6, 0.26, 1.00 respectively). Morris *et al.,* [138] found that a combination of low O_2Supp and high O_2Supp in a 'Live High, Train Low' approach resulted in significant performance improvements for junior elite cyclists. Each participant lived at altitude and then completed training with high oxygen availability, effectively living high and training low. Every participant was able to train at higher intensities and significantly improve their cycling time trial performance to 120 KJ of work and their maximum steady state power by as much as 20 W. However, the stimulus from living at altitude may be the main driving point for adaptation rather than the O_2Supp training sessions.

However, there is also evidence to suggest long term use of O_2Supp in recovery or training has no effect on performance measures. Perry *et al.,* [109], Ploulz-Snyder *et al.,* [139] and Kilding *et al.,* [125] found no improvements in either $\dot{V}O_2$max, PPO, maximum heart rate, maximum lactate accumulation and no increase in muscle fibre recruitment or size in response to training with supplementary oxygen. The duration of these studies ranged from 4-6 weeks, with HIIT as the main training stimulus, and used O_2Supp concentrations between 0.6-0.7.

In a later study, Perry *et al.,* [137] did find that after a 6-week O_2Supp interval training plan, PPO improved by 8% whilst at 90% of maximum heart rate. However, peripheral factors such as metabolic overload were attributed to this improvement rather than the O_2Supp itself. Despite similar methodologies and oxygen content of the inhaled air (FiO_2 0.60) in their two studies, Perry *et al.,* [109] found these conflicting results. In their second study they found that enzyme activity in skeletal muscle was the same as the Normoxia condition, and that O_2Supp was not a significant extra stimulus above Normoxia at evoking pronounced skeletal and whole-body oxygen uptake (measured using $\dot{V}O_2$max and muscle biopsies). However, they did attribute the findings of their second study to an untrained population. They suggested that the increase in stimulus from the training alone led to large changes, but which were not different between conditions.

Recently, research is starting to assess the mechanisms underlying potential effects of O_2Supp on chronic adaptations. Cardinale *et al.,* [140] are at the forefront of this. They have recently assessed the oxidative capacity of the peripheral muscle following O_2Supp training, using a muscle biopsy and high resolution respirometry. They found that a 3-session a week, 6-week O_2Supp training programme was not sufficient at eliciting

additional stimulus for chronic adaptation, compared with training in Normoxia. Although, Cardinale *et al.,* found little difference between conditions in oxidative capacity, Ploulz *et al.*, found that O_2Supp did improve the efficiency of the working muscles using oxygen consumption (VO_2) and mitochondrial enzymes (creatine kinase, 3-hydroxyacyl CoA dehydrogenase and cytochrome c-oxidase).

The research into the chronic effects of O_2Supp is scarce with varied results; from O_2Supp providing an additional positive training stimulus, to it having no effect on training at all. The research therefore highlights the need to establish answers to pressing questions: 1) Does O_2Supp provide an additional positive stimulus during training interventions with chronic exposure? 2) What are the mechanisms underlying the potential effects of O_2Supp during chronic use? 3) What training modality (HIIT or endurance) is most effective at eliciting positive adaptations following chronic use of O_2Supp?

2.8 Summary

Many new potential 'ergogenic aids', are available to the consumer, such as Boost Oxygen®, Oxy Sport and OXY 99™. However, there is currently insufficient evidence to support the long-term use of these products. The research conducted on the effects of O_2Supp is still equivocal. Studies have attempted to identify the effect of O_2Supp on performance using a range of concentrations from 0.30 to 1.00 with varying degrees of success. Studies have also used O_2Supp during different parts of a training session (before exercise, inter repetition recovery and inter exercise recovery) making it difficult to establish any strong consensus regarding results.

As shown by Table 2-5 and Table 2-6 there is a variety of concentrations that have been administered during O_2Supp, all with varying degrees of results. Therefore, this research

will apply maximum dosage (FiO_2 of 1.00) to each of the experimental chapters to build a narrative within the literature. Future research can look at lowering this once a clear theme has been established. Additionally, the administration procedures need standardising to minimise inter study differences. The research conducted in this thesis will apply a standardised methodology to each of the experimental chapters, as mentioned in chapter 3 - Methodology Chapter.

The research on chronic applications of O_2Supp is sparse, and the few existing studies using a variety of modalities (eg. cycling, swimming, hockey [7,8,141]) and concentrations (0.60 to 1.00) similar to acute supplementation research. It is also evident that researchers are to fully understand the mechanisms underlying the effects of supplementary oxygen.

It is evident that a minimum dose of FiO_2 value has yet to be identified, as this may be one of the reasons there are large discrepancies between results of studies with broadly similar methodologies. Future research should use an FiO_2 of 1.00 and identify its effects during different tasks, before reducing it to identify a dose response relationship.

It is now prudent to provide research to fill the gaps within the literature to maximise the potential benefits of O_2Supp on sporting performance. If O_2Supp is going to be used as an impactful ergogenic aid many of these areas need to be explored and answered - when is O_2Supp most effective during a training session? and Can a short-term training intervention influence performance or metabolic markers of performance?

2.9 Aims and Hypotheses of the Thesis

This thesis set out to investigate three main research questions.

- During which component of a repeat sprint cycling training session is O_2Supp most effective at increasing performance?

- Which physiological mechanisms does O_2Supp effect to allow for improvements in performance capacity?
- Can a short-term training intervention influence performance or metabolic markers of performance?

As there is yet to be a specific oxygen supplementation dosage recommended, this research will use an FiO_2 of 1.00 and maintain it throughout the experimental chapters, minimising the effect of dosage. There are a series of 5 experimental studies that aimed to add empirical information to the O_2Supp research field, specifically those areas identified in this section (Table 2-1).

Table 2-1 Identified gaps in the literature leading to experimental chapters' aims.

Chapter	Gap in the literature	Chapter Aims
1	- Lack of research within the area of short high intensity repeat sprint cycling during O_2Supp.	- To explore the effects of O_2Supp, both during recovery and during cycling, on sprint cycle performance. - To confirm the methodologies used throughout the latter thesis chapters.
2	- Lack of consistent understanding of the importance of timing of supplementation,	- To determine whether O_2Supp is more effective during or between repeat sprint intervals.
3	- Lack of research identifying the mechanisms underlying potential effects on performance.	- To develop the understanding of the effects of O_2Supp on repeat sprint cycling fatigue, using twitch interpolation technique.
4	- Lack of research identifying the mechanisms underlying potential effects on performance	- To further develop the understanding the effects of O_2Supp on repeat sprint cycling during using near infrared spectroscopy.
5	- Limited number of training programmes using O_2Supp within SIT. - Additional mechanisms underlying chronic O_2Supp adaptation.	- To explore the feasibility of using NIRS, arterial occlusions and repeated cycling sprints during a multi session training study with periods of supervised and unsupervised training. - To identify whether the proposed methodologies (NIRS) are sensitive enough to detect changes in muscle oxidative capacity using arterial occlusions.

Supplementary Tables

Table 2-2 Acute effects of Oxygen Supplementation.

Study	Participant Characteristics		Measure	Protocol	Result
	Sample size, Gender, Age	**Populations**			
Kyro *et al.,* (1999)	*n* = 6, Male (1), Female (5), 25 ± 4 years	Endurance trained runners	Arterial O_2 Sats, RPE, BLa	3 visits. 4 x 4 min treadmill running at 50,60,70,80% of max. 3 different conditions 0.15, 0.21, 0.29.	BLa is lowest during O_2Supp. O_2 sats are independent of FiO_2. O_2 sats decreased in hyperoxia.
Bonetti *et al.,* (2012)	*n* = 16, Male, 24.8 ± 9.1years	Well trained competitive individuals.	MPO, PPO, HR, OXH sat	2 groups, O_2Supp (0.60) and NORM (0.21). Baseline + fam. 12 x 2min with 2min recovery then 5 x 5min with 3min recovery.	MPO sig better in O_2Supp. S_PO_2 was higher in O_2Supp. Small change in BLa and $\dot{V}O_2$peak.
Croft *et al.,* (2013)	*n* = 10, Male, 22 ± 2 years	Endurance trained athletes	OXH sat, BLa, HR, PPO, MPO	3 visits. Baseline + fam. 10 x 3min at 85% Vmax with 90 s recovery. O_2Supp 1.00 or NORM 0.21 during recovery and 15mins post.	No Sig results. O_2Supp only helped improve OXH sats.
Andersson *et al.,* (2011)	*n* = 7, Male (5), Female (2), N/A	National level Kayak athletes	BLa, PPO, MPO, OXH sat, RPE	2 visits. 6 x 3min with 2min recovery. O_2Supp 0.99 or NORM 0.21 during recovery.	Only sig result – OXH sat improved. No sign result in PPO or BLa or RPE.

Legend: *FiO_2*- Fraction of inspired oxygen, min- Minutes, *n* – Sample size, *BLa* – Blood lactate, AT- Anaerobic threshold, PPO- Peak power, MPO- mean power, BV- Blood volume, RPE- Ratings of perceived exertion, Sats – Saturation, Haem – Hemoglobin, NMF- neuromuscular fatigue, RPM- Revolutions per minute, HR- Heart rate, OXH – Oxyhaemoglobin, HYPO- Hypoxia, O_2Supp – Oxygen supplementation, NORM- Normoxia, TT- Time Trial, TTE- time to exhaustion, Q- Cardiac Output, VMax- velocity at $\dot{V}O_2$max.

Table 2-3 Continued. Acute Effects of Oxygen Supplementation

Study	Participant Characteristics		Measure	Protocol	Result
	Sample size, Gender, Age	**Populations**			
Holmberg *et al.*, (2011)	*n* = 12, Male, 21 ± 3 years	Elite level swimmers	PPO, MPO, fatigue index, LT, NMF, RPE	3 visits. 1 Max measure + Fam. Visit 2-3 5 x 40 butterfly strokes with 6min recovery at 1.00 or 0.21.	PPO and MPO sig diff in 1.00. Fatigue index No change. Enhanced $^{p}O_2$ and haem sats with no change in BLa or PH.
Maeda *et al.*, (1997)	*n* = 14, Male, 21 ± 0.8 years	Healthy Individuals	BLa, AT	$\dot{V}O_2$max + AT prior to experiment. 5 x 5min at 70% $\dot{V}O_2$max at 60rpm with 6min recovery at 0.21, 0.30, 0.40, 0.60, 0.80 O_2 during recovery.	BLa sig lower during O_2Supp. O_2Supp improved recovery at high AT.
Amman *et al.*, (2006)	*n* = 8, Male, 22.5 ± 1.7 years	Healthy trained cyclists.	MPO, EMG, BLa, PPO	Baseline + fam. 4 sessions, 5km cycling TT at 0.21, 0.15, 0.24-0.30, 1.00 O_2.	Performance increased per O_2 content. PPO+ MPO increased with O_2 increases. MPO and time increased by over 20% in 1.00.
Peltonen *et al.*, (2001)	*n* = 6, Male, 24 ± 5 years	Endurance trained cyclists	TTE, FiO_2, Q, PPO, MPO, BLa	3 visits, Incremental test 100 W every 5min until exhaustion after 400 W up in 50 W increments at either HYPO 0.15, NORM ~0.21 and O_2Supp 0.31.	FiO_2 had a sig effect on PPO was 12.8% smaller in HYPO and 5.5% greater in O_2Supp then NORM. $\dot{V}O_2$max 13.6% greater in O_2Supp than NORM.

Legend: FiO_2- Fraction of inspired oxygen, min- Minutes, *n* – Sample size, BLa – Blood lactate, AT- Anaerobic threshold, PP- Peak power, MP- mean power, BV- Blood volume, RPE- Ratings of perceived exertion, Sats – Saturation, Haem – Haemoglobin, NMF- neuromuscular fatigue, rpm- Revolutions per minute, HR- Heart rate, OXH – Oxyhaemoglobin, HYPO- Hypoxia, O_2Supp – Oxygen supplementation, NORM- Normoxia, TT- Time Trial, TTE- time to exhaustion, Q- Cardiac Output, V_{max}- velocity at $\dot{V}O_2$max.

Table 2-4. Chronic Effects of Oxygen Supplementation.

Study	Participant Characteristics		Measure	Protocol	Result
	Sample size, Gender, Age	Populations			
Cardinale *et al.*, (2019)	*n* = 32, Male (24), female (7) 34 ± 7 years	Ultra-endurance cyclists	$\dot{V}O_2$max, MITO, BV,	6-week training intervention. 15 sessions supervised and 10 non supervised at 0.21 or 0.30.	Small increase in O_2Supp cycling performance. no change in $\dot{V}O_2$max, MITO, BV and Haem.
Murray *et al.*, (2016)	*n* = 15, Female, 28 ± 4 years	International hockey players	MAS, distance run, RPE, BLa HR.	6-week training program. 3 groups O_2Supp 1.00, NORM pressurized 0.21, Control 0.21.	MAS increased in O_2Supp, no change in RPE, BLa, or distance run. Lower HR in O_2Supp.
Perry *et al.*, (2005)	*n* = 11, Male (8), Female (3), 26 years (no SD given)	Active untrained participants	$\dot{V}O_2$max, MPO, Vmax.	6-weeks in each condition (0.60 or 0.21) with 12-week detraining in middle.	No change in Vmax or $\dot{V}O_2$max. 8% increase in MPO with O_2Supp.
Morris *et al.*, (2000)	*n* = 15, Male, 17 ± 1 years	Highly training cyclists	PPO, $\dot{V}O_2$max, BLa	3-week training. 3 sessions supervised and 3 non supervised Norm and O_2Supp equivalent to 0.26.	No Sig changes in PPO, positive changes in BLa and power at Vmax.
Ploutz-Snyder *et al.*, (1996)	*n* = 19, Male, 23 ± 2 years	Untrained cyclists	HR, BLa, muscle biopsies, Q.	5-week training intervention. 5 days a week (0.21 or 0.70)	Q, BLa and VO_2 significantly decreased for same workload in O_2Supp. Both groups showed significant increase in type II muscle fibers.
Burgomaster *et al.*, (2005)	*n* = 16, Male (14) female (2), 25 ± 2 years	Healthy untrained cyclists	$\dot{V}O_2$max, muscle biopsies, PPO, endurance capacity	6 Sprint interval sessions over two weeks	Endurance capacity doubled with no change in $\dot{V}O_2$max, or PPO during O_2Supp. Increase muscle oxidative capacity (muscle biopsy) with O_2Supp.

Legend: *FiO_2*- Fraction of inspired oxygen, min- Minutes, *n* – Sample size, *BLa* – Blood lactate, AT- Anaerobic threshold, PPO- Peak power, MPO- mean power, BV- Blood volume, RPE- Ratings of perceived exertion, Sats – Saturation, Haem – Haemoglobin, NMF- neuromuscular fatigue, RPM- Revolutions per minute, HR- Heart rate, OXH – Oxyhaemoglobin, HYPO- Hypoxia, O_2Supp – Oxygen Supplementation, NORM- Normoxia, TT- Time Trial, TTE- time to exhaustion, Q- Cardiac Output, VMax- velocity at $\dot{V}O_2$max, MITO – Mitochondria oxidative capacity, MAS- Maximal aerobic speed, SD- Standard deviation.

Table 2-5. Methodological Variations Within Oxygen Supplementation Studies.

Study	Participant Characteristics		Oxygen Percentage and Mixture	Administration Technique	Result
	Sample size, Gender, Age	Populations			
Kyro *et al.,* (1999)	*n* = 6, Male (1), Female (5), 25 ± 4 years	Endurance trained runners	FiO_2 0.15, 0.21 & 0.29 Medical grade air	Gas cylinder to 100 L Meteorological balloons. Maintained at ambient pressure and humidified before use.	BLa is lowest during O_2Supp. Little change in performance.
Bonetti *et al.,* (2012)	*n* = 16, Male, 24.8 ± 9.1years	Well trained competitive individuals.	FiO_2 0.21 & 0.60 Medical grade air mixed with nitrogen	Gas cylinder to 250 L Douglas bags. Humidified before use.	MPO sig better in O_2Supp. S_PO_2 was higher in O_2Supp. Small change in BLa and $\dot{V}O_2$peak.
Croft *et al.,* (2013)	*n* = 10, Male, 22 ± 2 years	Endurance trained athletes	FiO_2 0.21 & 1.00 Medical grade oxygen	Connected directly to medical oxygen cylinder, flow rate of 10 L per minute.	No Sig results. O_2Supp only helped improve OXH sats.
Andersson *et al.,* (2011)	*n* = 7, Male (5), Female (2), N/A	National level Kayak athletes	FiO_2 0.21 & 0.99 Medical grade oxygen	No data on procedure.	No sign result in PPO or BLa or RPE.
Holmberg *et al.,* (2011)	*n* = 12, Male, 21 ± 3 years	Elite level swimmers	FiO_2 0.21 & 1.00 Medical grade oxygen	Gas cylinder to 250 L Douglas bags.	PPO and MPO sig diff in 1.00. Enhanced $^PO_2$ and haem sats
Maeda *et al.,* (1997)	*n* = 14, Male, 21 ± 0.8 years	Healthy Individuals	FiO_2 0.21 0.30, 0.40, 0.60 & 0.80 Micro blended gases	Blended gasses (Bird 3800, microbrender), into 250 L Douglas bags. Humidified to 50% before use.	BLa sig lower during O_2Supp. O_2Supp improved recovery at high AT.

Legend: *FiO_2*- Fraction of inspired oxygen, *n* – Sample size, L- Litre, Peak power, MPO- mean power, RPE- Ratings of perceived exertion, Sats – Saturation, OXH – Oxyhaemoglobin, HYPO- Hypoxia, O_2Supp – Oxygen Supplementation, *BLa* – Blood lactate, AT- Anaerobic threshold.

Table 2-6. Continued Methodological Variations Within Oxygen Supplementation Studies.

Study	Participant Characteristics		Oxygen Percentage and Mixture	Administration Technique	Result
	Sample size, Gender, Age	Populations			
Amman *et al.,* (2006)	*n* = 8, Male, 22.5 ± 1.7 years	Healthy trained cyclists.	FiO_2 0.15, 0.21, 0.24-0.30 & 1.00 Medical grade air	No data on procedure.	PPO+ MPO increased with O_2 increases. MPO and time increased by over 20% in 1.00.
Peltonen *et al.,* (2001)	*n* = 6, Male, 24 ± 5 years	Endurance trained cyclists	FiO_2 0.21 & 0.31 Medical grade air.	Gas cylinder to 250 L Douglas bags. Humidified before use. And a taro oxygen concentrator to a 200 L Douglas bag.	FiO_2 had a sig effect on PPO 5.5% greater in O_2Supp. $\dot{V}O_2$max 13.6% greater in O_2Supp.
Cardinale *et al.,* (2019)	*n* = 32, Male (24), female (7) 34 ± 7 years	Ultra-endurance cyclists	FiO_2 0.21 & 0.30 Medical grade oxygen	Dosing unit used to send gas bolus into a face mask per breath using a gas cylinder.	Small increase in O_2Supp cycling performance. no change in $\dot{V}O_2$max, MITO, BV and Haem.
Murray *et al.,* (2016)	*n* = 15, Female, 28 ± 4 years	International hockey players	FiO_2 0.21, pressurized 0.21 & 1.00 Medical grade oxygen	Breathing directly from cylinder at 8 L per minute.	MAS increased in O_2Supp, no change in RPE, BLa, or distance run. Lower HR in O_2Supp.
Perry *et al.,* (2005)	*n* = 11, Male (8), Female (3), 26 years (no SD given)	Active untrained participants	FiO_2 0.21 & 0.60 Medical grade oxygen	Gas cylinder to 300 L reservoir bags. Humidified before use.	No change in Vmax or $\dot{V}O_2$max. 8% increase in MPO with O_2Supp.
Morris *et al.,* (2000)	*n* = 15, Male, 17 ± 1 years	Highly training cyclists	FiO_2 0.21 & 0.26 Medical grade oxygen	Gas cylinder to 120 L reservoir bags. Humidified before use.	No Sig changes in PPO, positive changes in BLa and power at Vmax.
Ploutz-Snyder *et al.,* (1996)	*n* = 19, Male, 23 ± 2 years	Untrained cyclists	FiO_2 0.21 & 0.70 Medical grade oxygen	Gas cylinder to a mixing chamber, then hydrated and humidified, then connected to 5000 L meteorological balloon.	Q, BLa and VO_2 significantly decreased for same workload in O_2Supp.

Legend: *FiO_2*- Fraction of inspired oxygen, *n* – Sample size, L- Litre, Peak power, MPO- mean power, RPE- Ratings of perceived exertion, Sats – Saturation, OXH – Oxyhaemoglobin, HYPO- Hypoxia, O_2Supp – Oxygen Supplementation, *BLa* – Blood lactate, AT- Anaerobic threshold.

3 - Methodology Chapter

The Main Methodologies Used Throughout the Experimental Chapters

3.1 Introduction

The research in this thesis was conducted in accordance with the Declaration of Helsinki. In all cases, ethical consideration, and comprehensive risk assessment documents were provided to and approved by the University of Essex Ethics Committee, prior to conducting experimental analysis. All participants provided written informed consent detailing that they were informed and happy to partake in every aspect of each study.

The experimental data collected throughout the experimental chapters (chapters 4-9) is numerical quantitative data in nature. Physiological data collected were used to represent primary physical outcomes (Power (W), NIRS and fatigue).

A large amount of experimental measures were replicated across each of the experimental chapters. The reoccurrence of these measures are outlined within this chapter. Other experimental measures are introduced within the experimental chapters during which they are used.

3.2 Reoccurring Methods

Several different methodologies were used to identify the effectiveness of O_2Supp within a sporting population. Various combinations of methodologies (NIRS, Rate of Perceived Exertion (RPE), and Iso kinetic dynamometer with twitch interpolation) were selected throughout the experimental chapters in order to give a comprehensive overview of O_2Supp on repeat sprint cycling.

Table 3-1 shows the measures included during each experimental chapter. The reoccurring measures (measures that appear in two or more experimental chapters) are discussed in more detail in the paragraphs that follow.

Table 3-1 Measures conducted in each experimental chapter

Measure	Experimental Chapter				
	1	2	3	4	5
Sprint Cycling	■	■	■	■	■
Blood Lactate	■	■	■	■	■
Rating of Perceived	■	■	■	■	■
Levels of Exertion	■	■	■	■	■
Near Infrared			■		■
Spectroscopy			■		■

3.2.1 Sprint Cycling

During the experimental chapters 1 through 5 the physical work was conducted on a Wattbike Pro model – B cycle ergometer (Wattbike, Nottingham, UK). Wattbike are cycle ergometers with varying degrees of resistance and functionality (exercise modes). Wattbike pro has various mechanisms for applying resistance to the cyclist. There are two main ways; air resistance flywheel and a magnetic resisted flywheel. The air resistance flywheel works by regulating the flow of air entering the flywheel by increase the size of the hole in which air flows into the flywheel (bigger the hole the more air flows through it). The air resistance flywheel is there to replicate the ‘gears’ on a road bike whilst cycling on flat road. Setting 1 (low) simulates the easiest gear, increasing until 10 (high) the hardest gear on the bike. The magnetic resisted flywheel on the other hand, is designed to replicate the ‘gradient’ of the road. Similar, to the air resistance, 1 (low) simulates a flat road and 7 (high) a steep gradient.

Wattbike Pro's strain gauge measures the force applied to the chain through each pedal revolution at 100 Hz. Due to the functionality of the Wattbike, over 40 variables (including peak power, mean power, cadence, work, and speed) can be collected at 100 Hz per pedal revolution. The accuracy of the Wattbike is a reliable power measure for trained athletes and has been shown to be accurate to within 2% across the full range of Watts (0-3760 W) with a high reproducibility (CV = 2.6%, 95% CI = 1.18-5.1%) [142,143]. During the experimental chapters, variables such as, mean power output (W), peak power output (W), relative mean power output and relative peak power output were used for statistical analysis. The conversion of peak and mean power output to relative power output was conducted to consider each participants body mass. The power output of the participant (peak and mean) was divided by their body mass to give a power output relative to their body mass. This allowed for classification of performance status (power to weight) and the ability to directly compare each participant with one another.

Mean power output was used to determine the extent of performance decline throughout the 10 repeated sprints. Performance decline was calculated taking the first sprint (highest) and then their sprint with the lowest power output and calculating the percentage decline. Percentage decline = [(highest– lowest) / highest] *100. The higher the number the greater the decline in performance.

During each experiment participants were required to complete a pre-set protocol programmed into the Wattbike display unit. Programme consists of three distinct sections: warm up, recovery, and sprints. Warm up consists of 5 min at an air brake of 4 and a cadence of 70-80 rpm. The recovery section was a 5 min static recovery seated on the bike. The sprint protocol consisted of 10 x 15 s sprints with 45 s of static recovery.

3.2.2 Oxygen Administration

The main independent variable of the thesis was the manipulation of the content of air the participants were required to inspire. Each study used the same underlying methodology and equipment during various time frames. Hyperoxic gas was used to fill up a rig of 4 x 200 L Douglas bags (Hans Rudolph, Shawnee, KS, USA) (see Figure 3-2). Each rig consisted of 4 x 200 L Douglas bags connected via four 34" long breathing tubes EVA with a diameter of 35 mm.

Oxygen supplementation was created using medical grade oxygen (British Oxygen Company, Surrey, UK) and stored in a size G cylinder prior to administration. The gas was administered to the participants via a face mask (Hands Rudolph 7400 Series Silicone Vmask™ Oro-Nasal Mask) which was attached to a T-shaped two-way non-rebreathing valve, via a 72" long breathing tubes EVA with a diameter of 35 mm. The 72" breathing tube had a dead space of 1.76 L. Therefore, it would take approximately 3 breaths to clear the air in the tubes. Administration of either mixtures was started and stopped 5 s prior to the start and end of the specific administration periods (sprint, inter sprint recovery), to ensure timings of the gas administration were accurate throughout. During the Normoxia sessions participants were administered normal atmospheric air, via the same rig set up in order to minimise placebo effect. Douglas bags were filled with atmospheric air was via an electric air pump (Bosch GmbH, Stuttgart, Germany). During pre-experimental pilot testing the medical oxygen was pumped into four Douglas bags, and left for 45 minutes to test the seals of the bags and three-way valves. The bag was monitored and tested for oxygen percentage every 5 minutes for 45 minutes. After 45 minutes the content of the bag was still 99.8% oxygen with less than 0.3 L of gas escaping in the 45minutes. This established an appropriate storage and administration strategy for medical grade oxygen.

Figure 3-2. Douglas bag rig set up

Figure 3-1. Hans Rudolph Mask and Two-Way Non-Rebreathing Valve

3.2.3 Blood Lactate

During exercise by-products are excreted due to the metabolic function of biological tissues. Lactate within the blood is a by-product that is widely measured within sport due to its relative ease to measure. Lactate is found within the blood and can be used as a measure of metabolic function. Capillary samples are typically taken from the ear lobule or the index finger. BLa has been shown to be consistently higher in the finger than the ear lobule. During this thesis the right ear lobule was used for EVERY sample.

During each specimen a 20 µl capillary BLa sample was taken from the right ear lobe. Each sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup (1:50 ratio). The haemolysing solutions prevents haemolysis (rupturing of red blood cells) during the sampling process. Once collected each specimen was analysed using a Biosen C- Line Clinic lactate analyser (EKF diagnostics, Cardiff, UK). The Biosen lactate analyser measures each sample by a process called enzymatic-amperometry using chip sensor technology. Biosen C- Line Clinic has been shown to accurate and reliable to a CV < 1.5%. Following the sampling all specimens were analysed and destroyed within 24 h of withdrawal from the participant.

3.2.4 Rate of Perceived Exertion

Rating of perceived exertion is the use of an ordinal scale where individuals can describe the physiological effect at which they perceive they are working at, at any given moment. The most widely recognised way of reporting this is through the use of the 'Rate of Perceived Exertion' (RPE) scale [144]. The scale consists of a fifteen-point vertical list of numbers which are supplemented by a descriptor every other point, from '6- No Exertion' to '20 – Maximal Exertion'. RPE has been shown to be strongly correlated with both heart rate and BLa, regardless of gender, age, testing modality and ability [145,146].

In order to investigate the relationship RPE has with exercise during O_2Supp it was included in chapters 1-5. RPE was included as a secondary focus of the research.

3.2.5 Near Infrared Spectroscopy

NIRS is a non-invasive method of measuring the presence of oxygen within biological tissue, especially muscle. NIRS technology works on the premise of transmitting infrared light into biological tissue and calculating how much is absorbed. NIRS

technology is based on two main principles; firstly, that biological tissue has been shown absorb light in the near infrared region (700-900 nm) and secondly that biological tissues are oxygen dependent on absorbing infrared light.

NIRS assesses the changes in oxygen concentration (oxygenated [O_2Hb]/ deoxygenated [HHb]) haemoglobin (Hb), myoglobin (Mb) and cytochromes. The changes in concentration of oxygen (thus light absorption) vary over time, which provide evidence on the status of tissue oxygenation. The current understanding is that NIRS signals cannot distinguish between the oxygenation status of Hb and Mb individually. Therefore, the NIRS output should be inclusive of Hb and Mb together (O_2Hb + O_2Mb) whenever a oxygenation value is mentioned, thus throughout this thesis for ease it will be referred to as only O_2Hb. The same occurs for the deoxygenation values as they are also inclusive of HHb + HMb and will therefore be referred to as HHb alone.

NIRS works by emitting an infrared light into a biological tissue, which penetrates the tissue, the light is absorbed in different quantities and the amount that is refracted back and detected by a light detector once leaving the tissue. The Beer Lambert Law is then used to identify the change in the light absorbed and measure changes in O_2Hb and HHb. The Beer-Lambert Law describes the absorption of light in a non-scattering medium. Human tissue on the other hand is a scattered medium, therefore a modified Beer Lambert law is used. The modified law takes into account the amount of light lost due to scattering and includes a differential pathlengths factor (DPF). DPF is different for different muscle groups. The DPF for the quadriceps is 4 as used by Ruiter *et al.,* [147]. Therefore, during this thesis a DPF of 4 was used throughout.

Spatially resolved spectroscopy (SRS) utilises several light sources at varying distances from the light detector. Light intensity from infrared is detected leaving the tissue and is measured at different distances from the source then it is possible to derive the absorption

and scattering coefficients of the tissue. Using this method ratios of oxygenated haemoglobin can be derived and scaled to identify absolute haemoglobin concentration [148].

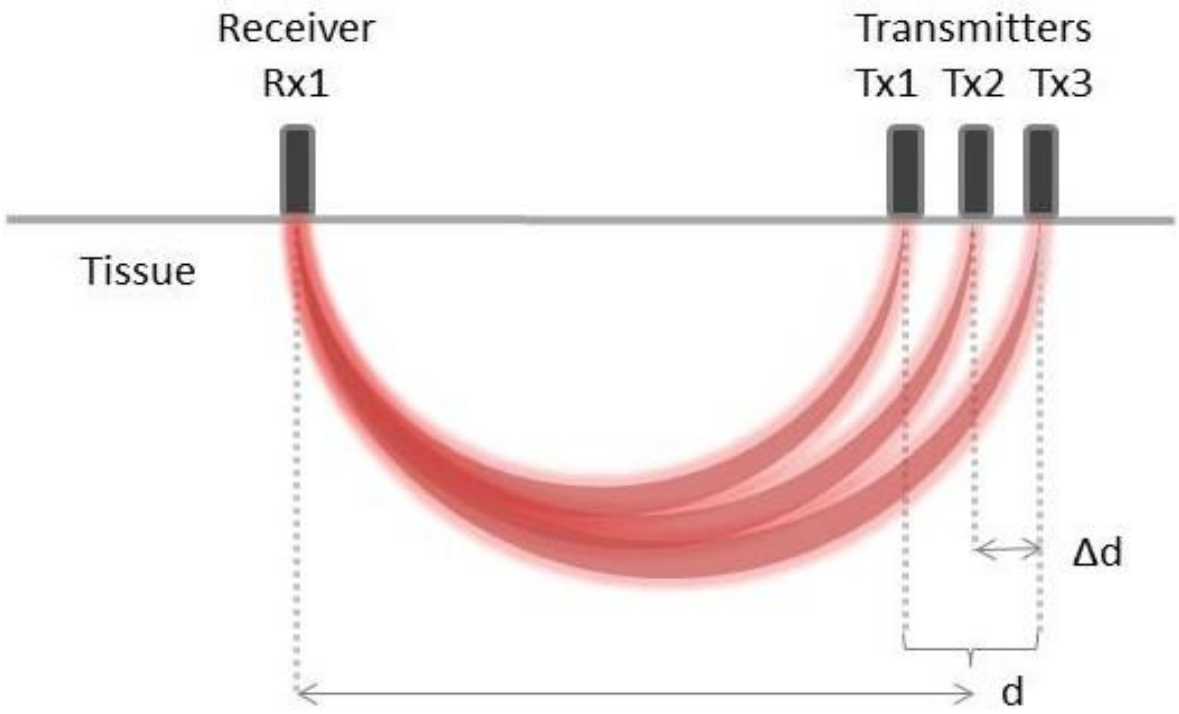

Figure 3-3. Diagram of spatially resolved spectroscopy (SRS) [149,150]

Participants wore the portable NIRS device on the belly of the right vastus lateralis (PortaMon, Artinis Medical Systems B.V., Elst, The Netherland). Any bodily hair was removed within the device placement area and cleaned with an alcohol wipe to remove any moisturiser or shaving cream residue.

The device was placed 3 cm anterior to the midpoint between the top of the greater trochanter and the lateral epicondyle of the right knee [151]. The device was subsequently taped with an adhesive wrapping and secondly wrapped with a blacked-out sports strapping to eliminate the chance of ambient light being detected.

The device was attached to the leg by the same researcher on every occasion, ensuring an external pressure of less than 20 mmHg on the device [152]. The external pressure was achieved during pilot testing using a MicroLab, PicoPress bladder placed beneath the NIRS device. Indelible ink was used to draw around the device to guarantee accurate NIRS placement during the subsequent visits. Throughout the experimental protocol the NIRS device was connected to a personal computer via the Bluetooth™ system for data acquisition (10 Hz), and conversion from analogue to digital data.

Throughout this thesis NIRS data was processed in line with the methodology suggested in Rodriguez *et al.,* [153] research. A one second moving average was applied to the data to attenuate the "noise" in the signal, whilst maintaining the integrity of the original data. This has been shown to be one of the most effective ways of smoothing the data ready for statistical analysis.

NIRS signals O_2Hb + O_2Mb, make up the oxygenation trace that is used to examine the rate of reoxygenation of the muscle following a desaturation period. Reoxygenation rate is the speed at which the body can recovery peripheral muscle oxygen levels. Additionally, HHb + HMb make up the deoxygenation portion of the NIRS trace, used to examine the extent of deoxygenation during periods of work.

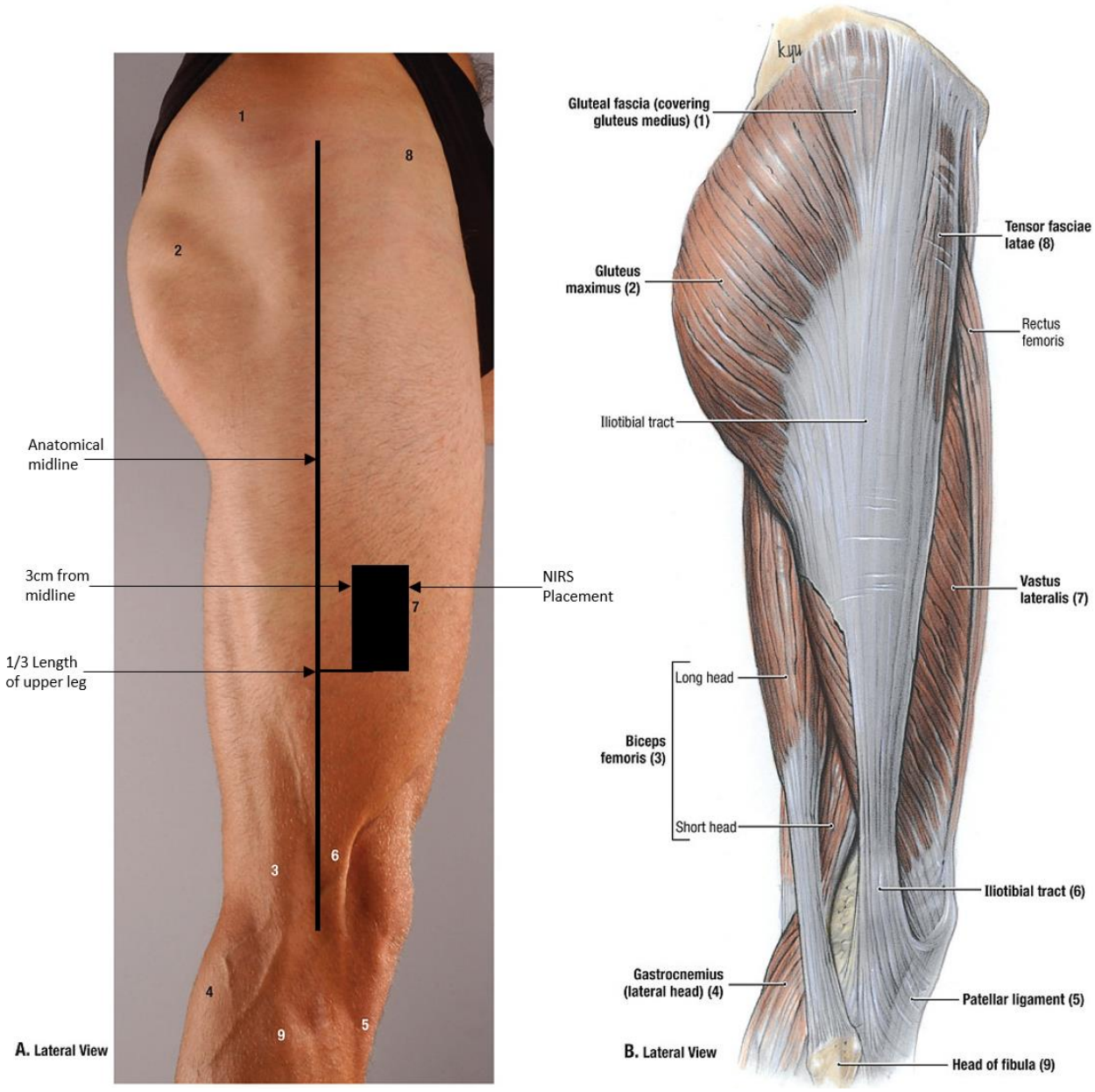

Figure 3-4. Diagram of Near Infrared Device Placement

3.3 Statistical Analyses

IBM SPSS version 21-25 software was used for all statistical analyses (IBM Corp. Released 2013. IBM SPSS Statistics for Windows). Experimental chapters use the newest version of the IBM SPSS available at the time. The algorithms used to run the statistical tests do vary slightly from version 23 to 25, although themes of data are the same. Small

variations in the physical number for significance may be different across each experimental study and is taking into consideration during thesis discussion [154–157].

Sample size was calculated for all experimental chapters and was established that samples sizes between 8 -12 were sufficient to determine a difference in the primary outcome measure (mean power output). A priori power analysis revealed that 8 participants would provide significant power to detect differences at an α-level of 0.05 for experimental chapter one (G∗POWER 3.1 Software, Düsseldorf, Germany). Means and standard deviations from the literature were used alongside meaningful differences (change in 30 W) to determine sample size for experimental chapter one. The subsequent chapters used the group means and standard deviations of chapter one due to the large effect size and power. The data was then deemed acceptable for further power analyses for subsequent experimental chapters.

Repeated measures ANOVAs were conducted in each experimental chapter. Where repeated measures ANOVA was used, sphericity was assessed using the Mauchley's test of sphericity. During One-Way ANOVA's and t-tests, heterogeneity of the data was assessed using Levene's test for equality of variance. ANOVAs were primarily used to answer each of the hypotheses during the experimental chapters

Pearson's correlations were conducted to test the relationships between two continuous variables. For example, - Experimental Chapter: Pearson's correlations were used between tissue oxygenation and power output between the two experimental conditions. A correlation coefficient between 0.1-0.3 was classified as a small correlation, 0.3-0.5 was classified as a medium correlation and 0.5-1.0 was classified as a large correlation. This is the same for both positive and negative coefficients. Correlations define the fit of the data, identifies the variation in the data and the relationships in the data (linear or nonlinear).

Paired samples t-tests were conducted during each experimental chapter for post hoc analysis of both primary and secondary measures. t-tests were used to identify mean differences in conditions for each variable (mean power, peak power, BLa) during sprints at an individual level.

3.3.1 Imputation of Missing Values

Data was imputed using 'Expectation Maximisation Imputation' following a missing value procedure to assess whether data was missing at random or not. Expectation maximisation is an iterative method to compute maximum likelihood estimates from incomplete data series [158]. Little's MCAR test was initially conducted on each data set to assess whether data was missing at random or whether there were patterns to the missing data (i.e. all the start or end values missing). The null hypothesis for Little's MCAR test is that the data is missing completely at random. If the results from the Little's MCAR are non-significant, estimation maximisation imputation can be run to complete the incomplete data series [159].

An α-level of 0.05 was set to signify statically significance across all experimental chapters. As well as the use of *p* values, Cohens *d* effect size and confidence interval statistics were used when appropriate to identify the magnitude of effects. Additional statistical analysis that is specific to that chapter alone is presented in the relevant section.

4 - Experimental Chapter:

When Is Oxygen Supplementation Most Effective for Sprint Cycling Performance?

A version of this chapter has been submitted for review as a research article. – European Journal of Sports Science.

4.1 Abstract

4.1.1 Objectives

Concurrent supplementation with oxygen enhances performance but this type of delivery is not feasible in performance situations due to the size of equipment and expertise needed. This study aimed to determine exactly when hyperoxic gas elicits the greatest effect on cycling performance. We aimed to determine whether O_2Supp given after a fatiguing task but prior to repeated sprints had a similar effect to when O_2Supp was given during the sprints alone.

4.1.2 Design and Methods

Eight trained male amateur level cyclists underwent four visits to the laboratory. Each session comprised a pre-fatiguing task (15 km cycling time-trial), 15 min recovery (100% oxygen (H) or Normal (N) air), followed by 10 x 15 s repeated sprints (during which time H or N was given). The 4 conditions were NN, HN, HH & NH. Repeated measures ANOVA's ($p < 0.05$) were used to examine difference between conditions in power output (peak and mean W).

4.1.3 Results

Peak and mean power output were significantly increased during full hyperoxic (HH, 4% and 3% respectively) compared to normoxic (NN) condition ($p < 0.05$).

4.1.4 Conclusion

O_2Supp (FiO_2 1.00) during exercise and recovery appears to elicit the greatest improvements in peak and mean power, without increasing lactate accumulation. This

shows that use of supplementary oxygen is beneficial during a maximal activity, and between set exercise recovery periods.

Keywords: Supplementary oxygen, maximal effort, sprinting, power

4.2 Background

Following the literature review (Chapter 1) several areas of research were apparent, one being the need to narrow down the specific timing of administration within O_2Supp research. Additionally, tables 2-5, and 2-6 highlight the need to standardise both the FiO_2 as well as the procedure used to administer O_2Supp within the research. Experimental chapter one has been designed to begin to answer when the most effective time to administer O_2Supp is and to standardise the methodologies used to achieve this.

4.3 Introduction

Supplementary oxygen was removed from the 'World Anti-Doping Agency' (WADA) [160] banned lists (2010), and has since gained significant attention from sport scientists [1,122] as both a performance and recovery enhancing tool.

O_2Supp has been administered at many different performance time points; before exercise, during and between exercise bouts [1], yet the exact timing of supplementary oxygen in relation to the greatest acute or chronic performance enhancement is not yet clear. At rest arterial haemoglobin is almost 100% saturated with oxygen, and the consumption of O_2Supp has little to no effect on subsequent performance [121]. As a result, the temporal focus of O_2Supp has moved away from before exercise, to during the performance and during recovery bouts between intervals.

Ergogenic aids are any aid that leads to a direct increase in performance capacity, some aids have been shown to increase this ceiling or reduce the BLa concentration for any given workload [117]. The performance improvements with O_2Supp are shown as increased physical work and changes in metabolism too. O_2Supp effects the accumulation of BLa by increasing the efficiency of the aerobic metabolic processes [113]. Increasing the efficiency of these pathways results in the sparing of fuel and attenuates accumulation of by-products.

BLa accumulation and increase of hydrogen ions (H^+) leads to the desensitisation of the cross bridges which subsequently causes peripheral fatigue and the termination of exercise.

Under the WADA guidelines administration of O_2Supp during competition is illegal, and not surprisingly several researchers have shown this is where the greatest performance benefits can be achieved [1,161]. Amman *et al.,* [81] found that the consumption of varied levels of O_2Supp (FiO_2 0.24-1.00) during 5 km cycling time trials resulted in significant increases in both peak and mean power (W), and faster times by up to 20%. O_2Supp has also been shown to improve dynamic knee extensor exercise during varied FiO_2 (0.21 or 1.0). In this case, O_2Supp induced a 10 W increase in power output during the final minute of dynamic leg extensions [111].

Several studies provide supplementary oxygen during the recovery periods between intense exercise bouts and this has been shown to be effective at both improving and maintaining performance during both long (> 3 min) and short (< 45 s) duration recoveries [3,7,126]. Research by both Sperlich *et al.,* [7] and Kay *et al.,* [126] found that the delivery of FiO_2 of 0.6-1.00 during 4-6 min recovery following repetitions of 30-60 s was sufficient to significantly improve peak power (4-6%) output in swimmers and cyclists alike. Similarly, pilot data conducted in our lab found that when O_2Supp was administered to cyclists during the recovery period between sprints (10 x 15 s sprints, 45 s recovery), they were able to elicit significant improvements in mean power output (4%).

However not all researchers have found O_2Supp during recovery intervals to be beneficial on subsequent performance. Sperlich *et al.,*[3] found that O_2Supp (FiO_2 1.00) given during 6 min recovery periods between 30 s sprints was not sufficient to evoke a change in peak

power, mean power or even BLa, despite athletes reporting a reduced rating of perceived exertion.

The available data directly comparing the influence of O_2Supp are sparse, both due to the lack of studies, the large variation in the FiO_2 given to participants and similar exercise modalities. It appears that the most effective time to use O_2Supp during short term high intensity exercise in order to improve performance, remains equivocal. Therefore, the aim of this study was to evaluate the effects of inhaling hyperoxic gas (FiO_2 1.00) during recovery periods and/or exercise on cycling performance. Previous work has researched the effects of O_2Supp during recovery or during exercise but not combined. This will be the first study to combine O_2Supp during both recovery and or sprints. This study will allow practitioners to identify the most effective time to administer O_2Supp in a training programme. Identifying the most effect time to administer O_2Supp will aid many sporting events that involve repeated high intensity intervals within training and competition.

It was hypothesised that the greatest increase in cycling performance would occur during the full hyperoxic condition compared with both partial O_2Supp or a control (Normoxia condition). Second, it was hypothesised that during the full O_2Supp condition, BLa would be compared with the other conditions.

4.4 Methods

4.4.1 Study Design

In order to identify the effects of O_2Supp on repeated cycling performance, four different conditions were identified. A combination of gases was administered in a recovery period following 15 km pre fatiguing cycle, and during a set of repeated sprints. The protocol replicates similar training undertaken by track points race cyclists, as they complete high intensity sprints after several laps around the track.

Eight male cyclists completed four sessions each in a randomised cross-over design whereby two participants conducted each study order. Each testing session occurred at the same time of day (± 2 h) for each participant. Performance variables (peak and mean power) were measured throughout each exercise task. BLa was measured at consistent intervals throughout the whole study.

Eight healthy male amateur level cyclists from University of Essex participated (26.5 ± 5.2 years; 85.1 ± 11.7 kg; 1.82 ± 0.01 m). To ensure homogeneity of ability, participants were required to evidence that they were able to complete a 10-mile cycling time trial (TT) between 26- 28 min prior to taking part in the study.

Participants were requested to refrain from alcohol and caffeine prior to participating, 24 h and 4 h respectively. Participants were informed of the procedure, completed written consent and a pre-exercise readiness health questionnaire. Ethical approval for the study was granted by the University of Essex ethics committee.

4.4.2 Experimental Protocol

Prior to completing the repeated sprints, participant completed 15 km TT as a pre-fatiguing task. This cycle was followed by 15 min ‘post TT recovery’ period where the

middle 5 minutes was active recovery to allow for warm up prior to intense repeated sprints. Finally, participants completed 10 x 15 s sprints, interspersed with 45 s recovery. Each participant completed 4 visits in a randomised counterbalanced order; Normoxia in the 15 min post TT recovery and during the 10 x 15 s sprints (NN), O_2Supp in both post TT recovery and sprints (HH), O_2Supp in the post TT recovery and Normoxia in sprints (HN) and Normoxia in post TT recovery and O_2Supp during sprints (NH) (Figure 4-1).

The four visits to the laboratory were separated by a minimum of 48 h and completed over a 3-week duration. Hyperoxic (O_2Supp - FiO_2 1.00) and normoxic (FiO_2 0.21) gas mixtures during recovery and/or sprints, were administered via a rig of 4 x 200 L Douglas bags, connected to a mask and head net (Hans Rudolph, Shawnee, KS, USA). O_2Supp was created using medical grade oxygen (British Oxygen Company, Surrey, UK) prior to administration. The washout period for medical oxygen is unknown, although the internal environmental changes take 15 s to detect changes in oxygen concentration, similar to hypoxia [162]. Therefore, training stimulus (fatigue) is purported to be the only reason for 48 h recovery period following each session.

Prior to the start of any physical activity, a pre-exercise 20 µl capillary BLa sample was taken from the right ear lobe. Each sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Subsequent lactate samples were taken every 2 min during recovery post TT. Further samples were taken during recovery from each sprint repetition. All samples were analysed within 1 h of withdrawal during which they were kept cool and stored in a secure biohazard fridge.

The 10 x 15 s maximal cycling sprints with 45 s of recovery (1:3 Work rest ratio) [52] were undertaken on a stationary cycle (WattBike, Nottingham, UK). Mean sprinting power for each of the 10 sprints was determined per individual. A 'group mean' sprinting power for each individual sprint, in each condition was also calculated using the average of each participant's sprint 1 to sprint 10. The same process was used to calculate individual and group peak sprinting power. Mean power output was used to determine the extent of performance decline throughout the 10 repeated sprints. Performance decline was calculated taking the first sprint (best) and then their sprint with the lowest power output and calculating the percentage decline. Percentage decline = [(best – worst) / best] *100. The higher the number the bigger the decline in performance.

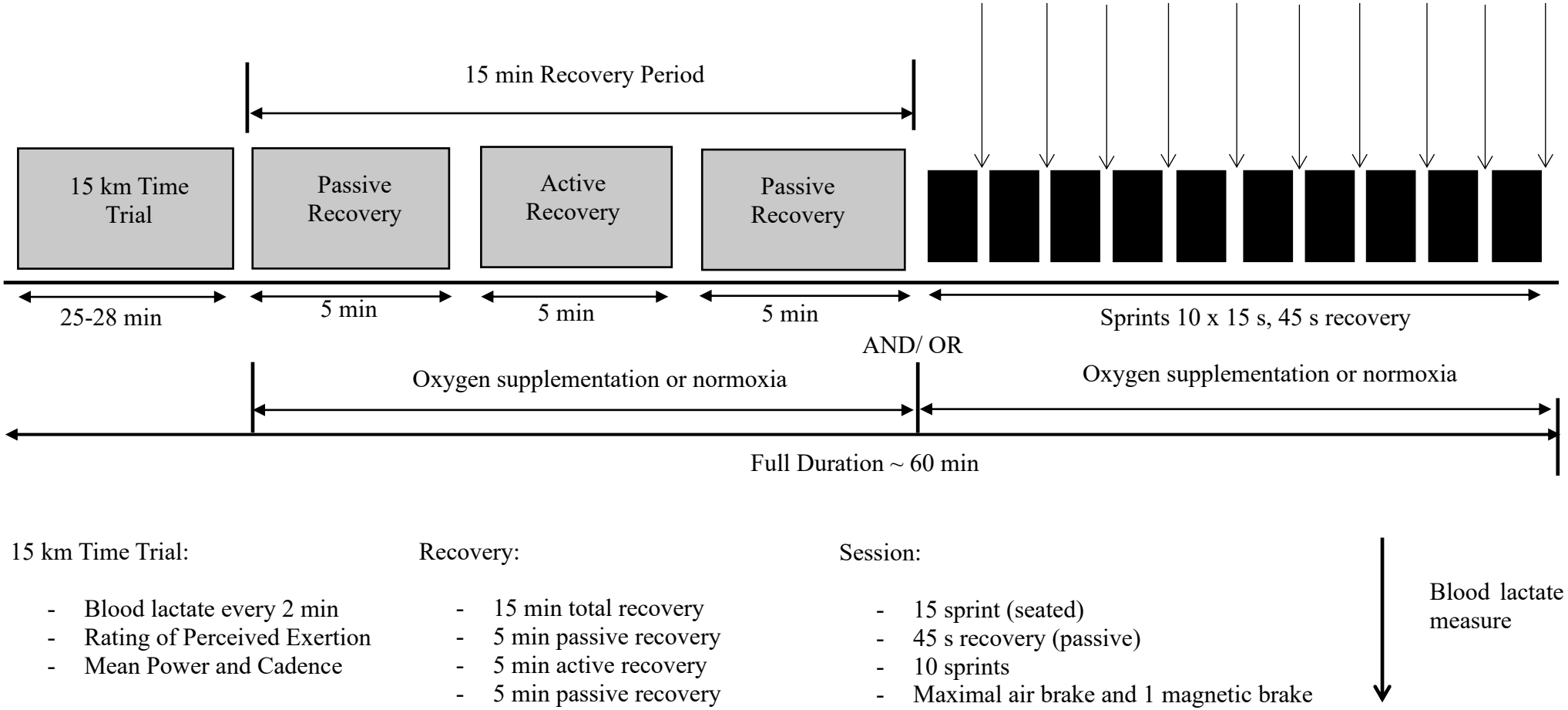

Figure 4-1. A schematic representation of the experimental methodology during experimental Chapter 1.

4.4.3 Statistical Analysis

All statistical analysis was performed using the statistical package, SPSS statistics version 21 for windows (SPSS, Inc, Chicago, IL, USA). Statistical analysis was performed using two-way within group ANOVA, to analyse the effect of condition on mean power (W), peak power (W), and BLa ($mmol \cdot L^{-1}$) and the effect of time across the 10 sprints. Partial eta squared values were calculated to determine overall effect sizes. In the case of large effect size, post hoc tests were used to look between conditions. Post hoc analysis was conducted using paired samples t-tests with Bonferroni correction on sprints at an individual level. α-level was set to $p = 0.05$ for all data analysis.

4.5 Results

Time Trial. There was no significant difference between the work (J) conducted in the TT prior to the sprints in any of the four conditions, F (3,21) = 2.98, p = 0.06. HN = 1471.78 ± 13.57 J, NH = 1586.69 ± 17.32 J, HH = 1482.68 ± 14.65 J, NN = 1462.01 ± 11.86 J.

Mean Power. There was a significant main effect of time across the 10 Sprints F (9,45) = 11.24, $p < 0.01$, $\eta_p^2 = 0.69$, but no significant main effect of condition F (3,15) = 3.05, p = 0.06, $\eta_p^2 = 0.48$ (Figure 4-2). No interaction effect was evident. The post hoc analysis showed that HH induced an average power 4.2% higher than HN ($p < 0.05$), which is (HH) also 3.2% higher than that of NN (p = 0.02) (Figure 4-2). No significance was shown following post hoc analysis at an individual sprint level $p > 0.05$.

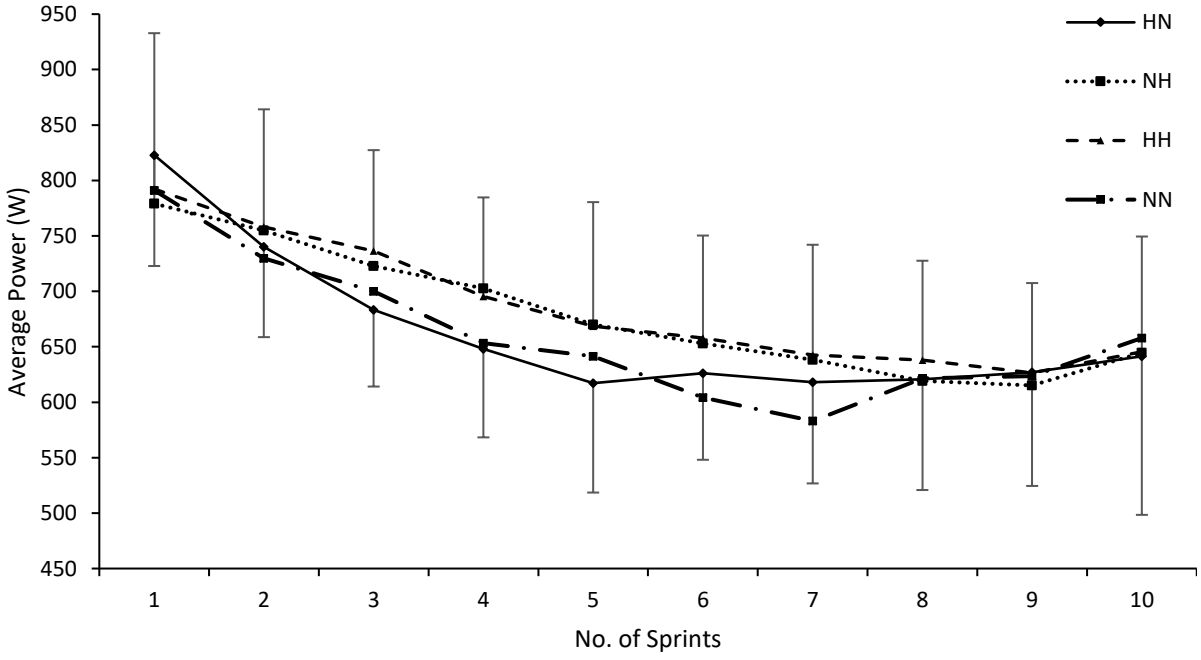

Figure 4-2. Mean cycling power output over 10 sprints in four conditions (n = 8).

Legend: O_2Supp -Normoxia; HN, Normoxia – O_2Supp; NH, O_2Supp- O_2Supp; HH, Normoxia – Normoxia; NN, Watts; W.

Peak Power. There was a significant main effect of time across 10 sprints F (9,45) = 7.30, $p < 0.01$, $\eta_p^2 = 0.59$. There was no main effect of condition in peak power F (3,15) = 0.83, $p = 0.50$, $\eta_p^2 = 0.14$. However, given the large effect size, further analysis was carried out and it was found that HH induced a peak power of 841.5 ± 60.7 W, which was 3.5% higher than the NN condition (810.3 ± 61.0 W, $p > 0.05$). There was also a non-significant difference (3.7%) peak power in the HN condition (840.1 ± 82.4 W) compared with NN (810.3 ± 61.0 W, $p > 0.05$). NH was not significantly different to any of the other three conditions (842.8 ± 61.0 W, $p > 0.05$). No interaction effect between condition and time was evident (Figure 4-3).

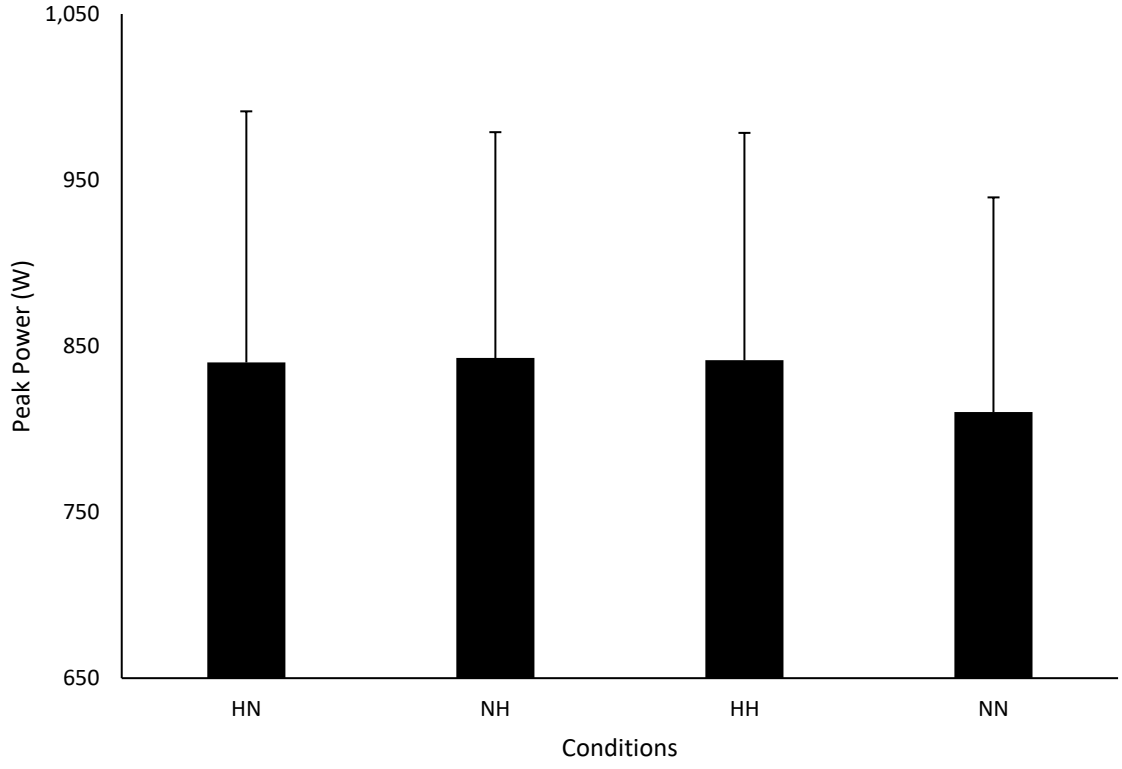

Figure 4-3. Peak cycling power output four conditions -M ± SD (*n* = 8).

Legend: O_2Supp-Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp - O_2Supp; HH, Normoxia – Normoxia; NN,

Lactate Accumulation. There was no difference in lactate accumulation during the recovery prior to the repeated efforts across the four conditions, F (3,15) = 0.23, p = 0.87, η_p^2 = 0.04, nor in the lactate accumulation during sprints across the four conditions, F (3,12) = 0.73, p = 0.55, η_p^2 = 0.15 (Figure 4-4).

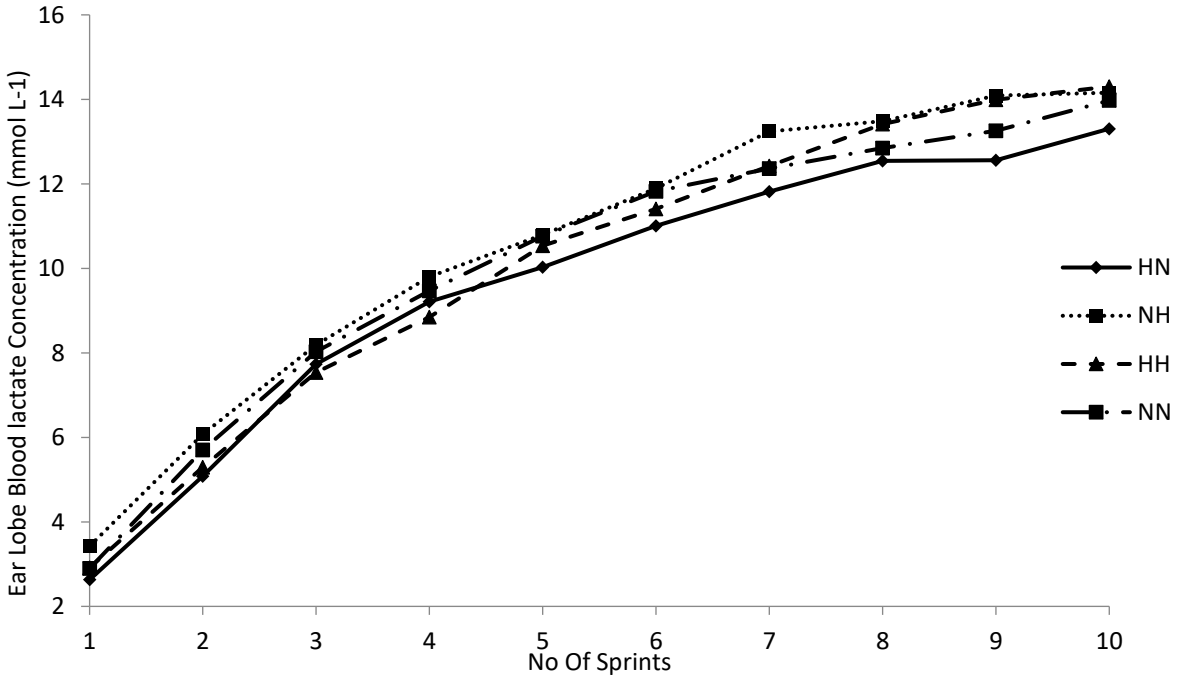

Figure 4-4. Mean lactate accumulation over 10 sprints in four conditions (*n* = 8).

Legend: O_2Supp-Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp - O_2Supp; HH, Normoxia – Normoxia; NN,

Performance Decline. There was no significant difference between the performance decline throughout the sprints in any of the four conditions, F (3,18) = 0.98, p = 0.43. HN = 30.59 ± 4.61%, NH = 22.54 ± 4.67%, HH = 26.04 ± 3.54%, NN = 31.14 ± 5.29%.

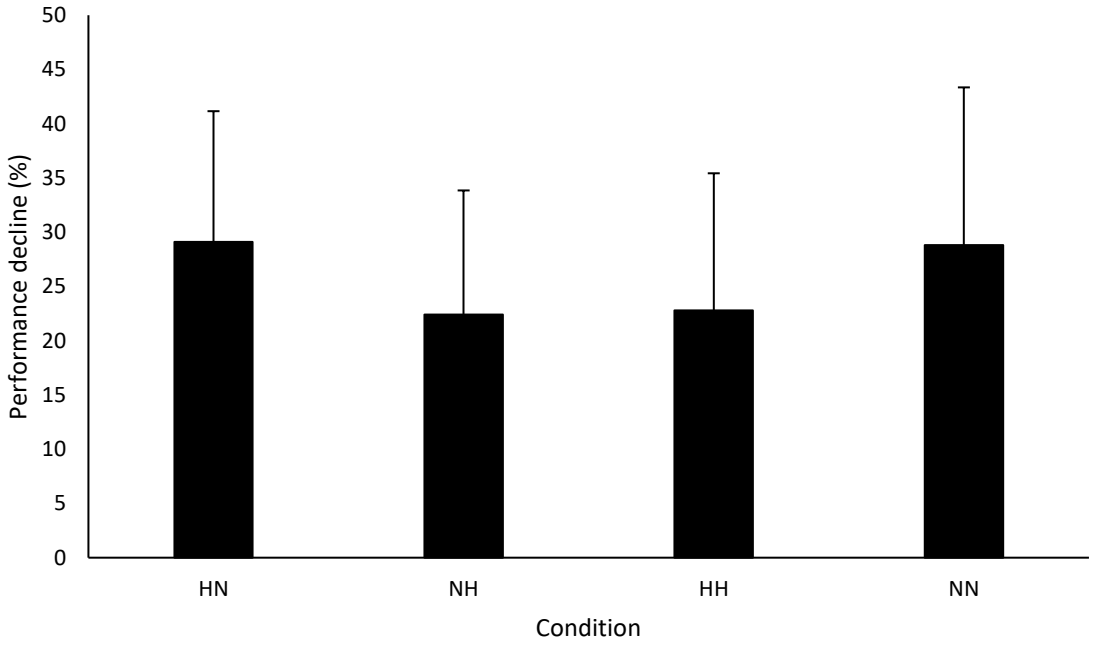

Figure 4-5. Percentage decline in cycling mean power output across the four conditions – M ± SD (*n* = 8).

Legend: O_2Supp-Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp – O_2Supp; HH, Normoxia – Normoxia; NN

4.6 Discussion

The aim of this research was to determine the effect of O_2Supp on sprint cycling performance, dependent on the timing of supplementation. The current study found that O_2Supp during recovery periods and exercise was most effective in eliciting increases in mean power output, HH was 4.2% higher than control condition NN. O_2Supp during exercise bouts alone (NH) was the second most beneficial administration time, with HH mean power output only being 1% higher than NH. Sprint cycling performance when O_2Supp was given during the pre-sprint recovery period was 3.2% higher than the mean power in NH condition.

The increase in mean sprint cycling performance seen during the HH and NH conditions can be credited to a number of different factors; the rate of PCr resynthesis, the efficiency of clearing metabolic by products, and an increased potential to use aerobic ATP regeneration. Although, PCr resynthesis was not directly measured within this study, the maintenance of BLa levels with increased power output, it can be speculated that this is as a direct result of increased PCr resynthesis [1,7].

Administering O_2Supp throughout the session likely influences the resynthesis of PCr. The effects of O_2Supp prior to exercise dissipate quickly due to the rapid diffusion of dissolved oxygen into/ out of the blood, and due to arterial haemoglobin (S_aO_2) being near fully saturated at rest [121]. However, during exercise there may be a drop in S_aO_2 of up to 15%, and concurrent O_2Supp has been shown to increase full body oxygenation, increasing S_aO_2 by as much as 7% [163]. This has been demonstrated when O_2Supp at an FiO_2 of 0.4 was used during and after 9 x 300 m running sprints [134].

The combination ATP and PCr provides the energy needed during high intensity exercise lasting between 10-15 s. Consequently, repeated sprint exercise continuously depletes PCr stores. A 45 s rest after 10 s of high intensity exercise only replenishes ~75% of total PCr stores [164]. Consequently, after the 5th successive sprint in a series, PCr stores will become significantly diminished resulting in a reduced sprinting ability. Even though, the PCr energy system is anaerobic in nature, its resynthesis requires oxygen. The resynthesis of PCr occurs using products from both the Krebs cycle and aerobic glycolysis, which have been shown to be enhanced following the use of O_2Supp [99]. Enhanced PCr resynthesis due to the consumption of hyperoxic gas [99,165] will allow performance to be maintained for longer – as seen in the current study.

There is a small (< 10%) aerobic contribution to energy production during a short (up to 30 s duration) maximal effort sprint and is suggested that this contribution increases as sprints are repeated, perhaps due to the alterations in oxygen kinetics [94–96]. This is part due to increased oxygen demand post sprints in order to re-establish the saturation of myoglobin with oxygen and PCr. This may result in subsequent sprints starting at progressively higher $\dot{V}O_2$. Additionally, Bogdanis *et al.*,[97] found that aerobic metabolism during the second sprint (30 s) provided ~49% of the energy yield. These values were estimated due to an increase in oxygen uptake by ~0.5 L·min^{-1} (increase of 20%). These findings can be explained by Wagner [98] who concluded that an increased FiO_2 raises the partial pressure of oxygen in the blood and resultantly increases $\dot{V}O_2$max (sparing anaerobic energy at higher workloads).

Accordingly, an increase in peak and mean power during repeated high intensity exercise can be attributed to an enhanced PCr resynthesis during O_2Supp, [52,93] and an increased

ability to respire aerobically. Glaister [52], suggests that repeated sprint exercise performance is determined by high energy yields from PCr resynthesis during exercise, but during recovery periods the processes are exclusively aerobic. The current study shows that O_2Supp is most effective at increasing peak and mean power output when used both after a fatiguing cycle AND during (15 s) and between (45 s) bouts of exercise, thus supporting the study hypothesis.

The effect of O_2Supp during exercise has been credited, in part, to the reduction in the accumulation of lactate in the blood [7]. O_2Supp during recovery has also been shown to aid enhanced metabolic clearing [2,54]. The reduced accumulation of lactate during exercise is strongly correlated with the cost of the anaerobic contribution during exercise. The present study measured BLa as a proxy of the anaerobic contribution of exercise. BLa was found to be similar across conditions, even with an increased power output in the O_2Supp conditions (HH and HN). It is suggested that the metabolic cost of producing more power was negated by the consumption of O_2Supp, confirming the hypothesises of this study. Therefore, it is to be assumed that the consumption of O_2Supp during both recovery and exercise is most effective in reducing the anaerobic cost and by products of repeated sprint training [166]. This work has shown that O_2Supp benefits acute performance outcomes, but the longer-term effects of O_2Supp on the training response need to be examined.

4.6.1 Limitations

Several limitations of this study, participants underwent a 15km time trial as a pre fatiguing exercise bout. This could have been standardised by controlling the amount of ‘work’ undertaken rather than a time or distance limit. This would have allowed each visit to be identical in nature, rather than the small differences in work between visits. Additionally,

no direct measure of fitness ($\dot{V}O_2$ max) was conducted to characterise the study population. Level of fitness is a potential mediator of response to O_2Supp.

4.6.2 Practical Implications

This research was set up with the aim of improving sporting performance and maximising the effectiveness of O_2Supp. Coaches and athletes can apply this relatively inexpensive training aid to provide short term increases in sprinting performance. Athletes will be able to produce more power for the same training stimuli resulting in greater potential for adaptation and acclimatisation.

This ergogenic aid can only be applied to training as it is banned in competition. Caution still needs to be applied when using O_2Supp as a training tool, particularly in relation to the longer-term effects.

4.7 Conclusion

In conclusion, the present study demonstrates that the most effective time to administer hyperoxic air is during recovery and exercise. Enhancements in performance with no meaningful influence of BLa concentration were apparent. As a result, O_2Supp should be considered as a meaningful tool for coaches to implement in training programmes (during intervals) enabling athletes to work harder during training without becoming more fatigued.

4.8 What Next?

Following the findings of this experimental chapter it is proposed that future research should look at trying to identify when during a training session is the most effective time

to administer O_2Supp. With that information coupled with the results of this study a strong practical application for O_2Supp in cycling will be evident.

5 - Experimental Chapter:

The Effect of Manipulating the Timing of Oxygen Supplementation on Repeat Sprint Cycling Performance

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A version of this chapter has been published as a research article. Citation as seen below.

Porter M, Reed K. The Effect of Manipulating the Time of Oxygen Supplementation on Repeat Sprint Cycling Performance. Journal of Human Sport and Exercise, in press. (2020)

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5.1 Abstract

5.1.1 Objectives:

The aim of this study was to determine the optimal time to administer oxygen during a repeat sprint protocol on cycling performance, when the oxygen delivery was changed rapidly during session.

5.1.2 Design and Method:

Ten male amateur trained cyclists took part. Testing comprised four visits to the laboratory in a counterbalanced design. Each session entailed; 5 min cycling warm up (~200 W), 5 min passive recovery, followed by 10 x 15 s repeated sprints interspersed with 45 s passive recovery, during which the air inspired was manipulated using a FiO_2 of 1.00 or 0.21 (normal air). The content inspired during the 15 s sprints and/or the 45 s recovery periods, comprised the four visits: NH, HN, HH, NN. It was hypothesised that the HH condition would evoke the largest performance improvements.

Outcome measures included mean power (W), BLa and performance decline. Repeated measures ANOVA were used to examine the difference between conditions in outcome measures.

5.1.3 Results:

There was no significant effect of oxygen supplementation on mean power (W), BLa ($mmol \cdot L^{-1}$) or performance decline (%) ($p > 0.05$). However, a common trend in HH condition (continuous) was evident, with lowest levels of lactate accumulation and the shallowest decline in performance across the 10 sprints. Performance was not enhanced when the oxygen supplementation was discontinuous.

5.1.4 Conclusion:

Oxygen supplementation during repeat sprint cycling has a net detrimental effect on performance when administered in short (15-45 s) discontinuous periods throughout a session and is not as effective at maximising performance compared with training with oxygen throughout.

5.2 Background

The findings of chapter 4 begin to identify the most effective administration time of O_2Supp. Chapter 4 found that O_2Supp was most effective during large duration administration. However, it has yet been established whether O_2Supp is effective during single components of a training session, such as the sprints and not the inter sprint recovery periods. This additional knowledge will highlight the practical application of O_2Supp; therefore, the forthcoming study will look to shed light on this.

5.3 Introduction

Hyperoxia occurs when cells, tissues and organs are exposed to an excess supply of oxygen or higher than normal partial pressure of oxygen [59]. Normoxia on the other hand is the natural body conditions that occur at sea level in absence of disease. To create hyperoxic conditions within the body one must breathe an oxygen enriched gas mixture (O_2Supp). Previously, O_2Supp was banned by the WADA due to its potential performance enhancing effect [161]. Recent evidence [114,136,163,167] has led to the reinstatement of O_2Supp within training for a competitive sport, resulting in its increasing application in exercise training [160].

O_2Supp is increasingly becoming a popular ergogenic aid within a range of sporting populations. Research has been conducted on runners, cyclists, swimmers and hockey players [7,8,134,168], most of which use the supplement during a continuous period of 30-240 s. These durations may elicit the biggest performance improvements, but they are not so easy to administer in a real-world setting (i.e. outside of the lab).

Companies such as Boost Oxygen© and Oxygen Plus© offer handheld oxygen canisters that contain between 25 to 220 breaths of pure 100% oxygen. These offer enough oxygen to complete short duration exercise or sporadic use during recovery periods between

exercise bouts. Oxygen has been administered at many different performance time points; before exercise, during and between exercise bouts [1], yet the exact timing of supplementary oxygen in relation to the greatest acute performance enhancement is not yet clear. However, chapter 4 has shed some light on this, showing that O_2Supp seems to be most effective during both sprints and recovery but is yet to be confirmed.

With the correct timing, supplementary oxygen given during a repeated sprint training session will be effective at increasing the resynthesis rate of PCr and subsequently performance [76,114]. It appears that the most effective time to use O_2Supp during short term high intensity exercise in order to improve performance, remains equivocal.

The aim of this research was to evaluate the effects of altering the timing of supplementary oxygen (FiO_2 1.00) (during recovery periods and/or exercise) during a single repeat sprint cycling session. It was hypothesised that the greatest increase in mean sprint cycling power output (W) would occur during the full O_2Supp condition (HH) compared with both partial O_2Supp (HN and NH) and a control (Normoxia condition, NN). Additionally, it was hypothesised that the HH condition would result in similar BLa concentration during each sprint, compared with the other conditions.

5.4 Methods

5.4.1 Study Design

Ten healthy university students were recruited to take part in the study. Participants (1.79 ± 0.05 m, 74.7 ± 10.5 kg, 22.8 ± 4.5 years) were amateur cyclists who all had previous experience using a cycle ergometer and repeated sprint protocols.

Participants were informed of the procedure and provided written informed consent prior to study commencement. Ethical approval for the study was granted by the University ethics committee in accordance with the Helsinki declaration.

This study was a single-blind, within-participant design comprising four counterbalanced assessments of repeat sprint performance using O_2Supp (FiO_2 1.00) or normal air (FiO_2 ~ 0.21).

Laboratory tests were completed at the same time of the day (± 2 h). Participants were asked to maintain normal activity and sleep pattern between testing sessions. Participants were asked to refrain from strenuous physical activity 24 h prior to participating.

5.4.2 Experimental Protocol

Participants undertook the same procedure on all 4 visits: 5 min warm up at a workload of ~200 W, 5 min passive recovery, and 10 x 15 s cycle sprints with 45 s recovery. Details of oxygen supplementation are detailed in chapter 3. In brief, O_2Supp and normoxic gas mixtures were administered via a rig of 4 x 200 L Hans Rudolph Douglas bags connected to a Hans Rudolph mask and head net (Hans Rudolph, Shawnee, KS, USA). The O_2Supp (FiO_2 1.00) condition used medical grade oxygen cylinder (BOC, Surrey, UK). In each condition, participants wore the mask and breathed from the Douglas bag during the repeated sprints and the recovery periods. Gas was administered to the participants through the Douglas bags after saturating and warming the compressed gas.

Gas administration was manipulated during the sprint and recovery to make up four conditions; O_2Supp in the 10 sprints and Normoxia in the 10 inter sprint recoveries (HN), Normoxia in sprints and O_2Supp in recoveries (NH), O_2Supp in both sprints and recoveries (HH), and finally Normoxia in both sprints and recoveries (NN) (Figure 5-1).

A pre-exercise 20 µl capillary sample was taken from the right ear lobe as a baseline measure. Each sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Subsequent samples were taken during the recovery period of each sprint repetition. All samples were analysed for BLa within ~1 h of withdrawal using a Biosen (EKF diagnostics, Cardiff, UK).

Following the warmup each participant undertook ten repetitions of 15 s cycling sprint followed by 45 s static recovery. Participants were instructed to stay seated to isolate leg power.

During the sprints the Wattbike (Watt Bike Ltd, Nottingham, UK) with the magnetic setting set to 1 and air brake set to 10. This protocol was set in accordance with pilot testing conducted prior to this study as it was determined this resistance allowed participants to generate their peak power whilst not exceeding their peak cadence. Performance data used for analysis were peak sprinting power (the highest Watts achieved in each cycle) and mean sprint power (the average Watts produced during each 15 s cycle)

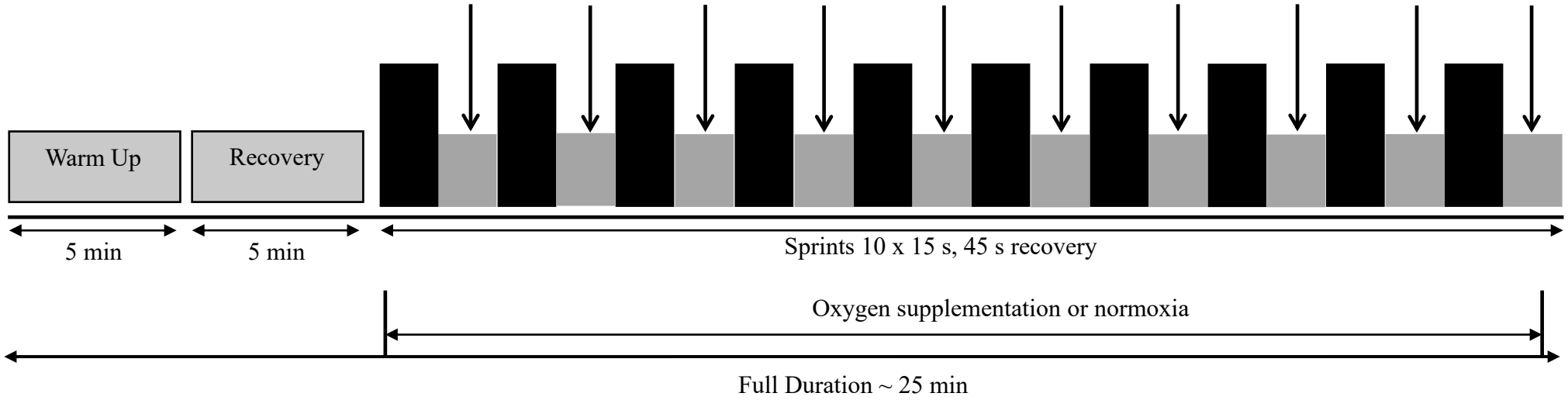

Figure 5-1. A schematic representation of the experimental methodology during experimental Chapter 2.

5.4.3 Statistical Analysis

All statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS Inc, Chicago, IL, USA). An a priori power analysis revealed that ten participants would provide significant power to detect differences in mean power by 3-4% at an α-level of 0.05 (G∗POWER 3.1 Software, Düsseldorf, Germany). Power analysis detected using data from previously published work with large differences in cycling power output [169].

Repeated measures analysis of variance (ANOVA) were conducted to look for differences according to condition (O_2Supp / Normoxia) for; peak power (W), mean power (W), and BLa ($mmol \cdot L^{-1}$) for each sprint.

α-level set $p = 0.05$ for all data analysis. Effect size for individual measures were calculated and reported as Cohen's *d* and interpreted using bounds as 0.2, 0.5, > 0.8, where they are small, medium and large respectively [170].

5.5 Results

Mean Power (Table 5-1). There was a significant main effect of time on sprint performance across the 10 Sprints F (9,27) = 35.73, $p < 0.01$, $d = 2.61$. There was no significant main effect for sprint performance according to condition F (3,12) = 0.21, $p = 0.99$, $d = 0.35$. No interaction effect was evident.

Peak Power. There was a significant main effect of time for peak sprinting power across 10 sprints F (9,27) = 16.78, $p < 0.01$, $d = 1.77$. There was no main effect of condition in peak power F (3,12) = 0.37, $p = 0.99$, $d = 0.46$. No interaction effect was evident.

Percentage decline in performance (Figure 5-2). There was a significant main effect of time on sprint performance across the 10 Sprints F (8,72) = 24.42, $p < 0.01$, $d = 7.22$. No significant main effect for sprint performance according to condition was found, F (3,23) = 1.19, $p = 0.33$, $d = 0.72$. No interaction effect was evident.

Lactate accumulation during sprint (Figure 5-3). There was no difference in lactate accumulation across the four different gas mixtures, F (3,12) = 1.22, $p = 0.21$, $d = 0.87$ (Figure 5-3). However, HH (7.15 ± 0.77 mmol·L^{-1}) had a lower lactate level than HN (9.45 ± 0.71 mmol·L^{-1}, $d = 3.11$) whilst HH was also meaningfully lower (change greater than 10%) than both NH (mean differences (MD) = -2.13 mmol·L^{-1}) and NN conditions (MD = -2.13 mmol·L^{-1}). (Figure 5-3).

On questioning, 70% of participants were able to correctly deduce the NN condition once they had completed all four visits, whereas only 40% of the participants were able to correctly deduce the HH condition. In the two other conditions were 50% and 60% respectively, participants correctly guessed the conditions.

Table 5-1 Mean cycling power over ten sprints in four conditions (M ± SD) (*n* = 10).

	Sprint 1 (W)	Sprint 2 (W)	Sprint 3 (W)	Sprint 4 (W)	Sprint 5 (W)	Sprint 6 (W)	Sprint 7 (W)	Sprint 8 (W)	Sprint 9 (W)	Sprint 10 (W)
HH	677.8 ± 128.0	615.4 ± 105.0	581.0 ± 111.6	550.3 ± 121.9	526.5 ± 122.9	500.3 ± 120.7	498.9 ± 117.0	504.2 ± 121.2	489.3 ± 93.5	510.7 ± 108.1
HN	735.8 ± 138.6	657.5 ± 118.1	584.8 ± 103.7	535.2 ± 94.4	514.5 ± 95.4	492.1 ± 102.5	462.9 ± 116.4	467.9 ± 129.7	463.5 ± 109.7	470.6 ± 122.4
NH	709.6 ± 125.0	616.4 ± 68.3	578.1 ± 96.5	540.0 ± 93.2	529.1 ± 100.9	502.5 ± 97.9	478.5 ± 106.8	475.1 ± 116.2	444.5 ± 98.4	456.8 ± 121.7
NN	709.9 ± 138.3	620.5 ± 106.8	567.6 ± 111.9	541.2 ± 91.4	517.2 ± 89.8	504.8 ± 82.2	474.1 ± 83.8	471.3 ± 95.6	490.9 ± 91.5	502.6 ± 99.8

Legend: O_2Supp -Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp – O_2Supp; HH, Normoxia – Normoxia; NN, Number; No, Watts; W.

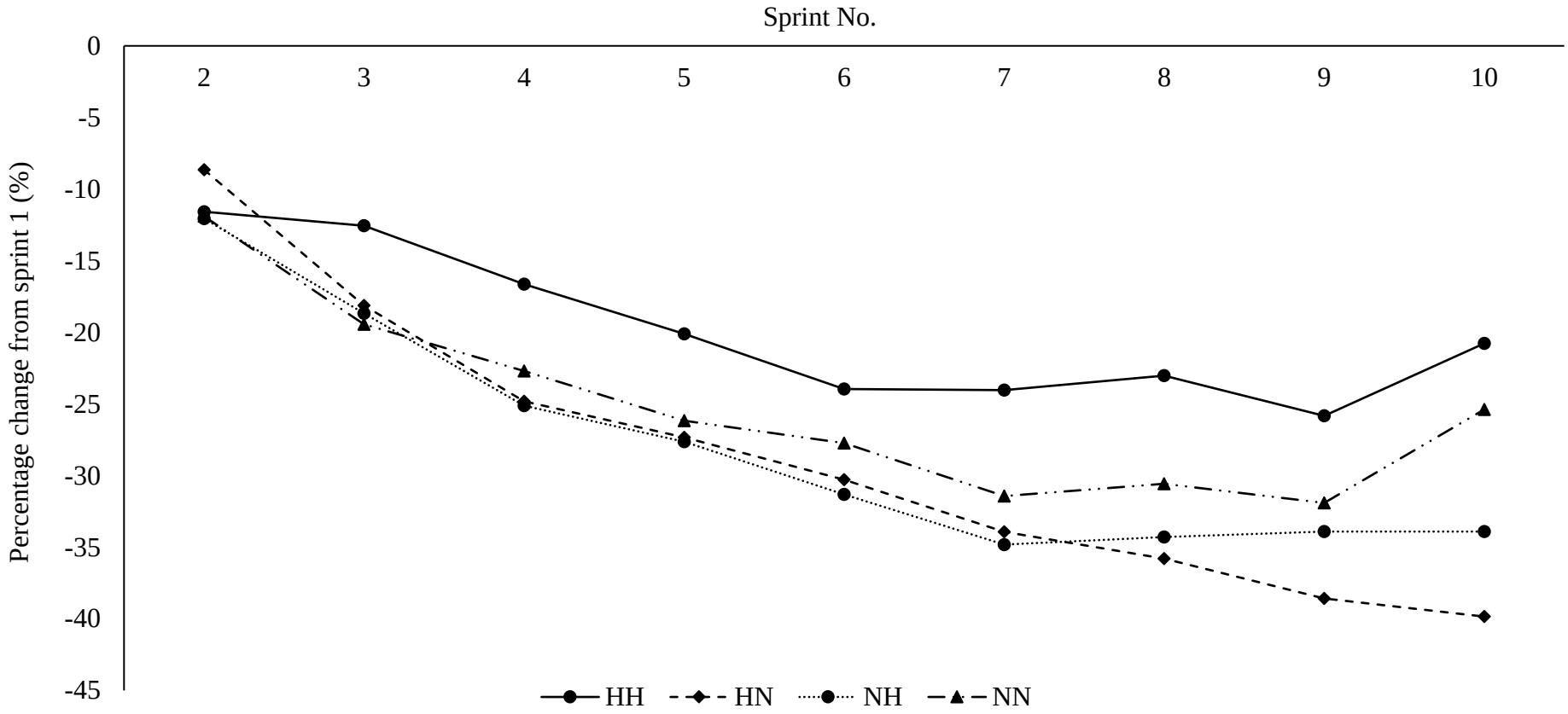

Figure 5-2. Percentage change in sprint performance from sprint one across nine subsequent sprints in four conditions (*n* = 10).

Legend: O_2Supp -Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp – O_2Supp; HH, Normoxia – Normoxia; NN, Number; No,

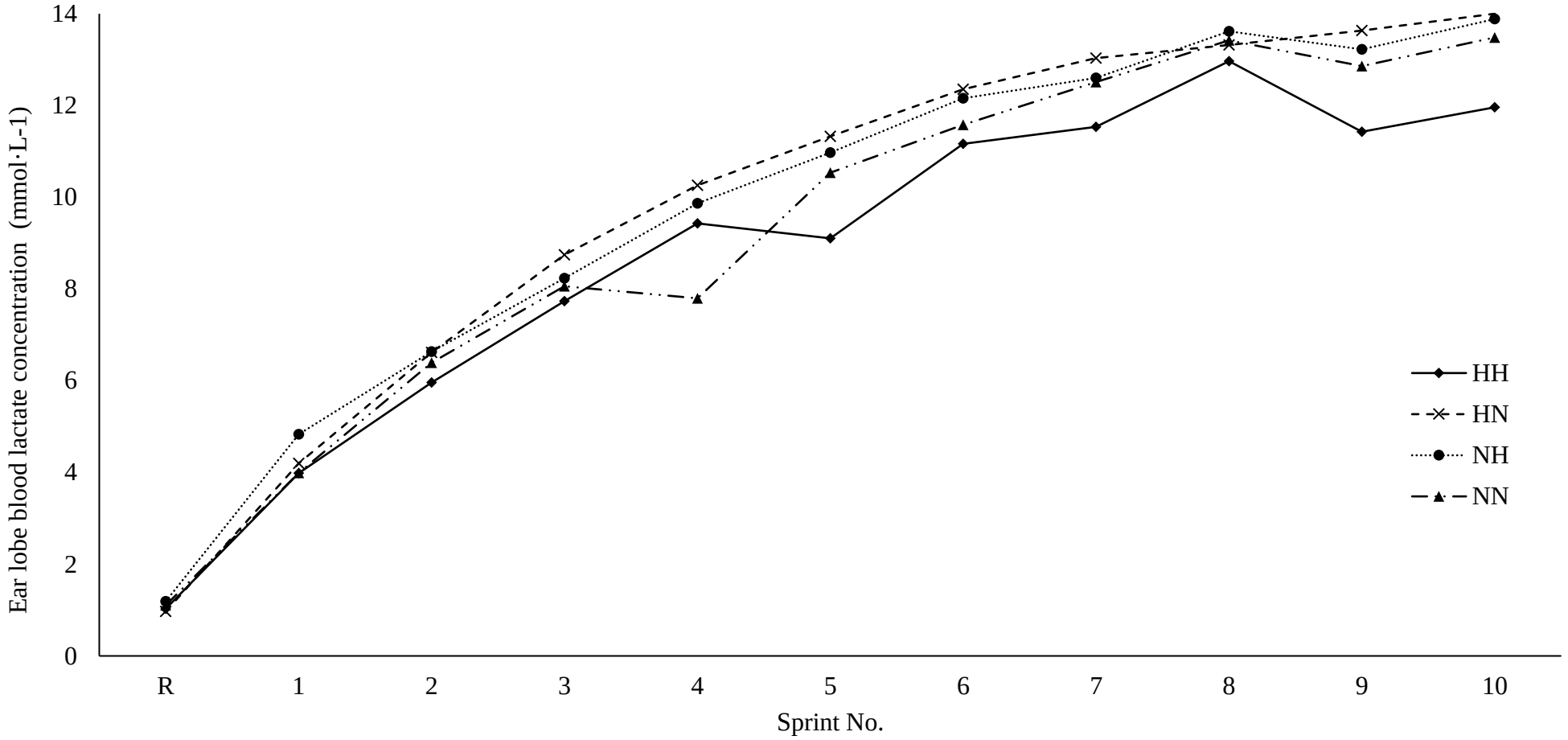

Figure 5-3. Mean lactate at rest and over ten sprints in four conditions (*n* = 10).

Legend: O_2Supp -Normoxia; HN, Normoxia -O_2Supp; NH, O_2Supp – O_2Supp; HH, Normoxia – Normoxia; NN, Number; No, Millimole per Litre; mmol·L^{-1}.

5.6 Discussion

The aim of this research was to determine the effect of O_2Supp on sprint cycling performance, with a specific focus on the timing of administration. The current study found that administering O_2Supp to a sprint cycling athlete has no statistically significant benefits for mean and peak power output (W), but a meaningful reduction in BLa concentration were evident in HH condition compared to the other conditions.

Primary findings revealed that mean sprint power output (15 W- 2.5%, d = 0.35) increased non significantly in the presence of extra oxygen (FiO_2 1.00). This change was replicated by the performance decline data that showed a shallower slope across each sprint in the HH condition.

Due to the relative early years of O_2Supp research many of the assumptions are developed to diametrically oppose the themes (reduced O_2 availability and utilisation) within hypoxia research (low O_2Supp). O_2Supp research, largely hypothesises that the opposites (to hypoxia) occur with little evidence to suggest why.

During the conception of the study we speculated that the two conditions that have discontinuous oxygen supplementation (NH, HN), would result in reduced performance capacity due to the continued alterations of the gas concentrations, this is evident within the current data. Participants performance deteriorated as their internal equilibrium is unable to effectively utilise rapid changes in oxygen concentration.

Changes that occur with additional oxygen include decreased blood flow [124], vasoconstriction [106,107], and increased arterial oxygen content [171]. During repeated changes in the oxygen consumption, and the continuous changes in the homeostatic responses, the individual cannot react effectively fast enough, thus performance capabilities are reduced as a result. The reasons why both the NH and HN conditions showed decreases in

performance compared to HH and NN conditions could be attributed fluctuating nature of the O_2Supp. NH and HN has changes in gas content every 15 s and 45 s respectively. In hypoxia research the detection of the internal change in oxygen occurs 15 s after its administration [162]. The timing of the response could be purported to be similar in O_2Supp. Resultantly the continuous manipulation in oxygen levels leave the medulla oblongata continuously trying to respond to fluctuations in the blood oxygen levels, by decreasing peripheral blood flow [124], and peripheral vasoconstriction [106,107]. Supplementary oxygen is appearing to be more effective when administered during longer periods of work/rest to allow for changes in internal equilibrium to occur and probably why we see large improvements in HH condition compared to the other three conditions (NH, HN, NN).

Performance changes (mean and peak power output) in the HH and NN conditions were as expected, whereby the full oxygen condition results in greater performance (albeit non-significant) than the Normoxia condition (NN) [140,166,169]. Performance decline followed a similar trend across each condition until sprint 6- this is where HH condition plateaus. This change in the slope of performance has also been suggested in previous studies [52,169]. Similarly, pilot work in our lab found that supplementary oxygen has significant performance benefits in particular following the first 5 sprints of a 10-sprint programme.

The performance enhancing effects of O_2Supp have been credited, in part, to the reduction in the accumulation of lactate in the blood [7]. Despite this study only resulting in small changes in performance during the HH condition, lactate was also lowest in this full oxygen condition. It could be suggested that the metabolic cost of producing more power was attenuated by the consumption of oxygen [166]. Hogan *et al.*, [113] suggest lactate accumulation can explain the differences in performance during varied oxygen conditions. O_2Supp can

also lead to an increased ‘lactate threshold’ or an increased ability to attenuate the accumulation of lactate, resulting in potential positive performance outcomes [2,54,113].

It is evident that O_2Supp is effective at improving performance during cycling, when administered over an extended duration. The duration appears to be an important variable in O_2Supp research. O_2Supp should therefore be applied to training and or recovery periods that last over 1 min in duration, with more investigation needed to identify the exact minimum time to elicit improvements in performance. This said the current marketed cannisters that only last for between 20-220 breaths may not be effective for use in a single training session, as each cannister may only last 200 s or less during periods of exercise or recovery.

The novel findings of this study open further avenues for investigation. The effects that occur during interrupted administration of O_2Supp, may lead us to understanding why we get performance improvements and what specific internal environments change. It would appear reasonable for further studies to identify the specific microvascular and chemical changes experienced by simultaneously exercising whilst manipulating the oxygen content for very short durations as was the case in this study.

5.6.1 Limitations

We acknowledge several limitations to this study. Participants pre study fitness level was not accounted for ($\dot{V}O_2$max) which could potentially mediate the response to O_2Supp. Additionally, the physical dead space in the breathing tubes, this meant two breaths were needed to attain the mixture in the reservoir bags even though oxygen would

have started to saturate (via diffusion) the space immediately. This may have stunted the response during the mixed conditions (NH, HN).

5.6.2 Practical Implications

O_2Supp has shown its ability to be applied to training programmes that can cater for continuous supplementation of oxygen, or programmes that have extended (> 1 min) periods of recovery. Both have sufficient administration duration to see internal changes and resultantly performance improvements. Over the counter O_2Supp cannisters should be used with caution and forethought within an exercise programme as they may not allow enough exposure before, they run out.

5.7 Conclusion

In conclusion, the present study demonstrates that when O_2Supp is used during longer duration (recovery and sprints) bouts it can be effective, though more conclusive evidence is needed. O_2Supp during short discontinuous periods is not effective at increasing cycling performance, if any it has a net detrimental effect on performance.
Similar performance characteristics are evident during HH and NN, whilst there is evidence of meaningful reductions in BLa concentration.

5.8 What Next?

The combination of the last two chapters confirm when the most effective time for administration of O_2Supp is. Additionally, chapters 4 & 5 have both shown positive performance enhancing effects as a direct result of O_2Supp, although the research has little understanding behind the mechanisms for why these are so evident. Future research

should look at what mechanisms cause such changes in performance. Is it a reduction of fatigue or is it a case of enhanced oxygen delivery to the working muscles?

6 - Experimental Chapter:

The Effects of Oxygen Supplementation on Repeated Sprint Cycling Performance and Muscle Fatigue

A version of this chapter has been published as a research article. Citation as seen below.

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6.1 Abstract

6.1.1 Objectives:

O_2 Supp (> 21% oxygen) can evoke performance improvements in aerobic and anaerobic exercise. The aims of the current study were to determine the effects of breathing hyperoxic gas (FiO_2 1.00) on repeated cycle performance, and to assess the nature and extent of fatigue after intermittent sprinting.

6.1.2 Design and Methods:

Testing (n = 14 males) comprised two visits to the laboratory. Each session involved 10 x 15 s repeated cycle sprints breathing FiO_2 1.00 (O_2Supp) or FiO_2 0.21 (Normoxia). Muscle fatigue was measured pre and post sprints using maximal voluntary contraction (MVC), voluntary activation (VA) and potentiated doublet twitch (PTF). BLa was taken between sprints.

Paired samples t-tests were used to examine difference between conditions in power output (peak and mean W) and BLa. Two-way ANOVA was used to examine fatigue variables pre and post sprints according to condition.

6.1.3 Results:

Mean power output was 4% greater in O_2Supp ($p < 0.01$), with no difference in peak power ($p > 0.05$). There was a significant increase in BLa in O_2Supp compared with Normoxia ($p < 0.01$) in sprints 4 and 8, as well as meaningful difference in sprints 4-10. There was no significant difference in fatigue factors (MVC, VA and PTF) ($p > 0.05$) in response to the cycling, although a large drop in PTF occurred in both conditions.

6.1.4 Conclusion:

O_2Supp can elicit improvements in mean cycling power, with no significant change in post exercise muscle fatigue. O_2Supp as a training aid may provide performance enhancing effects during repeated sprint cycling by reducing concurrent muscle fatigue, primarily via peripheral factors.

6.2 Background

The previous two chapters (chapters 4 & 5) highlighted the most effective time to administer O_2Supp during a repeated sprint protocol. The findings of these studies will be applied to the methodology of this and the coming studies. Additionally, chapter 1 highlighted that the research has yet to establish any mechanisms for why specific such large changes (4%) in cycling performance are evident. This coming study will begin to establish the fatiguing component within O_2Supp research, whilst standardising the administration procedure to mirror the previous studies.

6.3 Introduction

O_2Supp is the inhalation of air with an FiO_2 greater than that of sea level (~0.21). Supplementing high intensity exercise with FiO_2 > 0.21 allows the maintenance of performance when fatigue would usually become apparent, both during aerobic and sprint exercise [114,126]. The mechanism behind this attenuation of performance decline are multifactorial and include reduced production of BLa [114], enhanced clearance of BLa, prevention of muscle oxygen desaturation [54,78] the maintenance of blood pH and enhanced resynthesis of creatine phosphate [134]. These factors are associated with peripheral fatigue, i.e. the exercise induced decrease in muscle force production.

A reduction in neural drive from the motor cortex to muscle appears as a decrease in voluntary muscle activation (VA) during exercise [28]. This 'central fatigue', may also be influenced by the FiO_2. Indeed, research has shown that a reduced cerebral O_2 delivery resulting from hypoxia (FiO_2 0.18) results in curtailment of exercise performance due to fatigue [37]. Thus, whether O_2Supp can alleviate central fatigue in a sport situation is unknown.

Performance decline is likely a combination of both central and peripheral factors and the relative contribution of each depends upon the nature of the task [172]. Peripheral fatigue is likely the limiting factor in short, high intensity exercise, with central fatigue playing a greater role as the exercise bout continues. For example, a single 4 km TT lasting around 5 min was shown to be limited by peripheral fatigue, whilst a 20 km TT (lasting around 32 min) was primarily limited by central fatigue [172].

Repeated sprint efforts represent a short term high intensity exercise, which are likely to be limited primarily by peripheral fatigue [173]. However, the extent to which central fatigue contributes to performance decline in repeated sprint performance is equivocal. Racinais *et al.*, [174] determined that the ability to repeat short duration sprints was associated with both central and peripheral factors. The twitch interpolation technique is widely used and is considered a reliable method to estimate the origin of neuromuscular fatigue. Peripheral fatigue is measured by comparing the force responses to electrical stimulation pre and post fatiguing exercise. To determine the contribution of central factors the twitch interpolation technique is used, superimposing single or double twitches on MVC then comparing the superimposed response to the potentiated response obtained from the relaxed muscle

Thus, the aims of the current study were to determine the effects of O_2Supp on repeated cycle performance, and to assess the nature and extent of fatigue after sprinting.

It was hypothesised that repeated sprint cycle performance would decrease to a larger degree in the Normoxia condition compared with the O_2Supp condition. Second, it was hypothesised that both central and peripheral components of fatigue would be reduced to a greater extent in the O_2Supp condition.

6.4 Methods

6.4.1 Study Design

Fourteen male amateur level cyclists were recruited to take part in the study. Participants (1.81 ± 0.04 m, 77.6 ± 11.0 kg, 25.9 ± 7.3 years) were amateur cyclists who had all previously used a cycle ergometer. Participants were accustomed to cycling on a weekly basis, but none had ever competed at any cycling events. One participant had previous experience with O_2Supp.

Participants were informed of the procedure and provided informed consent. Ethical approval for the study was granted by the University ethics committee.

This study was a within subject's design with two visits to the laboratory in a counter balanced order, in a single blind fashion. Participants completed a series of sprints under two different conditions: O_2Supp (FiO_2 ~ 1.00) or Normoxia (FiO_2 ~ 0.21). Visits were separated by at least 48 h.

6.4.2 Experimental Protocol

Laboratory tests were completed at the same time of the day (± 2 h). Participants were asked to maintain normal activity and sleep patterns between testing sessions. Participants were requested to refrain from any caffeinated products or eating 3 h prior to participation. Participants were asked to refrain from strenuous physical activity 24 h prior to participating.

Participants undertook the same procedure on both visits; 3 x 5 s Maximal Voluntary Contractions (MVC) then 15 min relative intensity warm up at 52% of heart rate reserve using the rearranged Karvonen formula [175,176]. This was followed by 10 min passive recovery, 3 x 5 s MVCs (pre-sprint baseline) and 10 x 15 s cycle sprints with 45 s of recovery. Finally, a further set of 3 x 5 s MVCs (post-sprint). Gas administration occurred

at the commencement of the first sprint and continued throughout the sprints and the post sprint MVC's (Figure 6-1).

Hyperoxic and normoxic gas mixtures were administered via a rig of 4 x 200 L Douglas bags connected to a mask and head net (Hans Rudolph, Shawnee, KS, USA). The O_2Supp condition used medical grade oxygen (BOC, Surrey, UK). In each condition, participants wore the mask and breathed from the Douglas bag during the repeated sprints and the last set of MVC's.

Prior to starting the protocol, a pre-exercise 20 µl capillary sample was taken from the right ear lobe. Each sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Further samples were taken during the recovery period of each sprint repetition. All samples were analysed for BLa within 24 h of withdrawal using a Biosen (EKF diagnostics, Cardiff, UK).

Muscle fatigue was assessed prior to and after the repeated sprints using electrical stimulation of the right femoral nerve. The right leg was used regardless of dominance due to measurement restraints. The variables obtained to assess muscle performance were; maximal voluntary contraction (MVC), voluntary activation (VA) and potentiated doublet twitch force (PTF). Muscle fatigue was measured within 1 min of exercise cessation before the decline in force dissipates [177].

Knee extensor force (N) during voluntary and stimulated contractions was measured using a calibrated load cell dynamometer (Kin-Com, Chattanooga Group Inc., USA), attached to a custom-built chair. The participant's ankle was strapped to a load cell immediately superior to the right malleoli. Participants were instructed to maximally extend their leg against a static load cell at 90° for 5 s. Femoral nerve stimulation was delivered during the middle of each contraction and additionally ~5 s after contraction, to determine potentiated quadriceps twitch force and peripheral voluntary activation. PTF was measured as the

highest force produced during the three repetitions evoked by a paired pulse stimulus, administered to the resting muscle via the nerve at rest, 5 s post the MVC [178]. VA was determined using the interpolated doublet twitch technique and is estimated by the changes in the interpolated doublet twitch relative to the PTF (equation 1.). The force evoked by the imposed electrical stimulus on top of the MVC is the interpolated doublet twitch (IT).

Equation 1. Determining voluntary activation [179].

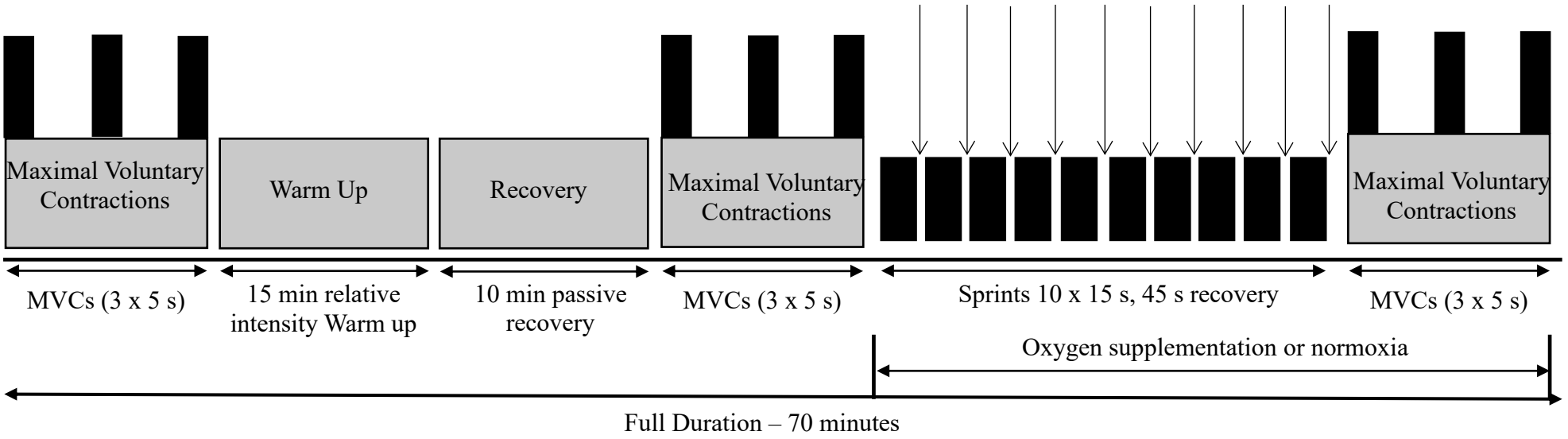
$$VA(\%) = \left(1 - \frac{IT}{PTF}\right) \cdot 100$$

Doublet- twitch electrical stimuli of 200 μs pulse width were delivered to the right femoral nerve via surface electrodes (Axelgaard ValuTrode) and a constant current stimulator (DS7AH; Digitimer Ltd., Welwyn Garden City, UK). A signal converter was used to convert the digital signals of the computer to analogue signals of the digitimer with a sampling rate of 2000 Hz (PowerLab/4st – ML760, AD Instruments, UK). The cathode was positioned on the femoral triangle. The anode was positioned 3 cm proximal to the base of the patella, whilst the knee was fully flexed [79]. Prior to application of the pads, the area was shaven. The electrode placement was marked with semi-permanent ink to ensure consistent placement between trials.

Prior to the MVCs, participants completed resting twitch stimuli in order to determine the maximal twitch amplitude and M-wave of the muscle at rest (the resting immediate response to an electrical stimulation). Doublet twitch stimuli were delivered starting at 100 mA and increasing to 150 mA then increasing in stepwise increments of 25 mA, until a plateau occurred in twitch amplitude. To ensure a full and optimal stimulus the last twitch

was increased by a further 30%. Offline analysis was enabled with the use of LabChart 7.0 software (AD Instruments, UK).

Following the warmup and two sets of MVCs each participant undertook 10 repetitions of 15 s cycling sprint (Watt Bike, Nottingham, UK) followed by 45 s static recovery. Participants were instructed to stay seated to isolate leg power. The air brake was set to 10 and magnetic brake set to 1 to allow sufficient resistance to generate peak force, whilst not exceeding peak cadence. During each sprint and recovery period the participants breathed either normoxic or hyperoxic air via the Douglas bag system. Data used for analysis were peak sprinting power (the highest W achieved in each cycle) and mean sprint power (the average W produced during each 15 s cycle). An overall peak and an overall mean were also calculated for each participant.



Maximal Voluntary Contractions:

- 3 x 5 s MVC's
- Isometric contraction 90 ° static load
- Doublet twitch during each contraction

Warm up:

- Sub maximal exercise
- 15 min
- Level 4 Wattbike (~200W)
- 52% of heart rate reserve

Session:

- 15 sprint (seated)
- 45 s recovery (passive)
- 10 sprints
- Maximal air brake and 1 magnetic brake.

Blood lactate measure

Figure 6-1. A schematic representation of the experimental methodology during experimental Chapter 3.

6.4.3 Statistical Analysis

All statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS Inc, Chicago, IL, USA).

Paired samples t-tests (Bonferroni corrected) were conducted to examine differences according to condition (O_2Supp / Normoxia) for; peak power (W), mean power (W) across each 15 s sprint, and BLa ($mmol \cdot L^{-1}$) for each sprint. Two-way analysis of variance (ANOVA) were conducted to test the differences between MVC, VA, PT before and after the repeated sprints, according to condition. α-level was set at $p = 0.05$ for all data analysis. Effect size for individual measures were calculated and reported as Cohen's *d* and interpreted using bounds as 0.2, 0.5, > 0.8, where they are small, medium and large respectively [170].

6.5 Results

There was no difference in peak sprinting power between the O_2Supp (753.3 ± 87.8 W) and Normoxia (761.0 ± 97.4 W) conditions across the 10 sprint repetitions: t (9) = 1.09, p =0.30, d = -0.08. However, average power was significantly higher (around 25 W) in the O_2Supp condition (654.6 ± 86.9 W) compared with the Normoxia condition (629.2 ± 96.2 W) across the 10 sprint repetitions; t (9) = -4.65, $p < 0.01$, d = 0.28 (Figure 6-2).

Mean BLa was higher in the O_2Supp condition (9.81 mmol·L^{-1}), although only by a small margin (0.43 mmol·L^{-1}) t (9) = 3.36, $p < 0.01$, ES = -0.13. When comparing sprints directly between conditions, it was only after sprints 4 and 8 that this difference reached significance (Figure 6-3).

MVC : As expected there was a main effect of time on muscle force, (F (1,12) = 34.47, $p < 0.01$, d = 4.14) with a decrease in MVC post the sprints (pre 774.4 ± 46.3 N vs post 587.9 ± 43.7 N) Despite a somewhat larger decline in MVC in the O_2Supp trial, there was no statistical difference between conditions (p = 0.66, d = 0.25) (Table 6-1). There was no interaction effect for condition x time (p = 0.08)

PTF: Again a main effect was found for time (F (1,12) = 53.03, $p < 0.01$, d = 8.66) with a smaller potentiated doublet twitch production post sprints compared to pre-sprint (pre 459.2 ± 22.2 N vs post 290.4 ± 16.5 N), but not for condition (p = 0.86, d = -0.03). There was no interaction effect reported for condition x time (p = 0.31) for PTF.

VA: A main effect was found for condition (F (1,12) = 8.23, p = 0.01, d = 2.23) with a higher voluntary activation in O_2Supp compared with Normoxia (O_2Supp 79.1 ± 2.2% vs Normoxia 74.3 ± 2.1%), but no effect of time (p = 0.14). Importantly there was no interaction effect reported for condition x time (p = 0.79) for VA.

Table 6-1: Neuromuscular function of the knee extensors; Maximal Voluntary contraction (MVC), potentiated doublet twitch (PTF) and voluntary activation (VA) (*n* = 14).

	Normoxia			Oxygen Supplementation		
	Pre-Sprint	Post Sprints	% Difference	Pre-Sprint	Post Sprints	% Difference
MVC (N)	762.6 ± 169.1	611.3 ± 150.6	19.8	786.1 ± 187.0	564.4 ± 204.6	28.2
PTF (N)	452.1 ± 86.0	295.4 ± 64.0	34.6	466.2 ± 95.5	285.4 ± 67.4	38.8
VA (%)	75.8 ± 10.0	72.8 ± 9.7	3.9	81.2 ± 10.3	77.1 ± 9.6	5.0

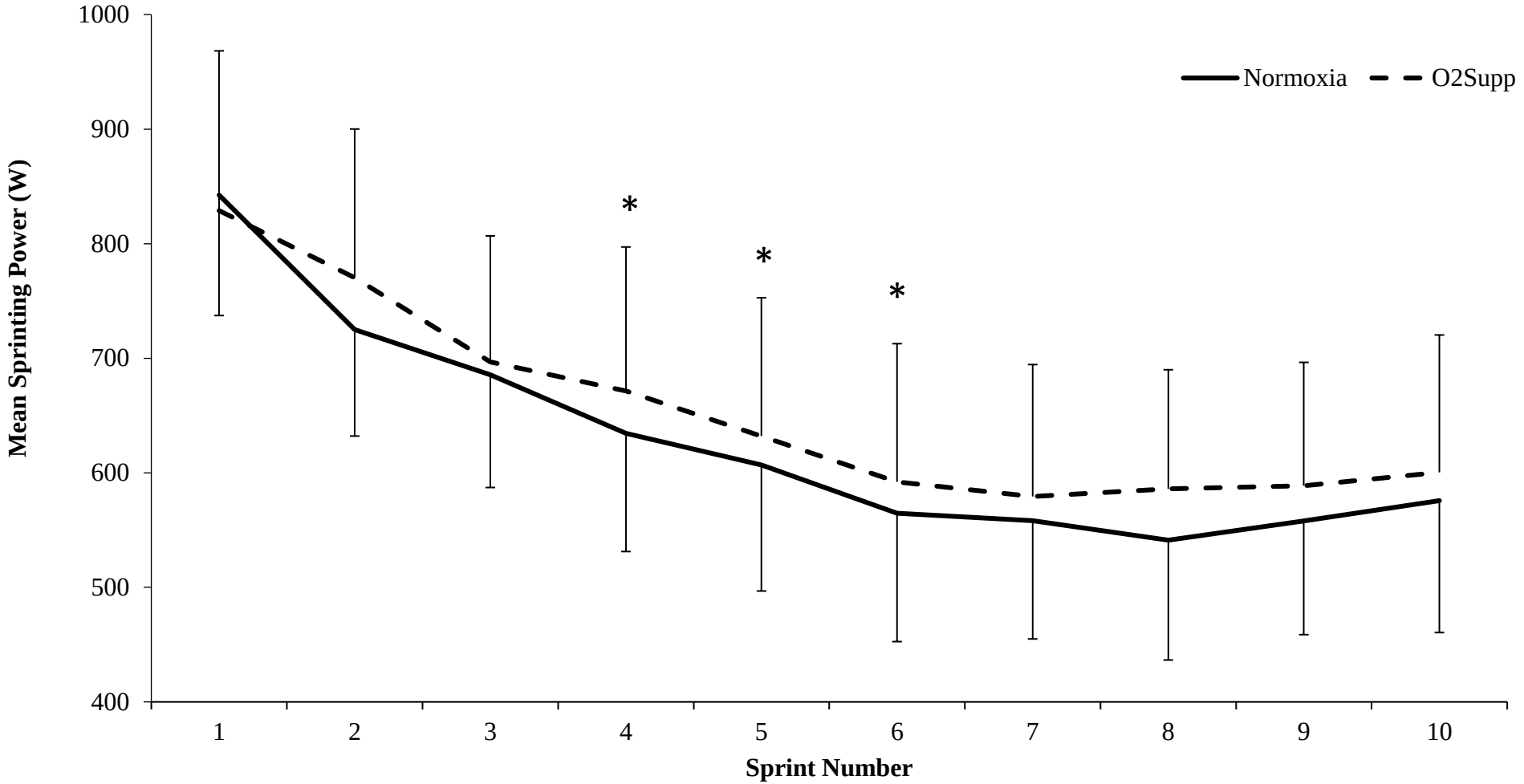


Figure 6-2. Mean sprinting power across 10 sprints (*n* = 14). * significant difference between conditions (O_2Supp & Normoxia) ($p < 0.05$).

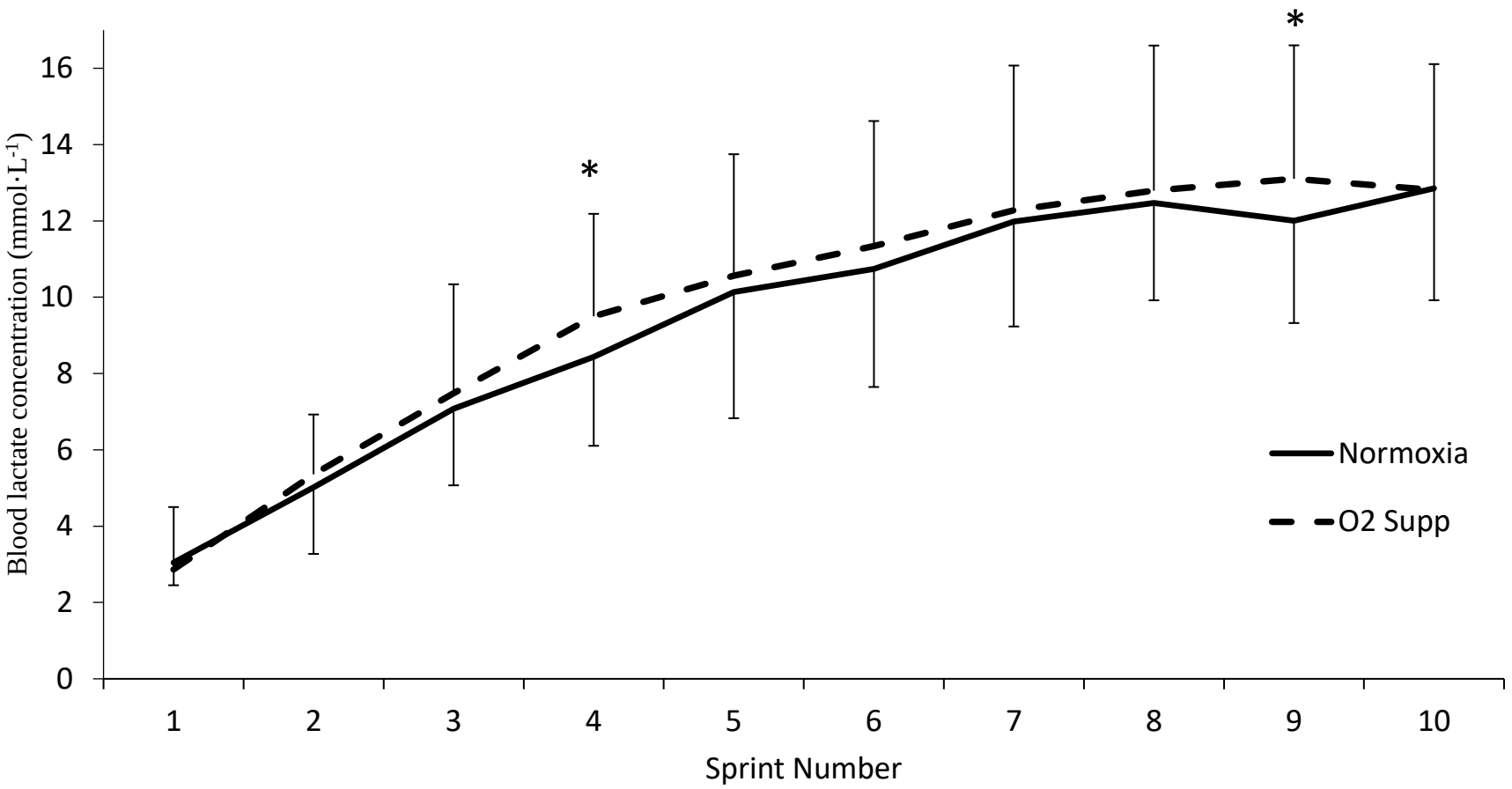


Figure 6-3. Mean blood lactate concentration (mmol·L^{-1}) over 10 sprints (n = 14). * significant difference between condition (O_2Supp and Normoxia) ($p < 0.05$).

6.6 Discussion

The aim of this study was to identify the effects of O_2Supp on repeated sprint ability and begin to examine its effects on muscle fatigue using interpolated twitch. Though peak cycling power was not different between conditions, average power was higher in the O_2Supp trials, by around 4%, indicating that a higher work output could be maintained in the presence of extra oxygen. This higher mean power was associated with a higher BLa level. There was no significant effect of condition on changes in muscle fatigue measures (assessed by MVC and electrical stimulation).

The current study found that breathing hyperoxic air during 15 s repeated sprint efforts led to a higher mean power output, (around 25 W), with no significant influence on peak power. Hauser *et al.*,[136] found that mean power was not different between the two conditions in their study (FiO_2 1.00 and FiO_2 0.21). However, their methodology of 3 x 3 min sprints meant their participants were potentially predominately using a different energy system to that used in a 15 s sprint. The lactic acid system and the aerobic system are the predominate sources of ATP production during maximal 3 min efforts, whereas the during 15 s sprints the majority of ATP is supplied by the ATP-PC system. Second, Hauser's participants experienced O_2Supp between efforts only. Timing of O_2Supp is potentially key. For example, Sperlich *et al.*, [3] also report no difference in mean or peak power across 2 sets of 5 x 30 s cycle sprints. Although nature of the exercise was more similar to the current study, in contrast, their participants only had supplementary oxygen in the 6 min recovery period between sets of cycle sprints.

O_2Supp has been shown to attenuate the onset of fatigue at a peripheral level whilst also maintaining cerebral oxygenation [127]. In the current study, MVC, a global measure of fatigue, dropped by around 20% in the Normoxia condition, and by around 28% in the O_2Supp

condition. This shows that there was greater (albeit non-significant) muscle fatigue as a result of the higher mean power output in the O_2Supp condition. The change in PTF and %VA were broadly similar across conditions. The drop in voluntary activation (%) was small and less than 1.5% different between conditions. The drop in PTF however was large, although again similar between conditions (34 and 38%, Table 6-1). These results support the findings of Thomas *et al*., [172], who report performance in shorter efforts is predominately curtailed by peripheral measures.

The ability to maintain power throughout repeated efforts has been attributed to several factors including the ability to maintain BLa, regulate pH, to maintain neural input[180] and importantly, to replenish PCr stores. According to Linossier *et al.*, [114] sprint capacity is not reduced to the same extent in O_2Supp due to the increased rate of cellular metabolic resynthesis of PCr, and ATP. PCr resynthesis during 45 s of recovery only replenishes ~75% of the stores [52]. Therefore, after sprint 5 in a series such as this one would expect to see a significant performance reduction. A continued depletion of ATP-PC stores and an inadequate resynthesis leads to a reduction in performance until additional energy systems aid in the resynthesis process (lactic acid system). An increase in peak and mean power has been attributed to an enhanced PCr resynthesis during repeated high intensity exercise by both Mendez- Villanueva *et al.,*[93] , and Glaister,[52]. Mendez- Villanueva [93] analysed PCr recovery rate during 10 x 6 s sprints with 30 s recovery and found that subsequent sprinting performance (peak power) during a final single sprint was increased corresponding with an 8% higher PCr resynthesis.

Increasing the percentage of inspired oxygen to 100% increases the rate of PCr replenishment from a half-life of 25 s to 20 s [163,181]. Haseler *et al.*, [163] used a plantar flexion exercise protocol with increasing workload till exhaustion. They found that an increased rate of PCr resynthesis

aided subsequent performance, and that O_2Supp maintained mean plantar flexion power further into the ramp test protocol (1 W increase every 2 min by pulley system). This is replicated in in the findings of the current study as it is shown that mean sprinting power during O_2Supp is similar to that during Normoxia until sprint 4 and beyond where the difference becomes statistically significant. Additionally, sprint 6 is where peak power in the normoxic group appears to decline at a greater rate than the hypoxia condition. Sprint 6 in a typical series is where it has been documented the reliance on aerobic metabolism increases [52].

Interestingly, the increase in sprint performance as a result of O_2Supp is similar to that seen with creatine supplementation, likely through the same mechanisms of PCr resynthesis [52]. Additional to this enhanced rate of resynthesis, O_2Supp has been shown to attenuate the build-up of metabolic by products of lactate, and inorganic phosphate (Pi) [167]. Pi in particular is detrimental to performance via inhibition of muscle afferents. Type III and IV muscle afferents relay exercise induced metabolic changes in the muscles to the central nervous system. The accumulation of metabolic by-products reduces the effectiveness of these afferents, subsequently leading to peripheral fatigue. However, both afferents are positively influenced by O_2Supp, by allowing the electrical feedback to be transmitted efficiently for longer [167], so attenuating fatigue.

O_2Supp elicits reductions in BLa at many workloads [114,122] and although O_2Supp given during recovery periods attenuates lactate accumulation, the effects are more variable [54]. Maeda *et al.,* [54] gave varying percentages of O_2Supp (FiO_2 0.30 to 1.00) in the recovery between sprints, and found that whilst overall increasing the FiO_2 resulted in reduced BLa after standardised exercise, the response was dependent on the subjects' fitness. In the current study, higher power outputs seen in the O_2Supp condition were associated with slightly increased lactate levels.

Knight *et al.*, [182] suggest that the increase in oxygen kinetics during O_2Supp is enough to attenuate the accumulation of lactate due to the increased diffusion of oxygen into the mitochondria. However, they add that that this attenuation can only last so long, and after a critical point lactate levels will increase exponentially. This could explain the increases in lactate that have been seen in the current study.

Therefore, it is suggested that O_2Supp results in a combination of increased PCr resynthesis and a slightly attenuated build-up of BLa, leading to a 'maintained' performance compared to the Normoxia condition seen in the current study. Acute exposure to additional oxygen appears to enhance repeated sprint performance, however, determining whether chronic physiological adaptation is blunted is crucial before its widespread use as a training tool can be advised.

6.6.1 Limitations

Several limitations of this study, participants were required to avoid strenuous exercise 24 h prior to testing but it is noted that the effects of training may be evident for 48/72 h. To minimise the effects of this, participants were requested to mimic the training three days prior to testing before both visits. Further, no direct measure of fitness ($\dot{V}O_2$ max) was conducted to characterise the study population. Level of fitness is a potential mediator of response to O_2Supp. Additionally, hydration status was not measured prior to testing, but participants were instructed to attend testing in a hydrated state.

6.6.2 Practical Implications

- Supplementary oxygen given during a single sprint-based cycling session can assist in reducing the extent that performance decreases.
- Peak power output cannot be increased with supplementary oxygen

- Long term effects of the use of oxygen during training are not known and therefore its use as a chronic training tool is not yet advised

6.7 Conclusion

Whilst supplementary oxygen does not increase peak power during repeated sprints, participants were able to maintain a higher mean power output (across the 10 sprints). Indices of fatigue (MVC, PFT and VA) changed to a similar extent across conditions in response to the cycling, but the largest drop was in PFT, suggesting fatigue to be predominately peripheral in nature.

6.8 What Next?

Combining the findings of chapter 4, 5 and 6 it is evident that O_2Supp is an ergogenic aid for repeat sprint cycling. It is also evident that other mechanisms are at play, other than peripheral muscle fatigue. Future research should begin to follow the pathway of oxygen after respiration to assess whether performance during O_2Supp is limited by delivery or utilisation oxygen to the working muscles.

7 - Experimental Chapter:

The Use of Acute Oxygen Supplementation Upon Muscle Tissue Saturation During Repeat Sprint Cycling

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A version of this chapter has been published as a research article. Citation as seen below.

Porter M, Reed K, & Jones B. The Use of Acute Oxygen Supplementation Upon Muscle Tissue Saturation During Repeat Sprint Cycling. Journal of Human Sport and Exercise, in press, (2020) https://doi.org/10.14198/jhse.2022.171.10

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7.1 Abstract

7.1.1 Objective:

O_2Supp (>0.21 oxygen) as an ergogenic aid is increasingly being investigated and utilised by athletic populations. This study examined performance and physiological responses (power output, tissue saturation index) to repeat sprint cycling with oxygen supplementation (fraction of inspired oxygen [FiO_2]1.00).

7.1.2 Design and Methods:

Fourteen male amateur level cyclists took part. Testing comprised two visits to the laboratory. Sessions entailed; 15 min relative intensity warm-up, 10 min of passive recovery, followed by 10 x 15 s repeated sprints, during which air inspired had FiO_2 1.00 oxygen or normal air.

Outcome measures include, mean power (W) and change in Tissue Saturation Index (ΔTSI%). Repeated measures ANOVA were used to examine difference between conditions in mean power output. Paired samples t-tests were used to examine differences between conditions in ΔTSI (%) and rate of muscle reoxygenation and deoxygenation (%·s^{-1}). The α level was set 0.05 *a priori*.

7.1.3 Results:

Mean power output was 4 ± 2.6% higher in the oxygen condition compared to Normoxia ($p < 0.01$). There was a significant positive correlation between power output and reoxygenation rate during O_2Supp (r = 0.65, $p = 0.04$). No correlation was seen between power output and reoxygenation rate during Normoxia (r = -0.30, $p = 0.40$). A significantly increased deoxy rate was seen in the O_2Supp condition compared to Normoxia ($p = 0.05$).

7.1.4 Conclusion:

O_2Supp (FiO_2 1.00) appears to elicit the greatest performance improvements in mean power, potentially facilitated by an increasing muscle reoxygenation rate. This evidences the utility of oxygen as an ergogenic aid to in cycling performance. This may have implications for the chronic application of oxygen as a training tool.

7.2 Background

The previous studies have highlighted the effectiveness of O_2Supp as a performance aid during repeated sprints, whilst standardising the overlapping methodologies. This study was established to further the previous research conducted within this thesis. The first two experimental chapters (4 & 5), established when O_2Supp is most effective during a repeat sprint protocol, and chapter 6 began to narrow down the specific mechanism behind why these findings are evident. Chapter 6 found that peripheral fatigue does not limit performance capacity during O_2Supp, therefore the forthcoming study was designed to assess peripheral muscle oxygen profile to try to establish a specific mechanism for such performance changes with O_2Supp.

7.3 Introduction

Hyperoxia occurs when cells, tissues and organs are exposed to a level of oxygen higher than that of sea level [59]. To create hyperoxic conditions one must breathe medical grade oxygen or an oxygen enriched gas mixture (O_2Supp). The creation of a hyperoxic condition can be viewed as an ergogenic aid, which is being explored within various sporting populations.

O_2Supp primarily functions on the premise of increasing the supply of muscle oxygen at exercise onset [76]. O_2Supp has been shown to aid repeated sprint performance through an increased resynthesis rate of cellular metabolic PCr [99,163]. Moreover, O_2Supp (FiO_2 0.7) has been shown to spare PCr degradation (by up to 55 s) over fixed workloads, compared with Normoxia [76]. It could be expected that O_2Supp during repeat sprint training would be effective at increasing the resynthesis rate of PCr and muscle oxygenation. Multiple sporting events rely on the ability to replenish and recover intramuscular stores (myoglobin oxygen saturation

[MbO_2], PCr, ATP) between high intensity efforts to enable continued high intensity performance [52,99].

High Intensity Interval Training (HIIT) simulates the energy demands of intermittent-sprint sports, with high intensity bouts followed by brief periods of recovery [13]. HIIT is categorised by peak ability during the first interval followed by decreasing performance in the subsequent repetitions [164,183]. Short duration sprints (< 15 s) interspersed with brief recoveries (< 60 s) result in near complete depletion of PCr during work periods, and incomplete resynthesis of PCr during the rest periods. This resynthesis is an oxidative process that requires free oxygen for rapid resynthesis. Performance during acute HIIT is reliant on reducing lactate accumulation and maintaining muscle oxygen status to resynthesise PCr rapidly [184].

HIIT based research has utilised NIRS extensively [14,185,186]. NIRS provides a non-invasive assessment of muscle oxygenation (tissue saturation) and haemodynamic status (peripheral blood flow) [187,188]. Distinct changes in tissue saturation have been observed by NIRS during HITT, characterised by large desaturation and restauration profiles. Similarly, the recovery time course of muscle oxygenation has been suggested to be correlated to PCr, at least following sub-maximal exercise, and resultantly NIRS is considered a proxy of PCr resynthesis [72,189]. Attenuating a decline in S_aO_2 with concurrent O_2Supp can increase full body oxygenation, by as much as 7% [163]. Recent evidence suggests that increasing the recovery rate of muscle oxygen can be correlated with improved performance in repeat sprint efforts [17,190]. These findings highlight the apparent importance of ‘enhanced’ muscle oxygenation profiles on subsequent performance.

Invasive measures of muscle oxygenation (muscle biopsies) have been previously used in O_2Supp research, however, this methodology has very little ecological application [140]. It is suggested the NIRS technique should be used within O_2Supp research to better characterise the peripheral muscle response to this ergogenic aid and more fully inform exercise practitioners in 'real world' settings.

A primary aim of training modalities such as, HITT and/or repeat sprint cycle training are to enhance the delivery and utilisation of oxygen [191]. O_2Supp has been shown to aid performance [169] within these exercise disciplines, intuitively NIRS could provide useful novel mechanistic insight for the response to O_2Supp.

The aim of this study was to assess the peripheral muscle oxygen response to this ergogenic aid using the NIRS technique. We hypothesised that both muscle oxygenation and performance (power output) would differ during oxygen supplementation compared with normal air.

7.4 Methods

7.4.1 Study Design

Participants were required to undergo two testing session in the lab over a week period. Sessions were completed with at least 48 h between sessions. Laboratory visits were conducted at the same time of the day (± 2 h) to minimise circadian effects [192]. Participants were asked to maintain normal activity and sleep pattern prior to and between testing sessions. Participants were requested to arrive at the laboratory adequately hydrated and to abstain from caffeinated products in the preceding 4 h of each visit. Additionally, participants were asked to refrain from strenuous physical activity 24 h prior to participating.

This study was a single-blind, within-participant design comprising two counterbalanced assessments of repeat sprint performance under: O_2Supp (FiO_2 1.00) or normal air (FiO_2 ~ 0.21). Each of the two sessions participants completed a 15 min cycling warm up, 10 min passive recovery, finishing with 10 x 15 s sprints with 45 s of passive recovery on a Wattbike cycle ergometer. All participants had previous experience with repeat sprint lab testing protocols. One participant had been included in previous oxygen supplementation research within the last 12 months. Performance measures such as: mean and peak power output were taken, along with NIRS measures of muscle oxygenation (resaturation and desaturation rates), as well as blood lactate concentrations.

Fourteen male amateur level cyclists for the study (1.81 ± 0.04 m, 77.7 ± 11.0 kg, 25.9 ± 7.4 years, thigh skinfold 10.5 ± 4.1 mm). Participants were healthy and were not taking any prescribed medications.

Ethical approval for the studies procedure was granted by the University ethics committee in accordance with the Declaration of Helsinki. Participants were informed of the procedure and asked to give written informed consent and complete a health questionnaire (PAR-Q).

7.4.2 Experimental Protocol

Participants completed the same procedure on both visits comprising; 15 min warm up 52% of heart rate reserve [175], 10 min passive recovery, finishing with 10 x 15 s sprints with 45 s of passive recovery, undertaken using a Wattbike Pro (Wattbike Ltd., Nottingham, UK) with the magnetic setting set to zero and air brake set to ten. The protocol was in accordance with pilot testing conducted prior to this study i.e. participants had sufficient load in order to reach peak power and not exceed max cadence (Figure 7-1).

Wattbike Pro was used to collect performance data (mean power), which was then used to calculate fatigue index (FI%) ((Best sprint – worst sprint)/best sprint) *100. No prior familiarisation was conducted in the current study as it was established that each participant was familiar with repeat sprint cycling on a cycle ergometer.

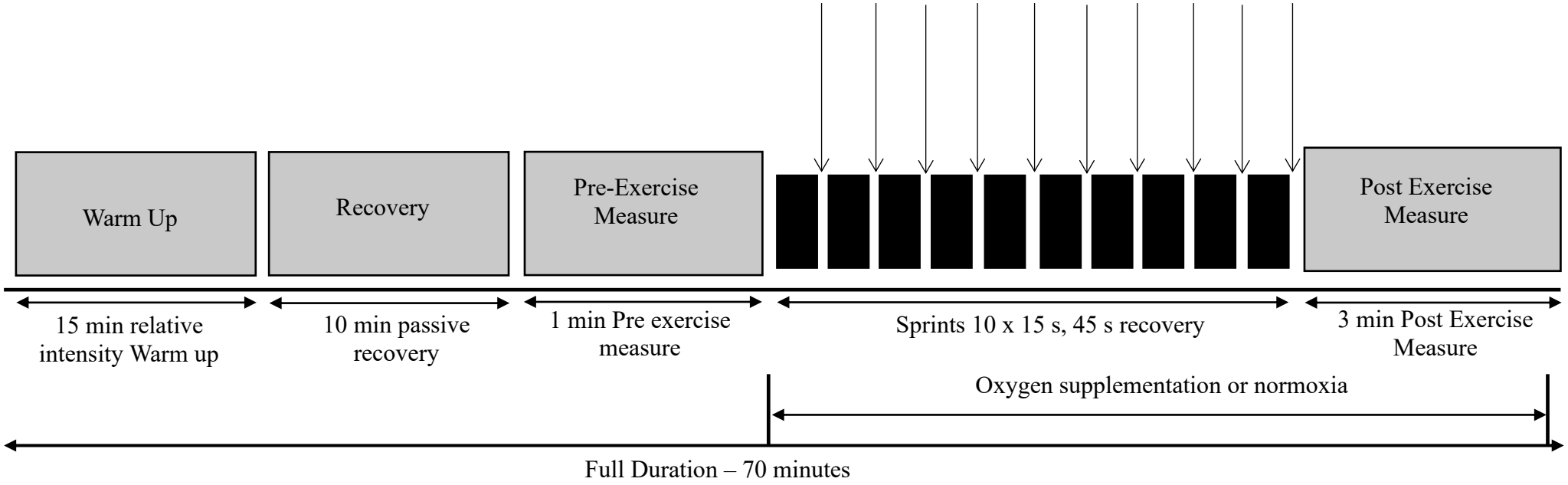
Hyperoxic and normoxic gas mixtures were administered throughout the repeated sprints. Gas mixtures were administered via a rig of 4 x 200 L Hans Rudolph Douglas bags connected to a Hans Rudolph mask and head net (Hans Rudolph, Shawnee, KS, USA) were used. O_2Supp was provided using Douglas bags filled from a medical grade oxygen cylinder (British Oxygen Company, Surrey, UK) prior to administration, as previously described. Hyperoxic air at FiO_2 of 1.00 was saturated and warmed to room temperature before administration.

Preceding to each trial, a pre-exercise 20 µl capillary blood lactate sample was taken from the right ear lobule. Sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Subsequent blood lactate samples were taken two minutes apart after the period between the warmup, and during the recovery period of each sprint repetition. All samples were analysed within using a Biosen (EKF diagnostics, Cardiff, UK).

Participants were required to wear a portable NIRS device to monitor oxygen saturation of the *vastus lateralis* muscle tissue (PortaMon, Artinis Medical Systems B.V., Elst, Netherlands). The NIRS device was fixed to the belly of the right *vastus lateralis*. Any bodily hair was removed within the device placement area and cleaned with an alcohol wipe to remove residue. The device was placed 3 cm anterior to the midpoint between the top of the greater trochanter and the lateral epicondyle. The device was taped with an adhesive wrapping and secondly wrapped with a black-out sports strapping to eliminate the entrance of ambient light. The same researcher attached the device on every occasion, ensuring an external pressure of less than 20 mmHg on the device. Indelible ink was used to draw around the device to guarantee accurate NIRS placement during the subsequent visits. Throughout the protocol the NIRS devices were connected to a personal computer via the Bluetooth™ system for data acquisition (10 Hz), and conversion from analogue to digital data.

The tissue haemoglobin saturation index (TSI), expressed in % and calculated as $([O_2Hb]/([O_2Hb + HHb])) \times 100$ (which demonstrates the O_2 supply and O_2 consumption) [193] was utilised. TSI was calculated using the SRS methodology and was used to assess muscle reoxygenation rate. Reoxygenation rate (reoxy rate) ($\%\cdot s^{-1}$) was calculated as the change in TSI (%) from the end of the sprint by fitting a linear model to the 45 s part of the TSI (%) recovery. The slope of the relationship was retained as an index of reoxygenation rate.

Similarly, deoxygenation rate (deoxy rate %·s^{-1}) was calculated as the change in TSI (%) from the beginning to end of the sprint by fitting a linear model to the 15 s part of the TSI (%) decline. Change (Δ) values were obtained during sprint and recovery periods. These were taken as; the difference between the baseline value (start sprint) and the one second average of the maximum value achieved during the sprint period and; the baseline value (end sprint) and the one second average of the maximum value achieved during the recovery period. NIRS data was processed using the methodology suggested by Rodriguez [153]. A one second moving average was applied to the data to attenuate the "noise" in the signal, whilst maintaining the integrity of the original data.



Warm up:

- Sub maximal exercise
- 15 min
- Level 4 Wattbike (~200W)

Session:

- 15 sprint (seated)
- 45 s recovery (passive)
- 10 sprints
- Maximal air brake and 1 magnetic brake.

Blood lactate measure

Figure 7-1. A schematic representation of the experimental methodology during experimental Chapter 4.

7.4.3 Statistical Analysis

An a priori power analysis revealed that 10 participants would provide significant power to detect differences at an α-level of 0.05 for the primary outcome measure (mean power output) (G∗POWER 3.1 Software, Düsseldorf, Germany). Statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS, Inc, Chicago, IL, USA).

A two-way repeated measure ANOVA (condition X time) was used to analyse differences in the 10 x 15 s sprints across the different protocols, followed by Turkey's post hoc tests where appropriate. Paired samples t-tests were conducted to examine the differences between the conditions for; change in tissue oxygenation (ΔTSI %), deoxy rate (%·s^{-1}) and reoxy rate (%·s^{-1}). Pearson's correlations were conducted to test the relationship between tissue oxygenation and power output between the conditions. Shapiro- Wilk normal distribution tests were conducted on all data. Data was screened for outliers outside of two standard deviations from the mean, no data were excluded. Effect size for individual measures were calculated and reported as Cohen's *d* and interpreted using bounds as 0.2, 0.5, > 0.8, where they are small, medium and large respectively. α-level set at $p = 0.05$ for all analyses.

7.5 Results

Figure 7-2. shows mean sprint power output (W) per sprint interval. ANOVA found a significant interaction effect F (9,13) = 2.66, p = 0.045, d = 0.98) and post hoc analysis shows a higher mean sprinting power for sprints 4-6. There was a significant increase (~4 ± 2.6%) in power output (W) in the O_2Supp group mean compared to the norm group (d = -0.28, 95% CI = 0.87 to 2.52; p < 0.01). Figure 7-2 shows a non-significant; t (13) = 1.735, p = 0.11, d = 0.23 reduction in Fatigue index (FI) following 10 cycling sprints under the two conditions. A 3% reduction in FI (%) is seen in the O_2Supp condition.

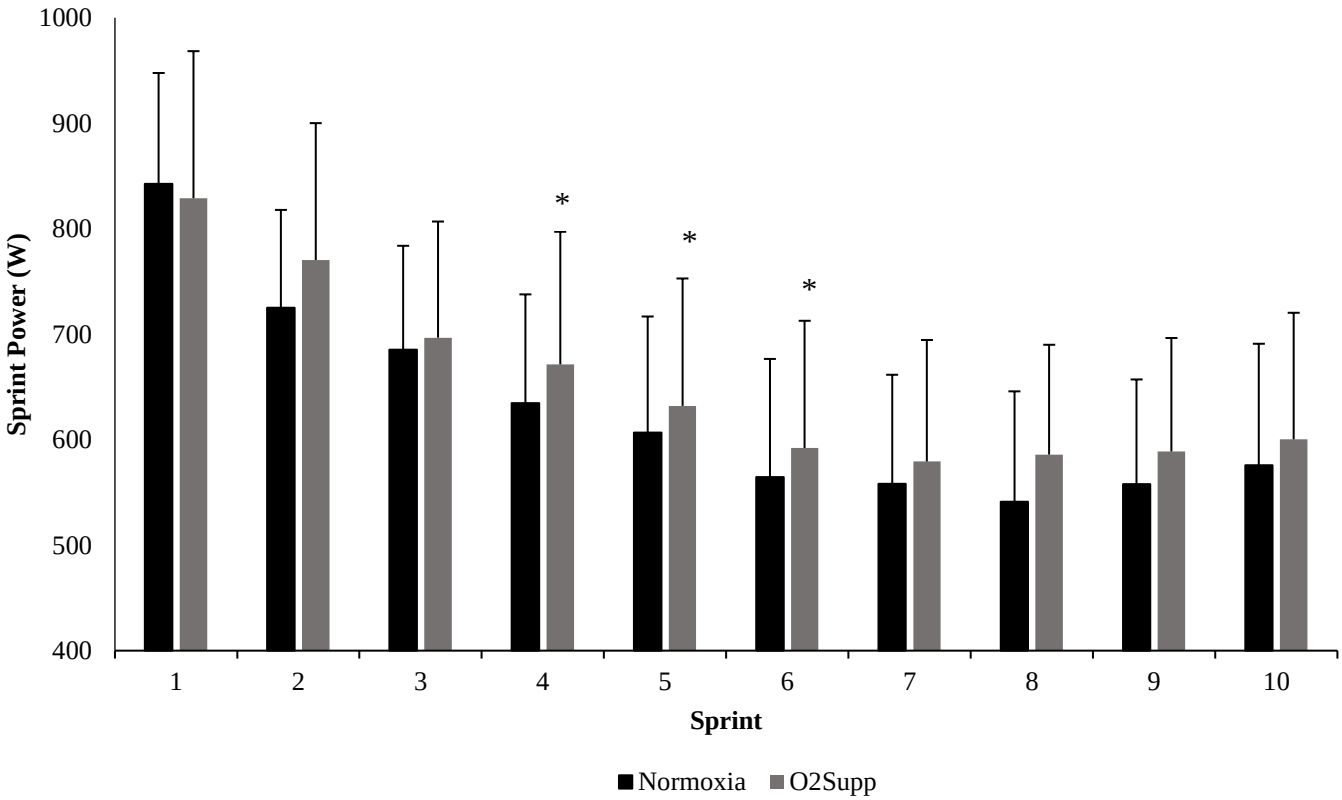


Figure 7-2. Mean sprinting power across 10 sprints * significant difference between condition (O_2Supp and Normoxia) (p < 0.05).

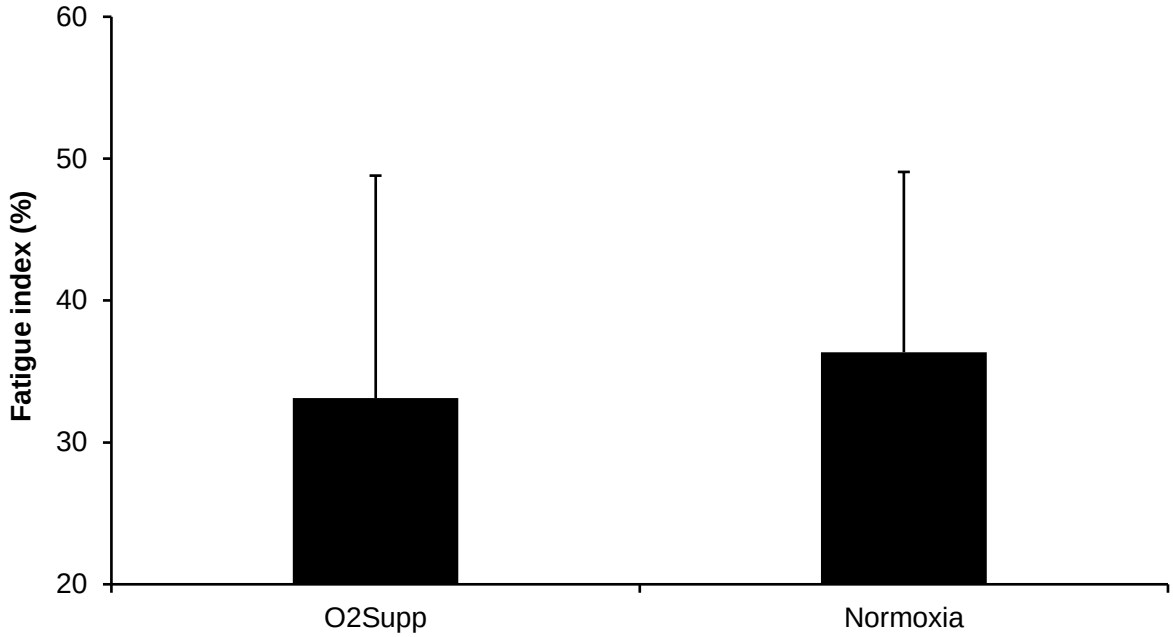


Figure 7-3. Fatigue index score under two conditions (*n* = 14).

Figure 7-4. displays the group average Normoxia vs. O_2Supp ΔTSI (%) data trace during (10 x 15 s with 45 s recovery) repeat cycling efforts. During the repeat efforts there was a rapid drop in TSI at the onset of each sprint with a nadir achieved approximately 10 s into each sprint. During each 45 s recovery period there was a trend of a rapid recovery of group TSI (first 20 s - Phase 1), followed by a slowing in the recovery rate (final 25 s – Phase 2). The rate and extent of TSI recovery in the O_2Supp condition is facilitating a quicker oxygen resaturation to baseline; this hyperoxic effect is clear for recovery periods 1-3. This response is attenuated as the sprints progress (sprints 4-10). After sprint 6 TSI fails to return to baseline as the sprints are completed.

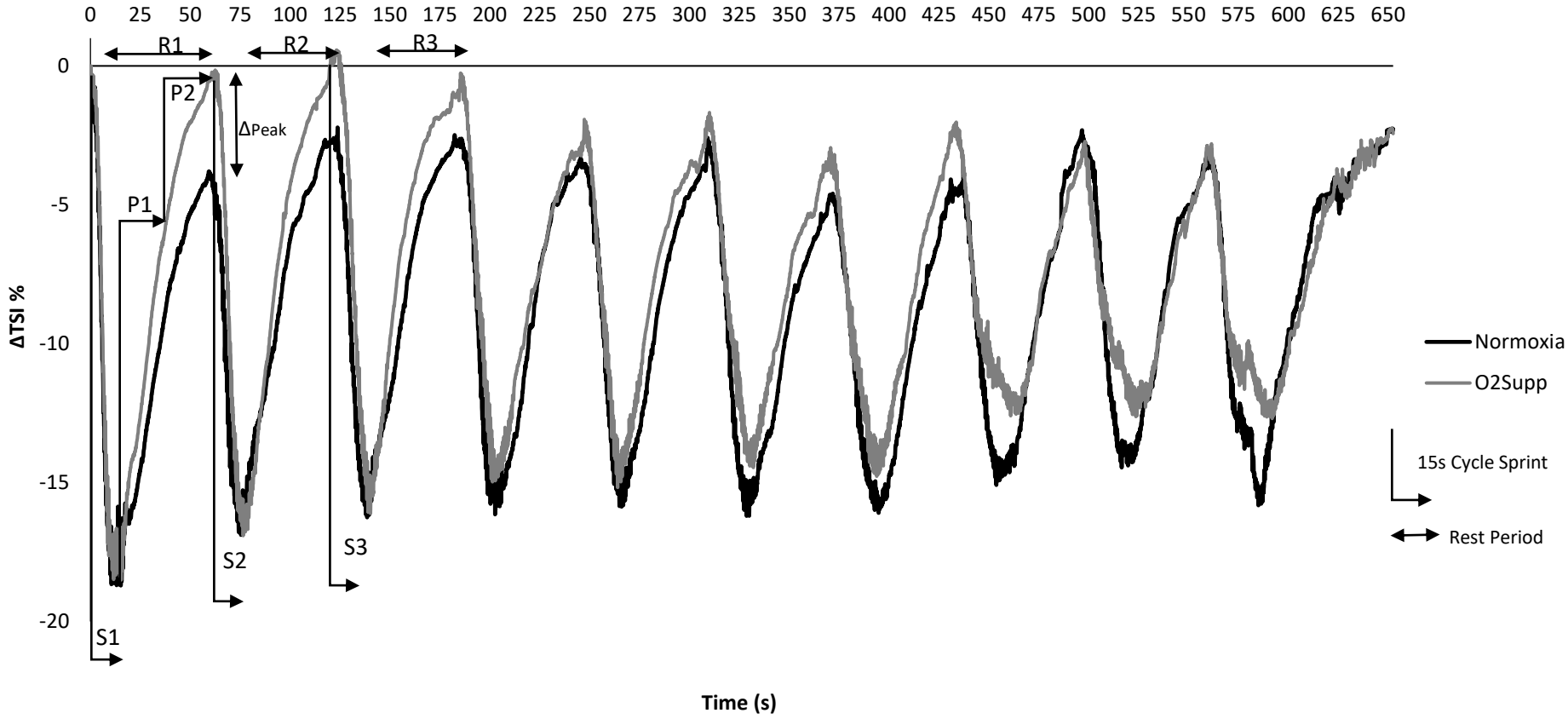


Figure 7-4. Tissue Saturation Index (TSI %) characteristic representation data for the group, during the repeat sprint cycling protocol. Normoxia and O_2Supp traces are overlaid.

Legend. ΔPeak – Change in peak resaturation, P1- Phase One, S1- Sprint One, R1- Recovery.

Mean blood lactate was higher in the O_2 Supp condition (9.81 mmol·L^{-1}), albeit by a small margin (0.43 mmol·L^{-1}), t (9) = 3.36, $p < 0.01$, $d = 0.13$. When comparing sprints directly between conditions, it was only after sprints 4 and 8 that this difference reached significance.

No significant difference was seen in the mean recovery amplitude (i.e. Δ) ΔTSI (%) in the O_2Supp group vs. Normoxia group (d = -0.33, 95% CI = -1.71 to 0.51; $p = 0.25$). A mean difference in individual sprints ΔTSI (%); 2.4%, 2.2% and 3.0% can be seen in Figure 7-4, for Sprints 4-6 O_2Supp vs. Normoxia group. No significant difference was seen within the group mean reoxy rate (%·s^{-1}) between the two conditions (d = 0.33, 95% CI = 0.01 to 0.04; $p = 0.26$). A significantly increased deoxy rate (%·s^{-1}) was seen in the O_2Supp condition compared to Normoxia (d = -0.61, 95% CI = -0.13 to -0.00; $p = 0.05$).

Figure 7-5. displays a positive correlation between reoxy rate (%·s^{-1}) and mean sprinting power during O_2Supp (r = 0.65, $p = 0.04$). No significant correlation was found between ΔTSI (%) and mean sprinting during Normoxia (r = -0.30, $p = 0.40$).

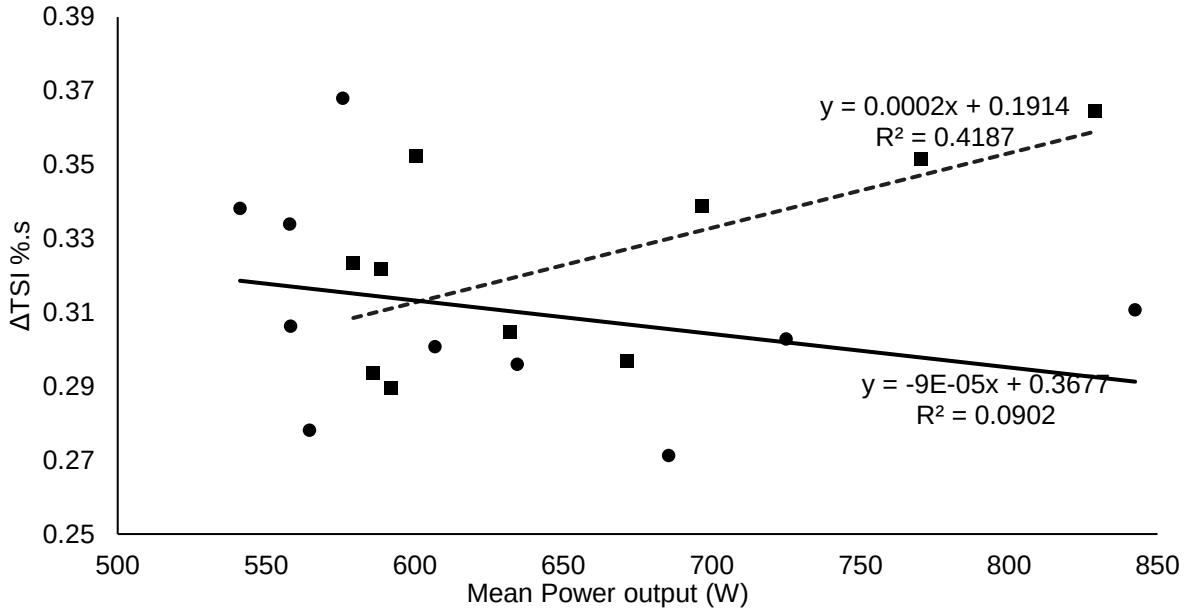


Figure 7-5. Relationship between muscle reoxygenation rate (%·s^{-1}) and mean cycling power (W) (*n* =14). ■ O_2Supp (---), ● Normoxia (____)

7.6 Discussion

The aim of this study was to assess the peripheral muscle oxygen response to this ergogenic aid using the NIRS technique. It was hypothesised that muscle oxygenation and performance would differ during oxygen supplementation compared to normal air. This is the first paper of its kind to assess changes in the rate of muscle oxygen resaturation, and desaturation during O_2Supp in repeat sprint cycling, using non-invasive 'real world' measures.

Primary findings revealed changes in muscle oxygen recovery (%·s^{-1}) rates in the presence of extra oxygen (FiO_2 1.00). This recovery rate was strongly correlated with preceding power output (W) (r = 0.65, p = 0.04) (Figure 7-2 and Figure 7-4). There was no correlation between recovery rate and power output during Normoxia (r =-0.30, p = 0.40) (Figure 7-5). Lactate levels were higher in the O_2Supp condition compared to Normoxia (p < 0.01). Additionally, there was an increased deoxy rate in O_2Supp compared to Normoxia across the 10 sprints (p = 0.05).

The rate of recovery of muscle oxygen is one of most important aspect of fitness to a sprint trained athlete [52]. The ability to flush out fatigue related metabolic by-products and the replenishment of fuel for successive sprints has obvious importance for performance. HIIT is highly reliant on the utilisation of PCr during exercise and exclusively upon aerobic processes (resynthesis) during periods of recovery [184]. O_2Supp has been shown to be beneficial for sprint performance due to the increased resynthesis rate of cellular metabolic PCr [114,163]. The significant increase in power output (3.95%), non-significant increase in the extent of ΔTSI (%) recovery and reoxygenation rate (%·s^{-1}) seen here are potentially indicative of an enhanced PCr resynthesis profile [72]. Though PCr resynthesis was not

measured directly, muscle (re)oxygenation has been suggested by others to be representative of this [72,189,190]. Additionally, the changes seen in performance variables (W) between O_2Supp and normal air, replicated previous research showing increases in mean power output (~4 ± 2.6% [30 W]) during O_2Supp (Figure 7-4).

Blood lactate data from this study have been previously published [169]. In brief, muscle oxygenation data is supported by the significantly reduced lactate response despite a higher power output. The ability to attenuate an anaerobic metabolic environment during repeat sprint work should allow for enhanced PCr resynthesis. Lactate and muscle oxygenation data highlight that despite higher intensity exercise (increased power output), there is a higher availability of oxygen. This suggests that in the initial sprints, oxygen availability is the primary mechanism for enhanced performance (Figure 7-2).

Studies by Jones *et al.,* [14,77] demonstrated a positive relationship between enhanced muscle oxygenation during recovery and increased subsequent performance following a training programme. Their study shows that a 3 s faster desaturation time contributes to increase in mean cycling performance by as much as 10%. This alteration in desaturation profile is suggested to occur as a result of positive peripheral muscle morphological adaptations such as increased mitochondrial density and efficiency, resulting in enhanced oxygen extraction [186,194]. The findings of the current study reflects potential adaptations due to similar desaturation profile, as seen in Jones *et al.,* [14,77]. These findings also mirror those of Buchheit *et al.,*[168] who also found that an increased rate of muscle deoxygenation (6%) during SIT elicits metabolic adaptations, such as increased citrate synthase activity, resulting in increased power output. The current study shows a significant increase in deoxy rate and non-significant increase in reoxy rate, similar to the previous studies [14,77,168]. Figure 7-5 highlights that both power and reoxy rate increases with O_2Supp although not

significantly different from control, they are significantly positively correlated- as power increases with O_2Supp so does reoxy rate. The increase rate of reoxygenation may likely cause the subsequent increase in performance as additional oxygen is available to the working muscle. This correlation is not evident during the Normoxia condition where reoxy rate has a weak negative correlation with power output. The simplest explanation for this is, due to an increased availability of oxygen as a result of the administered O_2Supp and not as a result of morphological adaptation to training.

Higher re-saturation rates occur from the first sprint, but it is not until sprint 4 onwards that performance increases are seen in the O_2Supp condition (Figure 7-4). The high availability of oxygen allows for rapid resaturation of myoglobin and haemoglobin, meaning subsequent sprints can start from a metabolically advantageous point. Inevitably there comes a point where, despite extra oxygen, recovery is not rapid enough to maintain performance.

Interestingly, the novel findings of this study open further avenues for exploration. Firstly, due to the acute nature (short term effects - minutes) of the current study, the changes in muscle oxygen and power output have only been evidenced acutely. Few long-term or short-term training studies using O_2Supp have examined the effects of 'chronic supplementation' on muscle oxygen response or peripheral muscle composition (increased mitochondrial density, increased baseline utilisation capacity of oxygen). Previous training studies [70,71] have demonstrated changes in tissue saturation profile and musculoskeletal adaptation i.e. increased mitochondrial biogenesis/ proliferation, increased enzyme activity (citrate synthases) following SIT protocols. It would appear intuitive for further studies to initially identify the effects of acute O_2Supp administration on performance using NIRS

(as shown here). An understanding of the effects of O_2Supp, whether this is duration and/ or dosage dependant, will be key to optimal administration.

Despite equivalent findings between repeat sprints, time trials (TT) and time trials to exhaustion (TTE) using O_2Supp, there is a lack of comparable studies with short duration recoveries following high intensity repeat sprints, even though this training approach is commonly used in sprint training programmes [13,52]. Using TT or TTE as a training approach for intermittent sports is not popular despite the performance benefits for TT and TTE [116,195]. As such the current study should pave the way for further studies exploring optimised training for high intensity repeat sprint training.

7.6.1 Limitations

Due to a technical issue, S_aO_2 was not ascertained during data collection. Having data on the individual response of S_aO_2 during O_2Supp, would have allowed the authors to determine whether changes in the muscle oxygenation were related to delivery or utilisation. Furthermore, ventilatory parameters would have better informed the results of this study. Unfortunately, collecting ventilatory gases whilst administering a manipulated gas content, posed logistical constraints that could not be overcome.

7.6.2 Practical Implications

Many cyclists incorporate some form of HIIT in their training, with the aim of improving cycling performance. The results of the current study show that in University level cyclists (amateur) when compared with a sea level condition, O_2Supp elicits immediate acute enhancements to performance measures via an improved muscle oxygenation status. This allows athletes to experience an immediate enhancement in their

training, allowing them to work harder in a single training session, with the aim of inducing greater resultant adaptation. NIRS is a functional tool in which skeletal muscle oxygenation data can be seen in 'real time', complimenting external power data , allowing coaches to make better informed decisions [191].

7.7 Conclusion

Supplementary oxygen elicits meaningful performance improvements whilst increasing muscle reoxygenation rate during repeat cycling sprints as shown in Figure 7-5. This increase in reoxygenation rate may likely cause the subsequent increase in performance. This study looks at the mechanistic approach to O_2Supp and demonstrates the potential utility of O_2Supp as an ergogenic aid within cycle and repeat sprint exercise. This novel study is the first to demonstrate changes in muscle oxygenation during O_2Supp using non-invasive 'real world' measures.

7.8 What Next?

This chapter further develops the narrative within this thesis. The first two chapters (4 & 5) assessed the timing of administration and enabled a standardised protocol for the following chapters. The previous chapter (6) explored a potential mechanism to justify the performance enhancements being shown. With this chapters results the narrative is now that O_2Supp is a performance enhancing aid that increases delivery of oxygen to the working muscles, which maintains peripheral levels of fatigue during the exercise task. Further research should assess whether O_2Supp is effective at eliciting chronic performance improvements following a period of multi acute sessions with O_2Supp.

8 - Experimental Chapter:

The Feasibility of Using Near Infrared Spectroscopy to Assess Muscle Oxidative Capacity Following Training with Oxygen Supplementation

8.1 Abstract

8.1.1 Objectives

The primary aim of this study was to assess the feasibility of using NIRS utilising arterial occlusions to measure muscle oxidative changes during a multi week training intervention with O_2Supp. Secondary aims were to assess recruitment and adherence to training and the ability to collect training data from both supervised and unsupervised training sessions of participants.

8.1.2 Design and Methods

13 amateur level cyclists, stature 1.77 ± 0.06 m, mass 77.4 ± 9.6 kg, age 38 ± 8.3 years, performed 3-week training block consisting of two supervised HIIT sessions a week and an additional unsupervised session 2-4 times a week. Participants were randomly allocated to either O_2Supp (FiO_2 1.00, n = 5) or Normoxia (FiO_2 0.21, n = 4) during the supervised training. An additional control group (n = 4) undertook only unsupervised training. Vastus lateralis muscle oxygenation and muscle oxidative capacity together with peak cycling power (W) and maximal $\dot{V}O_2$ ($ml \cdot kg \cdot min^{-1}$) were tested pre and post training intervention.

8.1.3 Results

Analysis shows that it is feasible to recruit, with large retention rates (77%), as well as being well received by participants. Adherence to both training sessions (supervised and unsupervised) was 100%, with some loss of data during unsupervised training. No participants reported any side effects of oxygen supplementation. The NIRS measurement used (along with arterial occlusions) were effective at detecting positive changes within muscle oxidative capacity. Additionally, O_2Supp led to small non-significant changes in

pre and post measure of mean power output (18.41 ± 30.43 W), peak power output and $\dot{V}O_2max$.

8.1.4 Conclusion

This study demonstrates that a five-week, O_2Supp repeat cycling exercise study with measures of NIRS is feasible. A measure of muscle oxidative capacity using NIRS has enough sensitivity to detect changes following O_2Supp training. The results highlight that O_2Supp elicits increases in muscle oxidative capacity and increases mean power output. Further, the training methodology and data collection techniques were effective as evidenced by the adherence rates. However, future studies will need to over-recruit to meet power requirements and account for the loss of data experienced during unsupervised training session. This study better informs the sports researcher to carry out robust, strongly evidenced O_2Supp training intervention-based research.

Keywords: Hyperoxia, Fraction of inspired oxygen, Cycling, Feasibility, NIRS.

8.2 Background

The previous four experimental chapters have all progressed a narrative with O_2Supp that leads into this chapter. Chapter 4 and 5 assessed the most effective component of training session for O_2Supp, chapter 6 assessed the fatiguing component of O_2Supp, and chapter 7 assessed a positive trend in muscle oxygen kinetics with O_2Supp. Each built a narrative to enable this forthcoming chapter to put each of the chapters components together to assess whether O_2Supp is feasible in a laboratory training study with novel measures of muscle oxygen.

8.3 Introduction

Intervention based training studies are an effective way to establish if a 'supplement' can enhance performance. However, training studies are notorious for being underpowered, lacking control groups, having small effect sizes, and using overly homogeneous participants (often male, university age students). Feasibility studies in principal, look at identifying the limitations that are associated with such intervention-based training programmes [196,197]. This chapter will aim to strengthen the rigor of intervention-based training studies in O_2Supp. It will do this by employing a three-group intervention with control, whilst also using a larger participant pool (other than just university students) and addressing issues with power and sample size. Although not all limitations of a training study will be addressed (male, small effect size, small sample size) in this feasibility study, although it will build on the research ready for future larger studies.

Most intervention-based training studies centre around establishing the performance outcomes following an aid, whereas few explore the sensitivity of measures used assess the mechanism that the training influences. This is a particular limitation within the O_2Supp

research, where the majority of research has focused upon performance changes [8,125,139]. Importantly, mechanistic understanding of performance changes can lead to more highly focused training i.e. minimal training need and greater performance enhancement.

The range of methodologies used to investigate the effects of oxygen supplementation on sporting performance has increased in recent years [1]. These methodologies which vary from twitch interpolation to assess muscle fatigue, to muscle biopsies to assess mitochondrial respiration, can provide further understanding on different mechanisms [140,169]. The determination of mitochondrial efficiency could be essential to understanding what occurs during O_2Supp, and the application of NIRS as a non-invasive measure may allow further understanding of this mechanism.

The non-invasive, real-time application of NIRS makes it desirable to coaches and athletes alike, who have identified the need for wearable technologies to provide useful biofeedback [198]. NIRS has recently been used to assess both delivery and consumption of oxygen to the working muscles. Further unpublished research from this institution has shown the effectiveness of NIRS as a measurement tool during O_2Supp and its sensitivity to detect acute changes. NIRS has also been shown to be effective at examining muscle oxygen consumption ($m\dot{V}O_2$) when combined with arterial occlusions to assess oxygen consumption rate [74,75]. $m\dot{V}O_2$ has been commonly used as a proxy/non-invasive measure of muscle oxygenation (mitochondrial respiration), whereas direct measure (muscle biopsy) can look at mitochondrial respiration, density, and concentration.

The combination of arterial occlusions and NIRS, are used to detect changes in $m\dot{V}O_2$ but are not without their own limitations (issues include movement artefact & detection of

blood flow). Unlike muscle bioposy, NIRS assesses $m\dot{V}O_2$ using arterial occlusion imposes no chronic pain on a participant, although it can impose temporary discomfort to some individuals. The arterial occlusions impose between 250-300 mmHg of pressure to the upper leg, 10 times that of sports compression garments [51]. Additionally, the process of arterial occlusions can add an extended duration to research protocols, as the measurement needs to be carried out following a rest period. In general, the benefits outweigh the negatives, as $m\dot{V}O_2$ can be assessed without any medical intervention, and is relatively painless when compared with muscle biopsies. Additionally, arterial occlusions can provide information related to the rate of mitochondrial respiration which biopsies cannot.

Feasibility studies are vital for researchers to understand the recruitment strategies needed to minimise the limitations of intervention studies, as well as identifying the adherence, and retention rates. El-Kotob *et al.,* [196] commented on the factors that need to be considered to ensure recruitment, adherence, and retention rates were high. These include travel duration, supervised vs unsupervised training, amount of sessions, use of activity monitors, and loss of interest. All factors were taken into consideration during the conception of this feasibility study.

We postulate that O_2Supp exercise allows an increase in both the delivery of oxygen to the working muscles, and the utilisation, therefore allowing a higher training load, potentially leading to significant changes in skeletal $m\dot{V}O_2$. This hypothesis is supported by Cardinale *et al.,* [140] and Przyklenk *et al.,* [199] who found that acute O_2Supp, increased O_2 delivery, enhanced performance and intracellular adaptation (mitochondrial density and efficiency). However, their studies varied in exercise repetition duration (30 s-30 min), intensity (sub max- maximal), fiO_2 (0.3-1.0), and modality (endurance vs HIIT). Each of these

methodological differences makes it hard to compare results due to the changes in the data being associated with these factors rather than the primary aim (O_2Supp). Additionally, this study will apply non-invasive measures to measure outcomes in m$\dot{V}O_2$, making it real world applicable via wearable biofeedback.

Additional, research is also needed to understand if a multi session training study with O_2Supp is feasible for participants (in relation to recruitment, retention, and adherence). High intensity interval training studies are notoriously under powered [200], most research has just two groups (intervention or control) with relatively small sample sizes (*n* = 6-10 per group). This sample size is only sufficient for powering for the primary measure, even though most research analyses involve 3+ measures.

This study was conducted to assess the feasibility of using O_2Supp during a multi-session training intervention with non-invasive measures of muscle oxygen response.

In order to explore this, a 5-week randomised controlled intervention was carried out, where participants were single blinded to the type of gas inhaled during the training. This study set out to determine whether a short sprint cycling training intervention with O_2Supp could influence muscle oxygen utilisation. Additionally, this study aimed to determine if the participants would tolerate and adhere to an 8-session training intervention with multiple non-invasive measures, for which they had to travel to the lab.

8.4 Methods

8.4.1 Study Design

This study used a 3-week single blind randomized controlled training study design with parallel groups stratified from participant's baseline $\dot{V}O_2$max. Participants were randomly assigned either an experimental group where they trained with oxygen supplementation (n = 5, FiO_2 1.0; O_2Supp), or normal sea level conditions (n = 4, FiO_2 0.21; Normoxia. A control group only partook in pre and post-tests ran (n = 4, Control). Participant data, 1.77 ± 0.06 m, mass 77.4 ± 9.6 kg, age 38 ± 8.3 years, thigh thickness 9.6 ± 1.2 mm.

Participants were recruited from Colchester Borough cycling clubs, who had previous experience with laboratory testing. Exclusion criteria included: Females, over 45 years old, diseased population. Due to the large uptake of male participants (95%), females were excluded from partaking in order to reduce the potential effects of menstrual cycle on results.

In weeks 1 and 5 (pre and post 3-week training study) participants undertook baseline and post-tests respectively. Training consisted of six supervised HIIT sessions within the middle 3-week period. Non supervised training was distributed across the 5-week period documented in Table 8-1. Training sessions were held on non-consecutive days with at least 48 h recovery – two times per week for three consecutive weeks (6 session). Every supervised session was conducted under the direct supervision of an exercise physiologist on a one-to-one basis.

Participants were informed of the procedure and provided written informed consent. Participants were asked to refrain from strenuous physical activity 24 h prior to

participating. The study design is shown in a diagram in Figure 8-1 and Figure 8-2. Ethical approval for the study was granted by the University of Essex ethics committee in accordance to the Helsinki declaration.

An a priori power calculation was not performed because the effect sizes and standard deviations were unknown for the primary measure ($m\dot{V}O_2$), and collection of such data was one of the aims of this study. The (pilot) data collected in this study can be used to inform power calculations for subsequent larger studies.

8.4.2 Recruitment, Retention and Adherence

Of the sixty participants that expressed interest in taking part in this study, only 15 attended pre-test screening, a recruitment rate of 25%. Of those 15 participants who attended pre-test screening, the study retained 87% of them (n = 13) with the other participants dropping out prior to the start of testing due to extenuating circumstances. The adherence rate was 100% of those that conducted the pre-test. Each of the thirteen participants conducted all of the sessions that had been randomly allocated to them (8 sessions for both experimental conditions and 2 sessions for the control condition). Participants who signed up but subsequently dropped out expressed that 8 sessions over 5-weeks was too many sessions in a short period of time, as well as the keeping each session at the same time of day and day of the week. No reward was provided to take part in the study, participation was of their own accord throughout.

8.4.3 Experimental Protocol

Pre and Post-test Measures

Testing Process

As documented in Figure 8-1 and Figure 8-2 the process of testing started with the pre-test measures. The order of these measures were; arterial occlusions following a planter flexion task, a cycling warm up, a 30 s cycling Wingate sprint, a recovery period and finally a cycling maximal incremental exercise test. This procedure was reproduced during the post-test measures.

30s Wingate Sprint

The Wingate test was performed following a 5 min 100 W warm up on a Lode cycle ergometer (Excalibur, Lode B.V, Groningen, The Netherlands). The 30 s all-out test started following 30 s of pedalling at 100 W resistance. Following a 5 s countdown the braking resistance was applied to the magnetic flywheel and remained the same throughout the test. Braking resistance was set to 0.8 Nm per kg·BM as determined by Lode instructions for well-trained cyclists. Participants remained seated throughout the test, with strong verbal encouragement throughout. Participants were instructed to pedal as hard and fast as possible throughout the whole 30 s and not to conserve energy for an end spurt [201].

Maximal Incremental exercise test

Following both a 5 min active recovery (100 W) and a 5 min static recovery. Participants completed a maximal incremental exercise test until volitional exhaustion on a cycle ergometer. This test has been described in detail elsewhere [202]. In short, participants cycled wearing a Hans Rudolf mask (Hans Rudolph Inc., Kansas, USA) which covered the mouth and nose for pulmonary oxygen consumption assessment (Vyntus, Vyaire Medical, Basingstoke, UK). The test was commenced with 1 min of cycling at a power output of 100 W, which was subsequently increased by 25 W every minute until exhaustion. Criteria for exercise cessation was a plateau in $\dot{V}O_2$, respiratory exchange ratio (RER) >1.10 and an

RPE greater than 18. $\dot{V}O_2$max was calculated as the average of the highest 30 s $\dot{V}O_2$ measurement.

Supervised Training

Subjects completed the same procedure on each of the 6 visits over 3 weeks, comprising; 5 min relative intensity warm up 52% of heart rate reserve [175,176,203], 5 min passive recovery, finishing with 10 x 15 s sprints with 45 s of recovery, undertaken using a Wattbike pro (magnetic brake set 1, air brake set to 10) (Wattbike, Nottingham, UK). During the supervised training sessions participants had either O_2Supp or Normoxia. Protocol in accordance with pilot testing conducted prior to this study. Participants have sufficient load that they are able to reach peak power and not exceed max cadence. The air brake and magnetic brake settings were based on participants characteristics, which were established in previous studies [169] and pilot testing.

Wattbike pro was used to collect performance data (mean power and peak power (W)). No prior familiarisation was conducted as prior reporting established that each participant was familiar with repeat sprint cycling on a cycle ergometer.

Gas administration

Hyperoxic and normoxic gas mixtures were administered via a rig of 4 x 200 L Hans Rudolph Douglas bags connected to a Hans Rudolph mask and head net (Hans Rudolph Inc., KS, USA). The oxygen supplementation (FiO_2 1.00) condition used medical grade oxygen cylinder (BOC, Surrey, UK). In each condition, participants wore the mask and breathed from the Douglas bag during the whole session (sprint and recovery). This was determined due to results from - Experimental Chapter: and - Experimental Chapter:

that showed a continuous supply of oxygen was most effective and increasing acute performance.

Blood lactate and Haemoglobin

Prior to each training session, a pre-exercise (baseline) 20 µl capillary sample of blood was taken from the right ear lobe to measure lactate. Each sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Subsequent BLa samples were taken during the recovery period between intervals. All samples were analysed for Bla within 24 h of withdrawal using a Biosen (EKF diagnostics, Cardiff, UK).

Additionally, haemoglobin was measured pre and post training intervention using a manual lancet to the right ear lobe. A single 50 µl capillary blood sample was collected using a microcuvettes and subsequently placed in a HemoCue Hb 201+ (HemoCue, Angelholm, Sweden). Haemoglobin was used to assist in the estimation of the extent of oxygen delivery to the working muscles [204].

Near-Infrared Spectroscopy (NIRS)

Full details of this methodology are presented in detail in methodology chapter 2. In brief, participants wore a portable NIRS device to monitor muscle oxygen saturation of the *Vastus Lateralis* (PortaMon, Artinis Medical Systems B.V., Elst, The Netherland). The NIRS device was fixed to the belly of the right *Vastus Lateralis*, regardless of limb dominance. The device was placed 3 cm anterior to the midpoint between the top of the greater trochanter and the lateral epicondyle of the right knee [151]. Indelible ink was used to draw around the device to guarantee accurate NIRS placement during subsequent visits. Throughout NIRS devices were connected to a personal computer via the Bluetooth™

system for data acquisition (10 Hz). NIRS data was subsequently filtered using the methodology detailed by Rodriguez *et al.*, [153].

Near Infrared Spectroscopy (NIRS) Analysis

A pre exercise baseline measure lasting 60s was taken prior to each test. Oxyhaemoglobin (O_2Hb), deoxyhaemoglobin (HHb) and total haemoglobin concentration (tHb) are reported as change from baseline The tissue haemoglobin saturation index (TSI) expressed in % and calculated as ($[O_2Hb]/([O_2Hb + HHb])) \times 100$, which demonstrates the O_2 supply and O_2 consumption [193], was calculated using SRS methods (see chapter 2) and used to assess muscle reoxygenation rate ($\%.s^{-1}$).

Muscle Oxygen Consumption ($m\dot{V}O_2$)

Arterial occlusions were conducted (at the start of all visits), following a short submaximal lower leg exercise (planter flexion using black TheraBand) using a rapid cuff inflator (Hockanson, SC12L/E20, PMS Instruments, UK). The cuff was placed proximal to the NIRS device. The cuff was inflated to a pressure of 275 mmHg and inflated/deflated in 0.3 s. The cuff was inflated for 30 s three times with 30 s rest between occlusions [74]. The primary measure, $m\dot{V}O_2$, was estimated by fitting the slope of the difference between O_2Hb and HHb (Hbdiff) signal during each occlusion [205]. Greater negative values represent higher muscle oxygen consumption.

Incomplete arterial occlusion was determined by the absence of pulsatility in O_2Hb signal and the direction of O_2Hb and HHb; under complete arterial occlusion these are expected to move in opposite directions [74]. If this does not occur the occlusion isn't correctly restricting blood flow and flow is still visible on the NIRS trace.

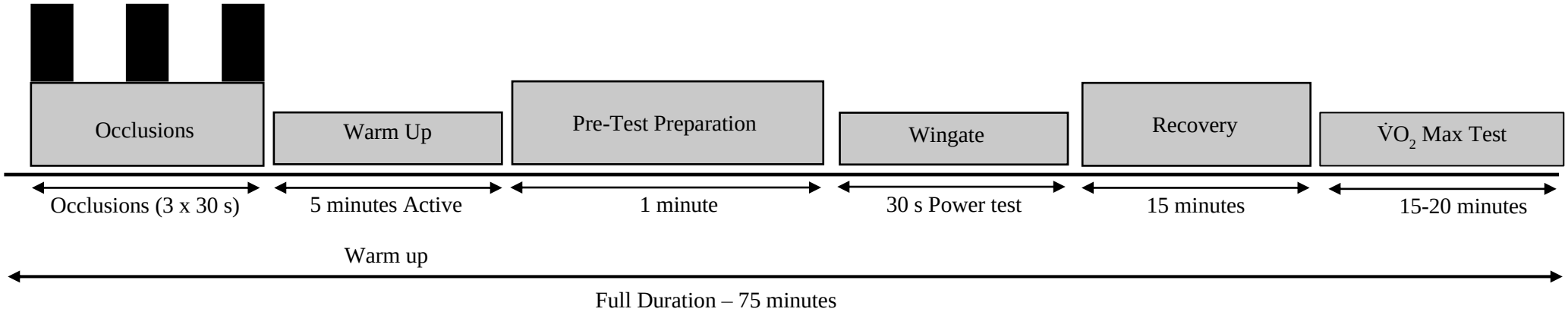
Acceptance and Perception

Throughout the course of the 8 sessions, multiple questions were asked of the participants, with specific focus of adherence, toleration and perception of the methodology. Participants were asked at the end of the study how much of an impact the study had on their normal lifestyle. Additionally, they were asked how tolerable the measures were and whether they would volunteer to partake in a similar study in the future. Lastly participants were asked if they had any side effects or discomforts directly from the oxygen supplementation. All participants were asked regardless of condition.

Table 8-1. Mean duration (HH: MM. SS) of non-supervised training performed during the total intervention period.

Intensity Zone	O_2Supp (*n* = 5)	Normoxia (*n* = 4)	Control (*n* = 4)
Intensity Zone I (>65% of HR max)	03:12.10	00:46.04	02:36.30
Intensity Zone II (66-82% of HR max)	06:26.43	03:01.41	07:11.26
Intensity Zone III (83-87% of HR max)	02:28.12	07:24.10	01:47.24
Intensity Zone IIII (88%-100 of HR max)	01:57.02	03:00.00	01:11.12
Total Training Duration*	16:10.07	13:57.55	13:29.50

*Zone data was unavailable for every participant. Unsupervised training could be conducted on 3 days (additional to the supervised sessions) during the week with rest days after each supervised training session.



Occlusions:

- Sub maximal exercise (planter flexion)
- Post exercise occlusions
- 30 s Occlusion with 30 s recovery, three times.
- 3 minutes in total
- Pressure of 275 mmHg

Warm up:

- Sub maximal exercise
- 5 minutes
- 100 W

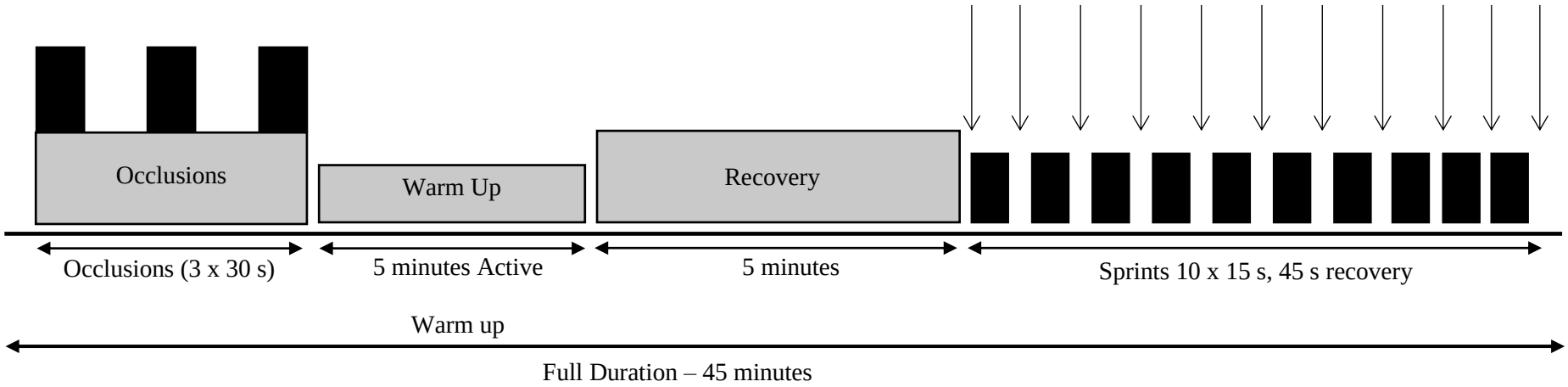
Wingate test:

- 30 s All out sprint test
- 7.5% of body weight

VO_2 Max Test:

- 5 minute WU
- 100 W start
- 25 W every 1 minute
- Test till exhaustion

Figure 8-1. A schematic representation of the experimental methodology during the pre and post-test.



Occlusions:

- Sub maximal exercise (planter flexion)
- Post exercise occlusions
- 30 s Occlusion with 30 s recovery, three times.
- 3 minutes in total
- Pressure of 275 mmHg

Warm up:

- Sub maximal exercise
- 5 minutes
- Level 4 Wattbike (~200 W)

Session:

- 15 sprint (seated)
- 45 s recovery (passive)
- 10 sprints
- Maximal air brake and 1 magnetic brake.

Blood lactate measure

Figure 8-2. A schematic representation of the experimental methodology during the training sessions.

8.4.4 Statistical Analysis

Statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS, Inc, Chicago, IL, USA).

Two-Way analysis of variance ANOVA was performed to examine the main effects of condition and time on; peak power (W), mean power (W), $\dot{V}O_2max$ ($ml \cdot kg \cdot min^{-1}$), haemoglobin concentration in blood ($g \cdot L^{-1}$) and $m\dot{V}O_2$ ($\mu M\text{-}HB\ diff \cdot s^{-1}$).

α level set $p = 0.05$ for all data analysis.

8.5 Results

This study primarily demonstrates that it is possible to design and implement a rigorous exercise study, whilst administering a blinded supplement during training sessions. Analysis shows that it is feasible to recruit, with large retention rates from the study commencement (77%), as well as being well received by participants. Although there was an initial pre-study retention rate of only 22%., adherence to training was 100%. The adherence of subjects wearing heart rate monitors during unsupervised training (Table 8-1) was 70% with training intensity data consequently not obtained.

As shown in Table 8-2, $m\dot{V}O_2$ was successfully obtained with 77% of the population, with the NIRS signals highlighting incomplete arterial occlusions (as documented in methods) during data analysis in 20 out of the 26 arterial measures. The incomplete arterial occlusions (33% of the total sample) was due to incomplete obtainment of NIRS signals (as documented in methods). No data was lost due to cessation of a measure due to discomfort with the arterial occlusions.

When asked about how much the study inconvenienced them, 80% expressed it substituted normal training, with 20% expressing it was a burden with travel and additional training stress. Additionally, 90% of participants found all measures to be tolerable with small discomfort felt during the occlusions. The other 10% expressed they would not partake again if they had to take part in occlusions due to the discomfort it caused. No participants reported any side effects with the oxygen supplementation or Normoxia and would take part again in oxygen supplementation study in the future.

Pre vs post-training represented in Table 8-2 shows positive changes in $m\dot{V}O_2$ (37%), haemoglobin concentration (~2 $g \cdot L^{-1}$), peak (4.1%) and mean (2.7%) cycling power (W)

following O_2Supp, whilst not being statistically different from change experienced with Normoxia ($p > 0.05$). There was no significant main effect for time, group or interaction for any measures ($p > 0.05$).

Figure 8-3 depicts representative data of the NIRS traces (O_2Hb, HHb and tHb) during the three submaximal exercise occlusion procedures.

Figure 8-4 shows individual m$\dot{V}O_2$ participant data at the pre and post training visits for O_2Supp and Normoxia. The trend in the data shows that m$\dot{V}O_2$ increases pre to post following oxygen supplemented training. Training in Normoxia results in a small decline in m$\dot{V}O_2$. The m$\dot{V}O_2$ findings follow a similar trend as the performance variables (mean and peak power), it is evident that cycling power improves alongside m$\dot{V}O_2$. However, the control group did experience an increase in m$\dot{V}O_2$ as a result of normal endurance training during the intervention period.

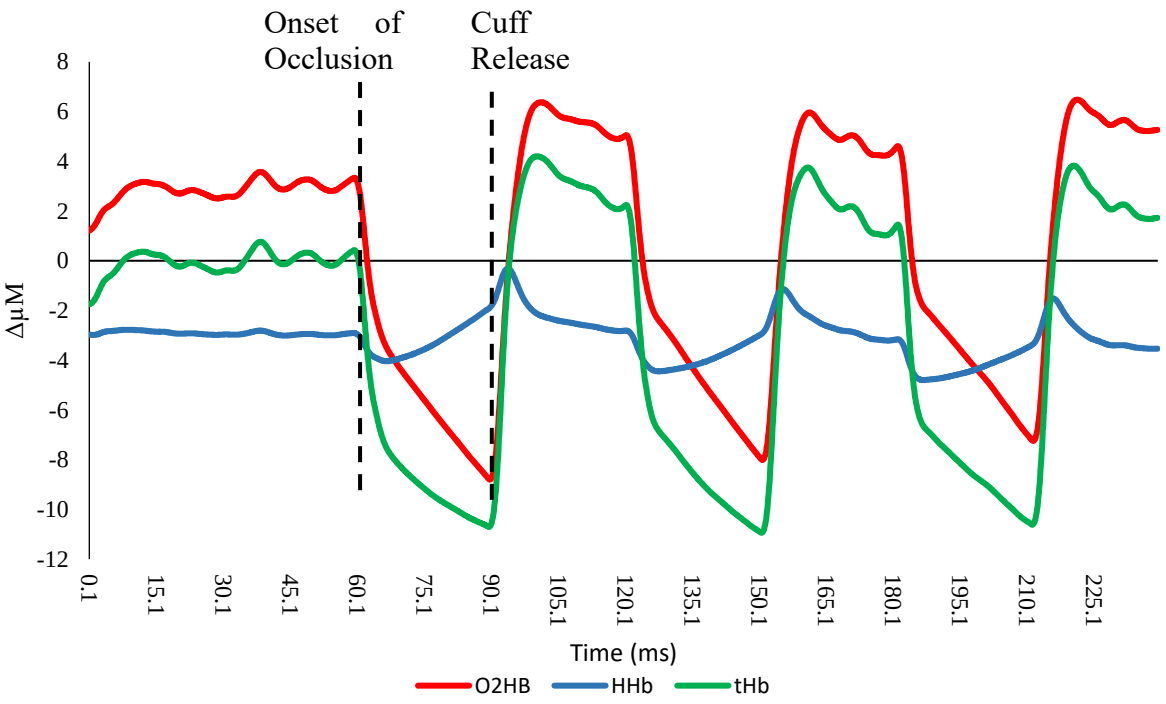


Figure 8-3. Representative data depicting the process of occlusions.

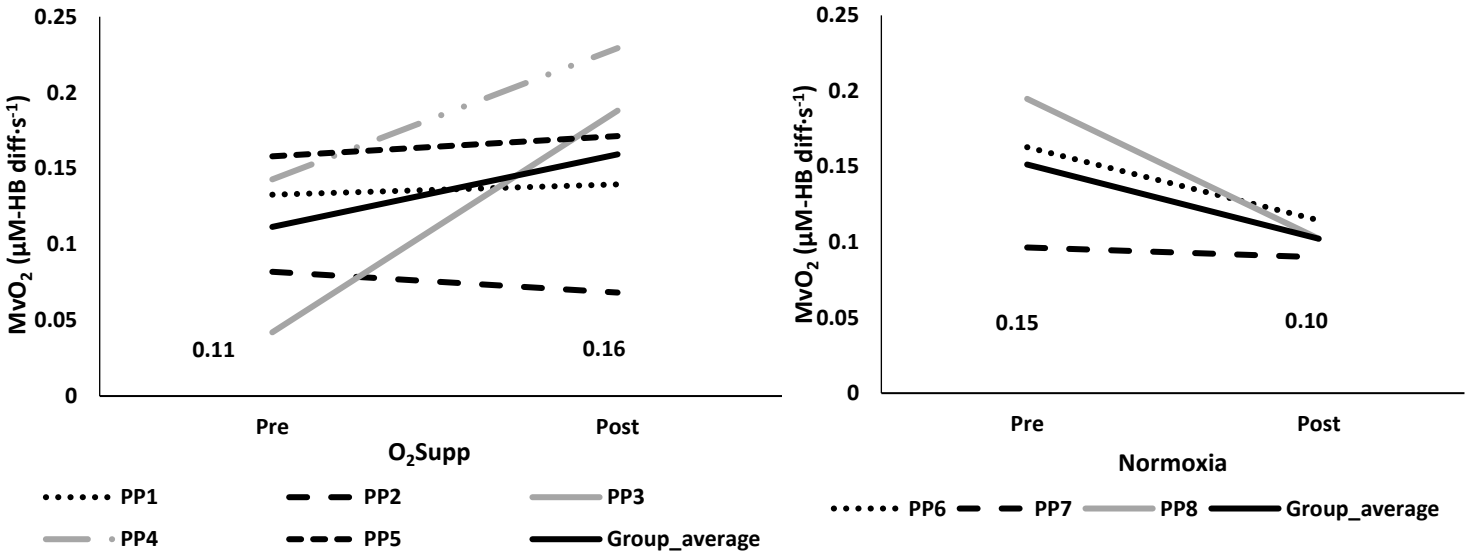


Figure 8-4. Individual participant changes in the slope of muscle oxygen consumption ($m\dot{V}O_2$) pre and post the training. Group average shown as solid black line.

Table 8-2. Data from maximal exercise test and Wingate tests pre and post training for cyclists doing sprint interval training with oxygen supplementation or normal conditions and the control.

	O_2 Supplementation (n=5)		Normoxia (n=4)		Control (n=4)			
	Pre	Post	Pre	Post	Pre	Post	F Stat	*p*-value
$\dot{V}O_2max$ ($ml \cdot kg \cdot min^{-1}$)	46.10 ± 6.36	46.65 ± 7.78	45.14 ± 3.96	46.11 ± 5.82	48.42 ± 2.63	49.47 ± 6.21	F (2) = 0.037	$p = 0.96$
Wmax (W)	340.00 ± 51.84	345.00 ± 44.72	343.75 ± 42.70	362.5 ± 32.27	331.25 ± 37.50	356.25 ± 51.54	F (2) = 2.151	$p = 0.17$
Peak power Wingate (W)	928.86 ± 79.06	967.32 ± 29.50	989.25 ± 137.88	1028.50 ± 133.29	1010.50 ± 97.48	1017.5 ± 7.32	F (2) = 0.256	$p = 0.78$
Mean power Wingate (W)	650.14 ± 41.24	668.55 ± 32.26	673.25 ± 33.23	692.00 ± 29.30	663.75 ± 67.91	663.50 ± 72.96	F (2) = 0.535	$p = 0.60$
Haemoglobin concentration ($g \cdot L^{-1}$)	155.40 ± 11.87	157.00 ± 14.00	156.75 ± 6.24	159.75 ± 8.14	177.75 ± 16.34	167.75 ± 13.74	F (2) =0.900	$p = 0.44$
$m\dot{V}O_2$ (µM-HB diff$\cdot s^{-1}$) †	0.11 ± 0.05	0.16 ± 0.06	0.15 ± 0.05	0.10 ± 0.01	0.10 ± 0.04	0.17 ± 0.01	F (2) =3.310	$p = 0.10$

Values are mean ± SD. † $n = 4$ in O_2Supp, $n = 4$ in Normoxia and $n = 2$ in control.

8.6 Discussion

This feasibility study demonstrates that the application of NIRS to assess muscle oxidative capacity, pre and post a 3-week O_2Supp intervention, is sensitive enough to detect changes in response to training and or supplementation. These findings were achieved following a mixed training intervention including supervised and unsupervised training. This study, therefore, provides support for the application of non-invasive biofeedback measures of muscle oxidative capacity within 'real world' training settings.

The adherence to training was 100% throughout the study, however, the adherence of subjects wearing heart rate monitors during unsupervised training (Table 8-1) was only 70% with training intensity data being lost in the process. It is reported that when people sign up to studies, they invest their time and effort, therefore the adherence to the proposed training is high. The participants that would have had lower adherence tend to drop out prior to the study's commencement. There is little evidence to suggest that participants will adhere to training better during supervised vs unsupervised training, however, there is evidence to suggest this is the fact with diseased populations [206–208]. It was found that adherence and training was elevated during supervised training sessions, even though supervised sessions were not always feasible (i.e. time consuming and expensive). These research studies also found that supervised training was much more effective during an intervention study than unsupervised training, although most report that improvements in unsupervised group are still greater than no training.

It is important to ensure that all participants are fully aware of the demands of the study prior to participation. This was why this study experienced lower initial up take of participants but managed to have very high retention rate, each participant was fully aware of the task in hand. Further incentives other than data and training should be made available

to participants during training interventions. An increase in incentives may result in subsequent increases in initial participant up take, unlike the current study.

There is a high requirement for unsupervised training within sport, as coaches are not always available to attend every session with every athlete. Coaches set training plans to include both supervised (typically HIIT) and unsupervised training (typically long duration endurance). This study trialled a reflection of this type of training practice, with the participants completing the high intensity work in the lab, then completing lower intensity, longer duration work 'at home'.

In future studies of this nature, we suggest further guidance and information need to be provided to participants to ensure that data collection is maintained. An increase in wearable technology is evident in the sports market, with the market's growth at around 20% per year [209]. This leads itself to a change in behaviour and routine of athletes, athletes are using more technology in sport to aid training and feedback [210]. Future studies may see an increase in adherence and retention due to athletes being more accustomed to wearing devices on their person.

As shown in Table 8-2, $m\dot{V}O_2$ was only successfully attained with 77% of the population, with the NIRS signals highlighting incomplete arterial occlusions (as documented in methods) during data analysis in 20 out of the 26 arterial measures. The incomplete arterial occlusions (33% of the total sample) was due to incomplete obtainment of NIRS signals (as documented in methods). No data were lost due to cessation of a measure due to discomfort with the arterial occlusions.

To account for the drop out and the sensitivity of the primary measure ($m\dot{V}O_2$ 1.5-2.5%)[152,211,212], future studies will need to overpower their sample size. The sample size will need to account for the 23% drop out (of data) and the coefficient of variation (~17%) of the primary measure [75,213]. A specific sample size analysis is problematic due to the novelty of the primary measure ($m\dot{V}O_2$) and the methodology applying that measure (6 session HIIT intervention with arterial occlusions). The research is yet to establish the minimal detectable change (MDC) or the minimal clinically important change in $m\dot{V}O_2$ using arterial occlusions (NIRS) following a training intervention.

The absolute reliability was used to calculate the MDC of each measure with the following formula: MDC = Standard Error of Measurement X 1.96 X √2. The MDC indicates the minimal amount of change that can be interpreted as a real change in the primary measure. The MDC for $m\dot{V}O_2$ was calculated as 47.12%, therefore a change greater than 48% would infer meaningful results. This feasibility study identified changes between 45% and as high as 70% for the primary measure. Therefore increasing the sample size of each group to align with the upper limit of the research (n = 8-15) would infer meaningful changes in $m\dot{V}O_2$, whilst accounting for a loss of data of up to 25% [214–216]. Additionally, using the data from this preliminary analysis to run a post hoc power analysis puts the sample size needed at n = 8 per group ($\alpha = 0.95$, $1-\beta = 0.05$, large effect size = 0.5 and medium correlation).

Additionally, the process of arterial occlusion within this study was adapted from Southern *et al.,* [75] and Jones *et al.,* [74], who both suggested that repeated short occlusions (3-8 s) with short rest periods was possible to attain a measure of $m\dot{V}O_2$, however pilot testing in this study suggested this to be not possible. Testing established that repeat short duration occlusions using a rapid cuff inflator by Hockanson (as referenced by both Southern and Jones) was insufficient in the aforementioned time scale (confirmed by manufacture-

Hockhanson). As a result, this study used a longer cuff inflation of 30 s to attain suitable $m\dot{V}O_2$ response using NIRS, which has also been used by Ryan *et al.,* [217] on collegiate athletes, sedentary individuals and people with spinal cord injuries.

The preliminary results of the effect of O_2Supp during a 5-week high intensity sprint cycling intervention on muscle oxidative capacity, mean power output and peak power output, has only been investigated by one other study [140]. Interestingly, the current study found that O_2Supp elicited a trend towards meaningful differences in $m\dot{V}O_2$, whereas Cardinale *et al.,* [140] found O_2Supp did not change haematological (venous blood sample) or $m\dot{V}O_2$ parameters following training. The changes in muscle oxygenation evident in this study were also replicated in unpublished work by the authors, this highlights that there are changes as a result of O_2Supp. These differences between studies is likely to be methodological differences (HIIT vs Endurance, venous blood vs capillary) rather than as a result of O_2Supp. Cardinale *et al*., also found that even though no significant changes in haematological or $m\dot{V}O_2$ parameters were evident, the 6-week training programme did elicit increases in mean power output (20 km TT). They believe that the changes in performance were related to neuromuscular measures that were not assessed and not haematological or cardiorespiratory. This study did not measure neuromuscular changes however previous work by the authors has highlighted that this may be a potential mechanism for changes as a result of O_2Supp (fibre recruitment) [169].

This study's findings also showed that 3-week HIIT with O_2Supp induced significant performance improvements (peak power output) compared to the control condition, with non-significant changes compared to Normoxia. Our findings contradict those of Kilding *et al.,* [125] who found that 8 sessions of O_2Supp HIIT was not sufficient at increasing

endurance performance any more than Normoxia condition. They highlight reasons for why they experienced limited findings; insufficient taper time, lack of neuromuscular measurements and reduced FiO_2 0.6. This differs from the current study due to the higher FiO_2 1.0, although the duration (less than 1 week) given for a taper is a limitation for this study too.

Our findings support the notion that O_2Supp during high intensity cycling is a strong stimulus to increase the rate of muscle oxygenation, subsequently increasing $m\dot{V}O_2$. It is also interesting that the control condition experienced a large increase in $m\dot{V}O_2$ across the 5-weeks, this data is shown within Table 8-1. Table 8-1 highlights that the control condition conducted a larger amount of low intensity endurance training without much high intensity training, which has also shown to increase muscle oxygenation [218]. This shows that the current training programme is feasible at increasing muscle $m\dot{V}O_2$ but maybe not more so than low intensity endurance training. However, research has reported that HIIT has a higher rate of adherence, with participants enjoying it more than longer periods of endurance training [219]. HIIT can therefore be applied to training studies where possible, to ensure retention and adherence, however the effectiveness of lower intensity training should not be forgotten and may be more appropriate for novice, older or less active population.

For subsequent larger studies it would be prudent to increase the geographic scope of inclusivity to enhance the number of participants enrolling in the study to meet a priori sample size calculation. This suggests that a specific small geographic location is not a practical method of recruitment. Although, further geographic scope can result in increased

athlete drop out due to extensive travel time needed to complete a multi session week training intervention.

To our knowledge this is the first study to report positive changes in $m\dot{V}O_2$ using NIRS during a multi week O_2Supp intervention. This may be due to the training methodology (10 x 15 s sprints 45 s recovery) eliciting a sufficient training stimulus for adaptation in muscle oxidative capacity, especially with the additional stimulus of O_2Supp. Although a 6-session training intervention has been shown to be sufficiently long to observe changes in muscle oxidative capacity using NIRS [14], a longer duration study (6 week with 12 sessions) may have shown further improvements in $m\dot{V}O_2$. Increasing the sample size and power by using a larger number of participants in a follow up study should be able to examine the relationship between $m\dot{V}O_2$ and power output in greater detail. This additional analysis may allow researchers to identify mechanisms behind such changes in both variables.

Additionally, with a short turnaround of 48 h between sessions within this study, increasing the period of rest (taper) between the last training session and the post-test would have allowed for fatigue levels to drop and therefore, greater adaptation may have been measurable. It has been shown that a taper period of 7 days is effective at diminishing the effects of fatigue induced by intense training, whilst maximising physiological adaptation and resultant performance [220]. Therefore, increasing the time between the last training session and the post test could have enabled adaptation to be measured in the absence of fatigue.

8.6.1 Limitations

This feasibility study has some limitations which are important to address in future larger study designs. Firstly, muscle oxidative capacity was measured by NIRS, a novel method to evaluate muscle oxidative capacity in this O_2Supp research, and not by muscle biopsies, which is regarded as the 'gold standard' method, although muscle biopsies can be used for multiple additional analysis. However, the non-invasive and real-time application of NIRS makes it desirable to coaches and athletes alike who have identified the need for wearable technologies to provide useful information [198].

Secondly, blinding of the exercise physiologist to reduce bias. The single blind nature of this study may have led to experimenter bias, influencing the results, however standardised instructions and encouragement was given equally throughout ALL testing sessions.

Finally, the lack of female participants incorporated within the study limits the scope of application of O_2Supp to a narrower audience (male dominated). In a larger study, female participants should be sought after. Further recruitment strategies (female only sessions, wider geographic inclusion) should be applied to increase the female participation for future studies. NIRS research currently focuses on male dominated studies due to a limitation in the depth of the near infrared signal – i.e. the depth of the infrared signal needs to be more than double the adipose tissue depth [69]. Women generally have more subcutaneous fat than men and therefore, the depth of the infrared signal is not sufficient in these populations, decreasing the sensitivity of the desaturation profile and overestimating the overall oxygenation response.

8.7 Practical Application

When combined with arterial occlusions, NIRS is a functional tool in which skeletal muscle oxidative capacity can be detected following training blocks, allowing coaches to make better informed decisions on the effectiveness of the training programme. Unsupervised training during HIIT is feasible for coaches to apply to training programmes to ensure adherence when used with the correct monitoring. O_2Supp has reported no additional side effects from training, when used in an unpressurised reservoir bag during HIIT.

8.8 Conclusion

This novel study demonstrates that a 5-week, O_2Supp repeat cycling exercise study with measures of NIRS is feasible. NIRS was shown to provide a measure of muscle oxidative capacity that was sufficiently sensitive to detect changes following O_2Supp training. The training and testing approach appear successful supported by the high retention and adherence rates of the participants. The results highlight that O_2Supp elicits increases in muscle oxidative capacity and increases mean power output. However, future studies will need to overcompensate the recruitment pool to consider trial uptake and loss of data.

8.9 What Next?

This chapter advances the research narrative within O_2Supp research. This research has established an effective time to administer O_2Supp (Chapter 4 & 5), it has ruled out potential mechanisms (Chapter 6) and it has started to evaluate other mechanisms (Chapter 7), and now it has shown the effectiveness of O_2Supp within a training study. Further research is needed to take the discussion points of this chapter and develop them into a

rigorous experimental study. This would further strength the narrative that O_2Supp is an effective ergogenic aid within repeat sprint cycling.

9 - Chapter Nine:

Combined Analysis

9.1 Abstract

9.1.1 Objectives

Each experimental chapter evidenced that O_2Supp permits a higher mean cycling power output during high intensity interval training (HIIT) than Normoxia. As each experimental chapter O_2Supp used similar methodologies, we conducted a combined analysis using the data from each experimental chapter to highlight the effectiveness of O_2Supp within a HIIT session, with particular focus on performance variables - mean and peak power output (W), and timing of performance decline.

9.1.2 Design and Methods

There was a combined sample size of 81 data sets (O_2Supp = 41, Normoxia = 40, *n* = 35), Age 26.6 ± 7.8 years, stature 1.81 ± 0.54 m, mass 78.8 ± 10.4 kg. Data used for analysis was taken from experimental chapters when O_2Supp and Normoxia were used throughout the entire session (sprints and inter sprint recovery) (experimental chapters 1 through 4). Each session consisted of 10 x 15 s repeated cycling sprints on a cycle ergometer, interspersed with 45 s recovery.

Repeated measures analysis of variance (ANOVA) were conducted to look for differences according to condition (O_2Supp / Normoxia) for; performance with sprint 1 as a baseline for mean and peak power, and BLa ($mmol \cdot L^{-1}$) for each sprint (10 sprints) (condition X time). Further, ANOVAs were conducted to assess the differences in performance decline, and between mean power output (W) and peak power output (W).

9.1.3 Results

O_2Supp training elicits positive meaningful improvements in mean sprint cycling performance using sprint one as baseline (increased by 16 ± 16 W, $p < 0.01$).

O_2Supp helps with the attenuation of lactate accumulation and performance decline. Small reductions in peak power was evident (25 ± 17 W, $p > 0.05$). BLa levels were on average 2 mmol·L^{-1} lower during O_2Supp compared with Normoxia. Performance decline was also 4% lower in O_2Supp.

9.1.4 Conclusion

This analysis showed that O_2Supp during repeated sprint cycling is beneficial for mean sprinting power and the attenuation of both performance decline and lactate accumulation. O_2Supp has shown positive meaningful benefits for repeated sprint cycling and could be applied to acute HIIT training sessions.

9.2 Background

Each of the previous chapters added small components to a larger narrative, 'O_2Supp is an effective ergogenic aid for repeat sprint cyclists'. Each of the previous studies have shown performance enhancing effects of O_2Supp, some way or another. However, due to the relatively small sample size of each of the previous chapters some questions will remain. This forthcoming chapter will try to eradicate them but looking at all the data collected within the studies sprints and assess common themes.

9.3 Introduction

Oxygen supplementation is considered as a relatively new area of focus within the sports science community. There is only a small amount of research conducted with large samples sizes, meta analyses comparing modalities or effectiveness or reviews that assess O_2Supp research as a whole [1,195,221]. The research conducted during the previous experimental chapters categorically falls outside of these too, hence the design of this study. The haemodynamic meta-analysis by Smit *et al.*, [221] focused on clinical populations (diseased patents) rather than sporting populations, therefore, the findings are not as relevant. Diseased populations by definition have sub optimum physiology therefore they react to stimuli (O_2Supp) differently to healthy individuals. Additionally, the meta-analysis assessed multiple methodological changes, whereas the current study will be focused on a specific population and methodology with lots of data sets, to enable strong inferences to be made.

The previous five experimental chapters were formulated to address specific research question within oxygen supplementation. The common factor between these chapters is that they all have a relatively small sample size. Each of the studies are sufficiently powered

for the primary outcome of each study (mean power output) but may not have sufficient power for analysis of secondary outcomes or for post hoc investigation.

This combined analysis using data from five previous experimental chapters was devised to highlight the effectiveness of oxygen supplemented repeat sprint cycling on performance outcomes (power output & performance decline) and BLa in trained cyclists, when the sample size is 8 times that of its power calculation (n = 8 to 10 for the primary outcome). We have already shown that oxygen supplementation would enhance cycling performance to an extent greater than that of Normoxia, and that O_2Supp would elicit reductions in BLa accumulation more so than Normoxia. This section therefore aimed to demonstrate the full extent of the effects.

9.4 Methodology

9.4.1 Study Design

Participant information from the experimental chapters was used for the combined analysis. Data sample size of 81 (O_2Supp = 41, Normoxia = 40, n = 35, Age 26.6 ± 7.8 years, stature 1.81 ± 0.54 m, mass 78.8 ± 10.4 kg)

Only data from the full oxygen supplemented condition and the full normoxic condition were considered for analysis during this combined chapter. Experimental conditions HN, NH in experimental chapter 1, 2, 3 and 4 were not included in the analysis due to their varying duration and methodology (pre exercise or inter-sprint recovery).

9.4.2 Experimental Protocol

Variables

The main three variables that were used for analysis were; mean power output (W), peak power output (W) and BLa concentration ($mmol \cdot L^{-1}$). Peak sprinting power (the

highest W achieved in each cycle) and mean sprint power (the average W produced during each 15 s cycle) were taken during the ten times 15 s repeated sprints. BLa concentration was taken during the inter-sprint recovery period (45 s). This is described in more detail in the literature review of this thesis (chapter 3.2.3).

Mean power output was used to determine the extent of performance decline throughout the 10 repeated sprints. Performance decline is described in more detail in section chapter 3.2.1.

Additionally, mean (MPO) and peak power (PPO) output variables were used to assess performance changes during each subsequent sprint from baseline [ΔS1_MPO & ΔS1_PPO]. Sprints 2 to 10 were used for the analysis, sprint 1 was used as a baseline to consider participants performance level.

Data handling

Data from each of the experimental studies was combined to produce one large data set. Data from experimental chapter 1 through 4 screening process is documented in each relevant chapter and added together to formulate a larger data set.

Experimental chapter 5 data was added to the large data set following a period of data handling. The data from participants training sessions was collated and sorted using the median of the six visits which provided one set per participant to use within the main data set. The data was subsequently rake weighted to consider the multiple visits from each of the participants (0.23 - NN and 2.63 - HH). Data was subsequently screened for outliers that were 2 standard deviations from the mean. Two values were removed from the data and imputed using estimation maximisation technique.

9.4.3 Statistical Analysis

All statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS Inc, Chicago, IL, USA).

Repeated measures analysis of variance (ANOVA) were conducted to look for differences according to condition (O_2Supp / Normoxia) for change in performance across 10 sprints (ΔS1_MPO, ΔS1_PPO) and BLa (mmol·L^{-1}) for each sprint (10 sprints) (condition X time). Additional, ANOVAs were conducted to assess the differences in performance decline between mean power output (W) and peak power output (W). Post hoc analysis was conducted using paired samples t-tests with Bonferroni correction on sprints at an individual level for change in mean power output, change in peak power output and BLa. α- level was set $p = 0.05$ for all data analysis. Effect size for individual measures were calculated and reported as Cohen's *d* and interpreted using bounds as 0.2, 0.5, > 0.8, where they are small, medium and large respectively [170].

9.5 Results

Change in Performance - Mean Power. There was a significant main effect on sprint performance across the 10 sprints (Time) when using sprint one as a baseline F (8,200) = 33.31, $p < 0.01$, $d = 2.31$. There was no significant main effect for sprint performance according to condition F (1,25) = 0.96, $p = 0.34$, $d = 0.39$. An interaction effect was evident for mean power output between the conditions across the sprints (Condition X Time) (F (8,200) = 7.93, $p < 0.01$, $d = 1.12$). Figure 9-1 shows that as the repeated sprints progress, mean power output in the oxygen supplemented condition begins to plateau and then slowly decline, whereas, the Normoxia condition shows a continued decline until sprint 9. Both conditions show an effect of motivation and an end spurt during sprint 10. Post hoc analysis shows a higher a change in mean power output from sprint 1 for sprints 4 through 10, $p < 0.05$.

Change in Performance - Peak Power. There was a significant main effect of time on sprint performance across the 10 sprints when using sprint one as a baseline F (8,200) = 18.43, $p < 0.01$, $d = 1.72$. There was no significant main effect for sprint performance according to condition F (1,25) = 2.50, $p = 0.13$, $d = 0.63$. An interaction effect was evident for peak power output between the conditions across the sprints (Condition X Time) (F (9,200) = 7.12, $p < 0.01$, $d = 1.07$). Peak power during the oxygen supplemented condition maintains at a consistent level following sprint 1, with a small drop after sprint 7-9. On the other hand, the Normoxia condition maintains a greater level of peak power throughout the first 7 sprints with small decrements throughout and then maintain through to the finish. Peak power is maintained for longer in Normoxia than the oxygen supplemented condition. Post hoc analysis shows no significant differences in peak power output from sprint 1 for any of the sprints, $p > 0.05$.

Overall Performance Decline. There was no significant difference in overall performance decline across the two conditions F (1,31) = 0.92, $p = 0.34$ (Figure 9-4). The decline in performance in the Normoxia condition was 4% greater than the O_2Supp condition.

Blood Lactate. BLa accumulation was taken during inter-sprint recovery (Figure 9-3). There was a significant main effect of time on lactate accumulation across the 10 sprints F (9,225) = 422.90, $p < 0.01$, $d = 8.21$. There was also a significant main effect for lactate accumulation according to condition (Condition) F (1,25) = 9.81, $p < 0.01$, $d = 1.25$. An interaction effect was evident for lactate accumulation between the conditions across the sprints (Condition X Time) (F (9,225) = 8.82, $p < 0.01$, $d = 1.19$). Figure 9-3 visually demonstrates the process of lactate accumulation across the 10 repeated sprints in both conditions (HH and NN). BLa in both conditions start off at a similar point but differs almost immediately. O_2Supp condition continues to increase by around mmol·L^{-1} after each sprint and peaks at 10 mmol·L^{-1}. The Normoxia condition peaks at around 12.25 mmol·L^{-1} and is on average 2 mmol·L^{-1} higher than O_2Supp from sprint 3 onwards. Post hoc analysis shows no significant differences in lactate accumulation from sprint 1 for any of the sprints, $p > 0.05$.

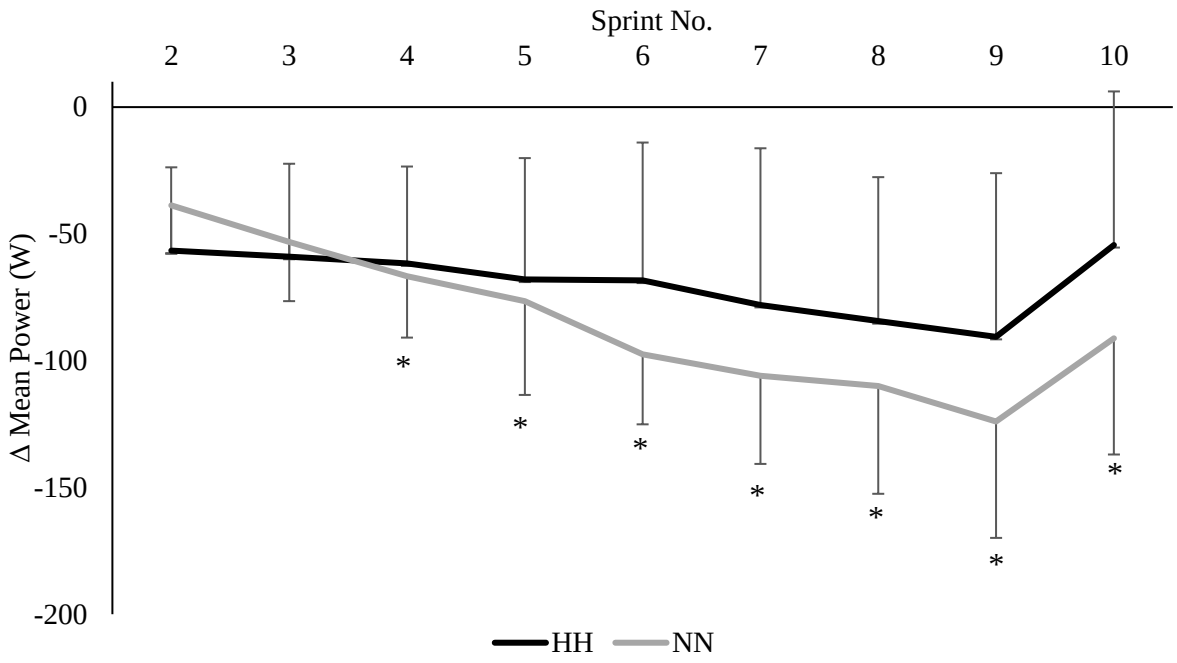


Figure 9-1. Change in Performance - Mean Power Output (W) across 10 sprints with sprint 1 as a baseline (81 trials). * significant difference between condition (O_2Supp and Normoxia) ($p < 0.05$).

Legend = O_2 Supplementation – HH, Normoxia – NN, Watts – W.

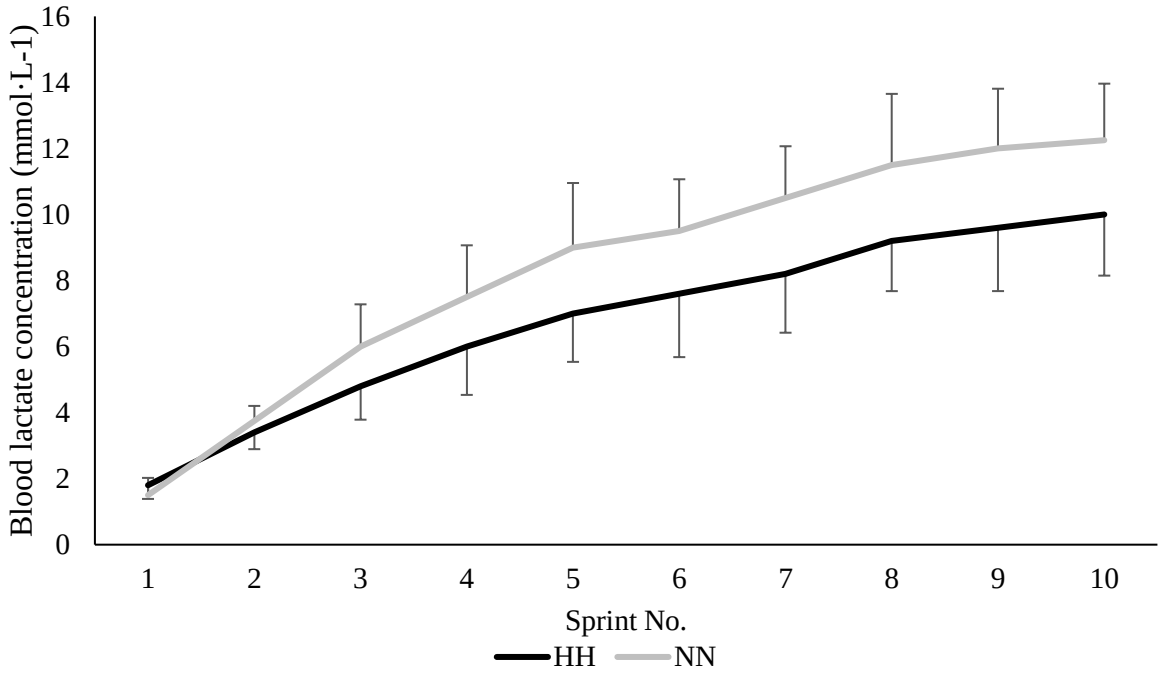


Figure 9-2. Mean lactate accumulation during the recovery periods interspersed between ten sprints (81 trials).

Legend: O_2 Supplementation – HH, Normoxia – NN.

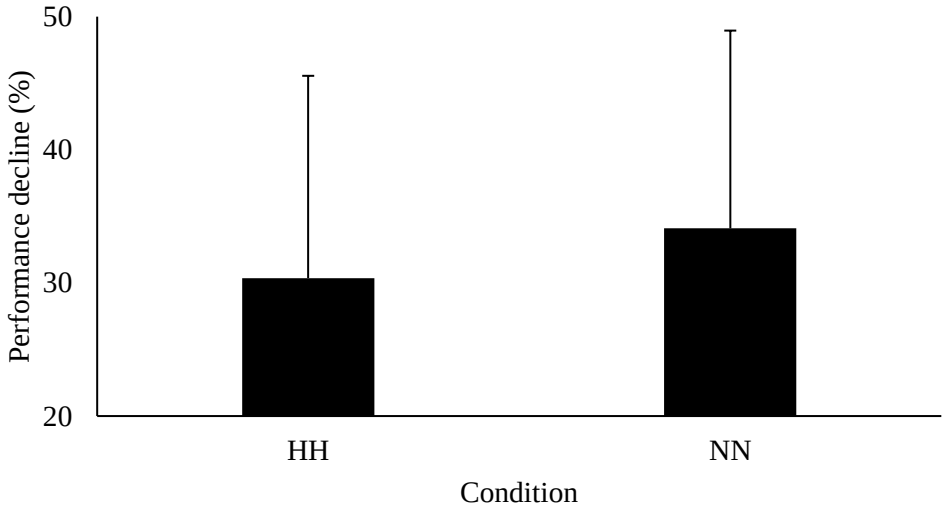


Figure 9-3. Overall Performance Decline throughout the 10 Sprints (mean power output) (81 trials).

Legend: O_2 Supplementation – HH, Normoxia – NN.

9.6 Discussion

The combined analysis of this study was a combination of data from the previous chapters. The aim of this chapter was to demonstrate the full extent of performance improvements with O_2Supp. The discussion points of this chapter inform the major discussion points of the thesis in its entirety. Therefore, the discussion is included in the following chapter (chapter 10).

10 - Summary:

Conclusions and Implications of Research

10.1 Summary of Experimental Chapters

Experimental Chapter	Chapter Aims	Chapter Findings
1	- To explore the effects of O_2Supp, both during recovery and during cycling, on sprint cycle performance. To confirm the methodologies used throughout the latter thesis chapters.	- 4% increase in mean power output during O_2Supp. - O_2Supp attenuated performance decline by up to 5%.
2	- To determine whether O_2Supp is more effective during or between repeat sprint intervals.	- Continuous O_2Supp appears to be more effective than discontinuous O_2Supp for performance. - Continuous O_2Supp condition attenuated decline in performance more than Normoxia.
3	- To develop an understanding of the effects of O_2Supp on repeat sprint cycling fatigue, using twitch interpolation technique.	- Mean power output increased by over 4% during O_2Supp. - No change in fatigue related measures (twitch interpolation)
4	- To further develop an understanding of the effects of O_2Supp on repeat sprint cycling using near infrared spectroscopy.	- Increased muscle deoxygenation and reoxygenation rate with O_2Supp (NIRS).
5	- To explore the feasibility of using NIRS, arterial occlusions and repeated cycling sprints during a multi session training study with periods of supervised and unsupervised training. - To identify whether the proposed methodologies (NIRS) are sensitive enough to detect changes in muscle oxidative capacity using arterial occlusions.	- NIRS is sensitive enough to detect small but meaningful increases mVO_2 in the O_2Supp group. - Training was well received by the participants with high adherence and retention rates. - All methods (NIRS, occlusions, training) were tolerable by 90% of the participants.

10.2 Summary

The main aims of this thesis were to identify the extent to which O_2Supp can be used as an ergogenic aid in both acute and chronic repeat sprint cycling training, and to begin to examine the underlying mechanisms. The literature review (chapter 2) identified that O_2Supp research has limited comparable studies (studies with similar dosages and methodologies). Chapter 2 enabled the conception of the methodologies and research aims used throughout this thesis. Chapter 2 also introduced a broader overview of the literature within O_2Supp research and addressed how O_2Supp can affect repeat sprint cycling and the potential mechanisms behind these effects. Research for this chapter allowed for the formulation of the research questions that would later be addressed in the 5 experimental chapters.

Research question 1 asked "during which component of a cycling training session is O_2Supp most effective at increasing performance?". Experimental chapters 1 and 2 were focused specifically on answering this question – when should oxygen supplementation be used? These experiments examined the three main periods that comprise a training session, the exercise period, the exercise recovery period between repetitions, and the longer period of recovery between main sets of work. It was reported that more components of a training session that are supplemented with oxygen, the greater the performance – i.e. supplementation during the recovery (s) and sprinting together was more effective than supplementation during the cycle or the recovery alone.

It has been suggested that the increase in performance evident following O_2Supp is due to the subsequent increase in PCr resynthesis following HIIT intervals [52,93]. Although PCr changes were not measured during this thesis it is thought that it may be one of the main contributing factors for increased performance during O_2Supp as stored ATP and PCr provides the energy needed during high intensity exercise lasting between 10-15 s.

Consequently, repeated sprint exercise continuously depletes PCr stores. A 45 s rest after 10 s of high intensity exercise only replenishes ~75% of total PCr stores [164]. Consequently, after several successive sprints in a series, PCr stores will become significantly diminished resulting in a reduced sprinting ability. Even though, the PCr energy system is anaerobic in nature, its resynthesis requires oxygen. The resynthesis of PCr occurs using products from both the Krebs cycle and aerobic glycolysis, which have been shown to be enhanced following the use of O_2Supp [99]. Enhanced PCr resynthesis due to the consumption of hyperoxic gas [99,165] will allow performance to be maintained for longer as evident during experimental chapter 1's results.

Administering oxygen supplementation during inter exercise recovery was more effective than no supplementation, but not as effective as supplementation throughout the whole session. This advances the oxygen supplementation research, and specifically the works by Sperlich *et al.,* Hauser *et al.,* and Zinner *et al.,* [1–3,7,121,136] by identifying that O_2Supp in longer durations and during exercise and recovery is more effective than either alone. Using the data from experimental chapters 1 through 5 show that increasing the duration of O_2Supp within a HIIT programme leads to increases in mean cycling performance and a reduction in both BLa accumulation and peripheral fatigue. This work can and should be applied to exercise modalities (running, field sports and rowing) and methodologies (HIIT with 1:3 work: rest ratio) similar to that of this thesis.

The second question posed was – research question 2 'Which physiological mechanisms does O_2Supp effect to allow for improvements in performance capacity?'. Experimental chapters 3, 4 and 5 were devised to further understanding regarding the mechanisms that underlie the beneficial effects of O_2Supp effects. Experimental chapter 3 looked at the

effect of O_2Supp on peripheral muscle fatigue. This allowed further understanding of how O_2Supp attenuates peripheral fatigue whilst maintaining power output. It helped provide evidence that the mechanism for increased power must lie within the peripheral muscle belly. This was demonstrated during this chapter, using MVC, a global measure of fatigue. The force applied during the MVCs dropped by around 20% in the Normoxia condition, and by around 28% in the O_2Supp condition. This shows that there was greater (albeit non-significant) muscle fatigue as a result of the higher mean power output in the O_2Supp condition. Interestingly, the change in PTF and %VA were broadly similar across conditions. The drop in voluntary activation (%) was small and less than 1.5% different between conditions. The drop in PTF however was large, although again similar between conditions (34 and 38%, Table 6-1). These results support the findings of Thomas *et al.,* [172], who report performance in shorter efforts is predominately curtailed by peripheral measures, rather than any central fatigue as proposed by Gandevia [28] and Nybo *et al.,* [35]. This was demonstrated in experimental chapter 3 when O_2Supp led to a 4% increase in cycling performance (mean power) without changes in peripheral muscle fatigue.

Using the findings from experimental chapter 3, experimental chapters 4 and 5 were devised to investigate mechanisms occurring within the peripheral muscle belly. These mechanisms would be identified using NIRS. Two significant findings occurred when investigating the effect of supplementary oxygen on muscle oxygen utilisation and reoxygenation. It was found during both acute (experimental chapter 4) and chronic (experimental chapter 5) exercise, muscle oxygenation and cycling performance increased more in the presence of O_2Supp. In experimental chapter 4 both increased muscle oxygen reoxygenation and increased utilisation were experienced by participants in O_2Supp

condition. This is the first research study that demonstrates increased muscle reoxygenation (NIRS) during O_2Supp.

The rate of recovery of muscle oxygen is one of most important aspect of fitness to a sprint trained athlete [52]. The ability to flush out fatigue related metabolic by-products and the replenishment of fuel for successive sprints has obvious importance for performance. HIIT is highly reliant on the utilisation of PCr during exercise and exclusively upon aerobic processes (resynthesis) during periods of recovery [184]. O_2 Supp has been shown to be beneficial for sprint performance due to the increased resynthesis rate of cellular metabolic PCr [114,163]. The significant increase in power output (3.95%), non-significant increase in the extent of ΔTSI (%) recovery and reoxygenation rate (%·s^{-1}) seen here are potentially indicative of an enhanced PCr resynthesis profile [72]. Though PCr resynthesis was not measured directly, muscle (re)oxygenation has been suggested by others to be representative of this [72,189,190].

In future studies, NIRS could be applied to O_2Supp research more frequently to definitively determine whether delivery or utilisation of the additional oxygen is the most important factor. From our research, we hypothesise that it appears to be increased utilisation, as evident through increases in resaturation, and larger desaturation profiles. Appling NIRS in conjunction with other mechanisms (such as peripheral fatigue, muscle biopsies and ventilation kinetics) will give insightful information into specific mechanisms to further develop O_2Supp research.

Experimental chapter 5 set out to identify the feasibility of O_2Supp being applied to a 3-week training intervention answering research question 3 - “Is O_2Supp effective during chronic supplementation?”. The findings of this chapter highlighted that O_2Supp is more

effective during single acute HIIT sessions, although small increases in $m\dot{V}O_2$ and performance were experienced with chronic O_2Supp. These findings oppose those of Cardinale *et al.,*[140] who found that O_2Supp did not change haematological or $m\dot{V}O_2$ parameters following training. Cardinale *et al.*, also found that even though no significant changes in haematological or $m\dot{V}O_2$ parameters were evident, the six-week (8 session) training programme did elicit increases in mean power output (assessed via a 20km time trial). They state that the changes in performance were related to neuromuscular measures that were not assessed rather than haematological or cardiorespiratory alterations. Although, neuromuscular measures were not assessed in experimental chapter 5, chapter 2 showed that peripheral muscle fatigue was not a significant factor in reducing performance following O_2Supp. Thus, this thesis does currently not support the hypothesis of Cardinale *et al.*, who believe neuromuscular factors to be a primary factor in performance change.

It is also evident (experimental chapter 5) that O_2Supp can be applied to multi session research (and training) with very high retention rates, with no immediate adverse side effects. Additionally, experimental chapter 5 evidences that NIRS is a sensitive measure for non-invasive detection of $m\dot{V}O_2$ during O_2Supp. This chapter is the first of its kind to assess the feasibility of applying O_2Supp to a short term HIIT intervention with sensitive non-invasive measures of biofeedback. This chapter lends itself nicely for a follow up study with increased sample size to assess the effectiveness of O_2Supp as an ergogenic tool during chronic use.

It can be shown throughout this thesis that O_2Supp is a useful ergogenic aid for both acute and chronic repeat sprint cycling. O_2Supp increased mean sprinting power, muscle oxygen

utilisation and reoxygenation, whilst attenuating performance decline and BLa concentration.

It is suggested that an increase in muscle oxygen kinetics, PCr resynthesis, resulting in lower BLa concentration is why O_2Supp elicits such large improvements in performance capacity. Therefore, if used during a full exercise session and administered at normal atmospheric pressure (1 ATM) oxygen supplementation is likely to become a popular training supplement.

10.3 Key Findings

The key findings of this thesis are:

- O_2Supp offers a new and effective ergogenic aid to cycling HIIT programmes.
- This thesis is the first to consistently show supplementation with oxygen increases mean cycling power output by up to 4% compared to normal atmospheric conditions.
- This thesis includes experimental chapters that show O_2Supp increases the rate at which oxygen is utilised by the working muscles during exercise and subsequently these muscles reoxygenate faster during periods of recovery. This is a novel finding in the literature
- The findings of this thesis highlight that O_2Supp attenuated performance fatigue experienced during a HIIT session.
- This thesis contains a novel first study to assess the feasibility of using O_2Supp in a multi session training programme combined with non-invasive measures of muscle oxidative capacity.

10.4 Limitations

This research has several limitations that could be potentially addressed in future research. Firstly, the rate of PCr resynthesis O_2Supp is a key component of HIIT research, and a direct measure of PCr was not possible in the current methodology. A direct measure of PCr (via muscle biopsies or phosphorus nuclear magnetic resonance) would have supported the discussions during each of the experimental chapters and would have allowed slightly less speculation related to its response to supplementary oxygen. Ideally this measure would have been included in experimental chapter 2 through 5, however, methodological constraints in our lab meant this was not possible. Future research should incorporate a direct measure of PCr to detect changes in PCr resynthesis, further elucidating the changes due to O_2Supp.

Another limitation of this thesis is the exclusion of S_aO_2 measures from experimental chapters 2 through 5. S_aO_2 was measured during experimental chapter 1 with a very low degree of success. The common equipment used to measure S_aO_2 is a pulse oximeter typically placed on the index finger of a participant. This location is problematic for a cycling HIIT session, the participant grabs and squeezes the handlebars, as well as it being in a direct path for surface run off of sweat from the upper body. Both of these problems lead to the pulse oximeter detecting erroneous data on over 85% of the participants. Pulse oximeters were removed from experimental 2-5 due to the lack of consistent viable results, allowing participants to be non-restricted in holding the handlebars. Further research should incorporate a measure of S_aO_2 to understand the arterial oxygen fluctuations with O_2Supp. It is recommended not to use finger based pulse oximeters, instead use forehead sensors [222]. This method of pulse oximetry was not available during the course of this thesis.

Another, limitation of this thesis comes from experimental chapter 5 with the use of NIRS to measure muscle oxidative capacity, a novel method to evaluate muscle oxidative capacity in this O_2Supp research, and not by muscle biopsies, which is regarded as the 'gold standard' method. However, the non-invasive and real-time application of NIRS makes it desirable to coaches and athletes alike who have identified the need for wearable technologies to provide useful information [198].

Additionally, the single blind nature of the study is not the 'gold standard' for supplementation research. The single blind nature of this study may have led to experimenter bias, influencing the results, however standardised instructions and encouragement was given equally throughout ALL testing sessions. To increase the rigor of this study a double or triple blinding of the gas administration should be considered.
Another limitation of this thesis is that all experimental chapter (1-5) were conducted using male participants only, thus reducing the ability to generalise. During the conception of experimental chapter 1 participants were recruited independent of sex, although no females partook in the research. All future experimental chapters subsequently recruited male participants only. Including females into experimental chapters would have led to further limitations as some researchers suggest exercise response differences during menstrual cycle [223,224], due to hormonal fluctuations. Future research should identify the effectiveness of O_2Supp on females' exercise performance during different stages of their menstrual cycle however this was beyond of the scope of this thesis.

10.5 Future Research

The research in O_2Supp as an ergogenic aid is still in its infancy. The research conducted within this thesis has both provided answers and more questions. Following on

from this thesis and addressing some of its limitations the following research questions could be addressed:

- What is the primary mechanism that causes such significant increases in power output when O_2Supp is used in HIIT programmes?
- How can medical grade oxygen be administered in a safe and effective manner in a range of sporting environments?
- How does the dose and duration of oxygen supplementation influence performance and recovery?

Many hypotheses related to O_2Supp are formulated using findings from the research conducted in high altitude studies, or medical pressurised oxygen settings. It is unlikely that the response of the body to hyperoxia is the exact opposite as its response to hypoxia, but the knowledge that altitude studies provide is a good starting point. For example, low oxygen tension stimulates the production of EPO in order to maximise available oxygen. The long-term effects of hyperoxia in relation to EPO and other blood components are, as yet, unknown. Similarly, whilst findings taken from studies on patients with respiratory disease who utilise hyperbaric hyperoxia are useful, it is likely that the nature of their disease reduces the transferability of any findings to athletes.

Another important factor to consider is how to administer medical grade oxygen safely and effective to a wider sporting population. Research into the mechanisms underlying the success of O_2Supp is increasing, therefore, the focus may begin to move toward how we make it widely accessible. Understanding the potential limiting factors in administration of oxygen will enable companies to produce suitable products. Companies such as Boost Oxygen© and Oxygen Plus© are already producing handheld aids, but their cannisters only hold enough for 1-2 min' worth of oxygen. This thesis explicitly shows that O_2Supp needs

to cover an entire cycling HIIT session lasting up to 15 min for it to be of any measurable benefit.

10.6 Conclusion

The work throughout this thesis addresses the effectiveness of O_2Supp during a cycling HIIT programme and has highlighted most effective time to administer additional oxygen; it has also identified potential mechanisms involved (Figure 10.1). A key aspect of this thesis has been to identify the usability of O_2Supp within a cycling HIIT training programme.

The findings of this thesis suggest that O_2Supp is most effective for performance during both the work and recovery durations within a HIIT training programme, with potential mechanisms being increased utilisation of oxygen at a muscle level. Another key aspect of this thesis is that O_2Supp can be feasibly applied to a short-term training intervention with non-invasive measures of $m\dot{V}O_2$ being sensitive enough to detect changes, as evidenced by experimental chapters 3 through 5. The findings of this thesis emphasise the importance of O_2Supp as an ergogenic aid, whilst highlighting that further work needs to be done before advocating its use to the wider sporting world.

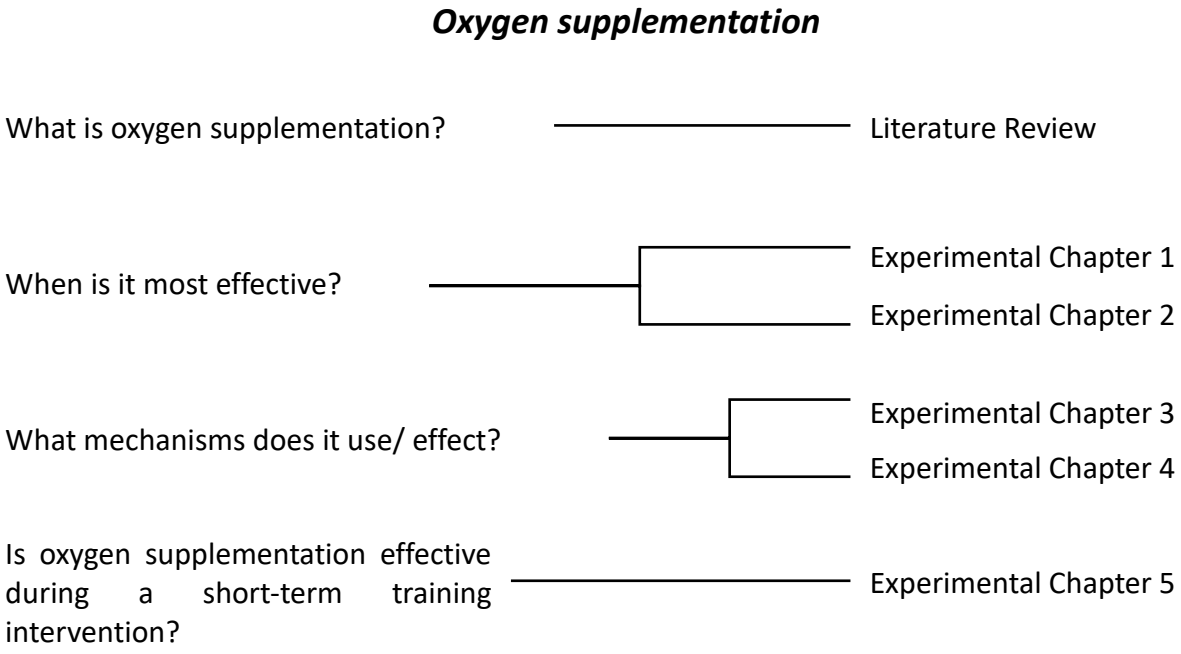


Figure 10-1. Questions answered within this thesis.

11 - References

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12 – Appendices

Additional information used throughout this thesis

12.1 Outline of Literature Review

The literature review (chapter 2) provides a comprehensive overview of each aspect of this thesis. The literature review will focus on O_2Supp– commonly referred to as hyperoxia, and its role within a high intensity interval training session, as well as the mechanisms it effects (such as muscle oxygenation and muscular fatigue). The literature review is followed by the detailed aim and hypotheses of the thesis.

A methods section is included as chapter 3. This section details many of the methods, equipment and statistical considerations used repeatedly throughout the thesis.

12.2 Outline of Experimental Chapter 1 – When is Oxygen supplementation Most Effective for Sprint Cycling Performance?

Concurrent supplementation with oxygen (O_2Supp) enhances performance but this type of delivery is not feasible in performance situations due to the equipment size (compressed gas cylinders) and expertise needed to administer it safely. Laboratory administration is needed to administer the gas in a controlled environment. This study aimed to determine when hyperoxic gas elicits the greatest effect on cycling performance. Specifically, we aimed to determine whether O_2Supp given after a fatiguing task but prior to repeated sprints had a similar effect to when O_2Supp was given during the repeated sprints alone.

Eight trained male cyclists underwent four visits to the laboratory. Each session comprised a pre-fatiguing task (15 km cycling time-trial), 15 min recovery (F_iO_2 1.00 (H) or Normal (N) air), followed by 10 x 15 s repeated sprints (during which time H or N was given). Thus, the 4 conditions were NN, HN, HH & NH. Repeated measures ANOVAs (α set to $p < 0.05$) were used to examine difference between conditions in power output (peak and mean Watts).

Peak and mean power output were significantly increased during full hyperoxic (HH) compared with normoxic (NN) condition [4% and 3% respectively] ($p < 0.05$).

O_2Supp (FiO_2 1.00) during recovery and exercise appears to elicit the greatest improvements in peak and mean power. This shows that use of supplementary oxygen is beneficial during maximal activity, and in recovery periods between exercise blocks.

12.3 Outline of Experimental Chapter 2 – The Effect of Manipulating the Timing of Oxygen Supplementation on Repeat Sprint Cycling Performance

The aim of this study was to determine the optimal time to administer oxygen during a repeat sprint protocol on cycling performance.

Ten male amateur University students participated. Testing comprised four visits to the laboratory in a counterbalanced design. Each session entailed; 5 min cycling warm up (~200 W), 5 min passive recovery, followed by 10 x 15 s repeated sprints interspersed with 45 s passive recovery, during which the air inspired was manipulated using a FiO_2 of 1.00 or 0.21 (normal air). The inspired air during the sprints and/or the recovery periods, determined the four conditions: NH, HN, HH, NN. For example, in HH, participants had supplementary oxygen during the 10 sprints and the 45 s recovery periods, whilst in HN, participants had supplementary oxygen during the sprints, but normal air during the recovery periods. It was hypothesised that the HH condition would evoke the largest performance improvements.

Outcome measures included, mean power (W), performance decline (%) and BLa ($mmol \cdot L^{-1}$). Repeated measures ANOVA were used to examine differences between conditions in outcome measures.

There was no significant effect of O_2Supp on mean power (W), BLa (mmol·L^{-1}) or performance decline (%) ($p > 0.05$). However, a common trend in HH condition was evident, with lowest levels of lactate accumulation and the shallowest decline in performance across the 10 sprints.

However, it appeared that non constant O_2Supp during repeat sprint cycling has a negative effect on performance when administered in short (15-45 s) interchangeable bouts during a training session. Therefore, oxygen supplemented training during short durations of a training session (HN, NH) is not as effective for maximising performance compared to training with oxygen throughout.

12.4 Outline of Experimental Chapter 3 – The Effects of Oxygen Supplementation on Repeated Sprint Cycling Performance and Muscle Fatigue

Oxygen supplementation ($F_iO_2 > 0.21$) can evoke performance improvements in aerobic and anaerobic exercise. The aims of study 3 were to determine the effects of breathing hyperoxic gas (F_iO_2 1.00) on repeated cycle performance, and to assess the nature and extent of fatigue after intermittent sprinting.

Testing (n = 14 males) comprised two visits to the laboratory. Each session involved 10 x 15 s repeated cycle sprints breathing FiO_2 1.00 (O_2Supp) or FiO_2 0.21 (Normoxia). Muscle fatigue was measured pre and post sprints using maximal voluntary contraction (MVC), voluntary activation (VA) and potentiated doublet twitch (PTF). Blood lactate (BLa) was taken between sprints.

Paired samples t-tests were used to examine differences between conditions in power output (peak and mean Watts) and BLa. Two-way ANOVA was used to examine fatigue

variables pre and post sprints according to condition. Effect sizes were also calculated and reported as Cohen's *d*.

Mean power output was 4% greater in O_2Supp ($p < 0.01$), with no difference in peak power ($p > 0.05$). There was a significant increase in BLa in O_2Supp compared with Normoxia ($p < 0.01$) in sprints 4 and 8, with large but non-significant differences in sprints 5-7 and 9-10. Despite the greater mean power achieved, there was no significant difference in measures of muscle fatigue (MVC, VA and PTF) ($p > 0.05$) in response to the cycling, although a large drop in PTF occurred in both conditions implying an decrease in the potentiation of the muscle (fatigue).

O_2Supp can elicit improvements in mean cycling power, with no significant change in post exercise muscle fatigue. O_2Supp as a training aid may provide performance enhancing effects during repeated sprint cycling by reducing concurrent muscle fatigue, primarily via peripheral factors.

12.5 Outline of Experimental Chapter 4 – The Use of Acute Oxygen Supplementation Upon Muscle Tissue Saturation During Repeat Sprint Cycling

This study examined performance (power output) and physiological responses (Tissue Saturation Index- a measure of muscle oxygenation status) to repeat sprint cycling with O_2Supp (FiO_2 1.00).

Fourteen amateur male cyclists participated. Testing comprised two visits to the laboratory. Sessions entailed; 15 min relative intensity warm-up, 10 min of passive recovery, followed by 10 x 15 s repeated sprints, during which air inspired had FiO_2 1.00 oxygen or normal air.

Outcome measures included mean power (W) and change in TSI (ΔTSI%). Repeated measures ANOVA were used to examine difference between conditions in mean power output for each sprint. Paired samples t-tests were used to examine differences between conditions in ΔTSI (%) and rate of muscle reoxygenation and deoxygenation (%·s^{-1}). Pearson's correlation tests were used to identify the relationship between power output and muscle reoxygenation rate between conditions.

Mean power output was 4% higher in the oxygen condition compared to Normoxia ($p < 0.01$). There was a significant positive correlation between power output and reoxygenation rate during O_2Supp ($r = 0.65$, $p = 0.04$). No correlation was seen between power output and reoxygenation rate during Normoxia ($r = -0.30$, $p = 0.40$).

O_2Supp (FiO_2 1.00) appears to elicit the greatest performance improvements in mean power, whilst increasing muscle reoxygenation. This novel research is the first to show positive increases in muscle oxygenation with O_2Supp and to suggest the mechanisms behind benefit of supplementary oxygen on sprint performance.

12.6 Outline of Experimental Chapter 5 – The Feasibility of Using Near Infrared Spectroscopy to Assess Muscle Oxidative Capacity following 3 Weeks Training with Oxygen Supplementation

The primary aim of this study was to assess the feasibility of using near infrared spectroscopy (NIRS) utilising arterial occlusions to measure muscle oxidative changes during a multi week training intervention with oxygen supplementation (O_2Supp). Secondary aims were to assess recruitment and adherence to training and the ability to collect training data from both supervised and unsupervised training sessions of participants.

13 amateur level cyclists performed 3-week training block consisting of two supervised HIIT sessions a week and additional unsupervised sessions 2-4 times a week. Participants were randomly allocated to either O_2Supp (FiO_2 1.00, n = 5) or Normoxia (FiO_2 0.21, n = 4) during the supervised training. An addition control group (n = 4) undertook only unsupervised training. Muscle oxygenation and muscle oxidative capacity together with peak cycling power (W) and $\dot{V}O_2max$ ($ml \cdot kg \cdot min^{-1}$) were tested pre and post training intervention.

Analysis shows that it was feasible to recruit to this type of study, with large retention rates (77%), and that the intervention well received by participants. Adherence to both training sessions (supervised and unsupervised) was 100%, with some losses in data during unsupervised training. No participants reported side effects with the oxygen supplementation. The measurements used (NIRS with arterial occlusions) were effective at detecting positive changes within muscle oxidative capacity. Additionally, O_2Supp led to small non-significant changes in pre and post measure of mean power output (18.41 ± 30.43 W), peak power output (38.46 ± 77.22 W) and $\dot{V}O_2max$ (0.45 ± 2.52 $ml \cdot kg \cdot min^{-1}$).

This study demonstrates that a short cycling exercise intervention using NIRS to assess change is feasible. This is supported by the high retention and adherence rates. The results show that O_2Supp training elicits increases in muscle oxidative capacity and small increases in mean power output. Our measure of muscle oxidative capacity using NIRS and arterial occlusions was sensitive enough to detect positive changes to short term training. However, future studies will need to overcompensate the recruitment pool to consider trial uptake and loss of data.

12.7 Chapter Combining Experimental Data

Each experimental chapter has evidenced that O_2Supp permits a more sustained cycling power output during high intensity interval training (HIIT). As each experimental chapter O_2Supp used during similar methodologies, we hypothesised that a combined analysis using the data from each experimental chapter could provide greater statistical power to explore the effectiveness of O_2Supp within a HIIT programme, with particular focus on performance variables - mean and peak power output (W), and performance decline.

There was a combined data set of 81 (O_2Supp = 41, Normoxia = 40). Data used for analysis was taken from experimental chapters when O_2Supp and Normoxia were used throughout the entire session (sprints and inter sprint recovery) meaning experimental chapters 1 through 4. Each session in these chapters consisted of 10 x 15 s repeated cycling sprints on a cycle ergometer, interspersed with 45 s recovery.

Repeated measures analysis of variance (ANOVA) were conducted to look for differences according to condition (O_2Supp / Normoxia) for change (from sprint 1 to 10) in mean and peak power and BLa ($mmol \cdot L^{-1}$) for each sprint (10 sprints) (condition X time). t-tests were conducted to assess the differences in performance decline between mean power output (W) and peak power output (W).

O_2Supp training elicits positive meaningful improvements in mean sprint cycling performance using sprint one as baseline (increase by 16 ± 19 W). Small reductions in raw peak power was experienced (25 ± 23 W) with O_2Supp. Performance decline was also 4% lower in O_2Supp.

O_2Supp helps attenuate lactate accumulation and performance decline. BLa levels were on average 2 $mmol \cdot L^{-1}$ lower during O_2Supp compared with Normoxia.

This analysis showed that O_2Supp during HIIT is beneficial for mean sprinting power and the attenuation of both performance decline and lactate accumulation. O_2Supp has shown positive and meaningful benefits for cycling HIIT and could be applied to acute HIIT training programmes.

12.8 Systematic Literature Search

This literature summary followed the PRISMA guidelines [225] that were deemed acceptable for this review. PRISMA has four main sections, it was deemed that the methods section (structured search terms, and inclusion and exclusion criteria) and results section (study selection) were only relevant to this literature summary. Identifying funding, risk ratios, data handling, synthesis of results and the analysis of key groups, were not suited for this review.

Studies were identified for literature summary using a variety of electronic and paper databases: Web of Science (performed on 24th November 2016), Pubmed (performed on 24th November 2016), and Google Scholar (performed on 24th November 2016) with the search criteria; 'Post exercise hyperoxia', 'hyperoxia exercise recovery', 'recovery', 'fatigue', and 'central and peripheral fatigue'. In the region of 600 studies were identified for review. Only journal articles and abstracts published between 1990 till September 2016 were considered for this literature analysis. Relevant journals published between December 2016 and 2019 were added for detailed ongoing review of the literature.

Procedures for selecting and evaluating the effectiveness of the articles were adapted using the Quality of Reporting of Meta-analyses (QUOROM) statement where applicable [226]. Both titles and abstracts were screened for suitability, and any duplicates were removed. Those papers that cleared scrutiny based on their title and/or their abstract, were subsequently read in full to confirm their suitability.

600 articles were identified following the literature search, with only 68 studies meeting our inclusion criteria. This is due to them not meeting all our inclusion criteria. As well as this a number of 240 papers were excluded due them being off topic when reviewed in

detail. Figure 12-1. shows the process of eliminating articles and finalising the journals to be reviewed.

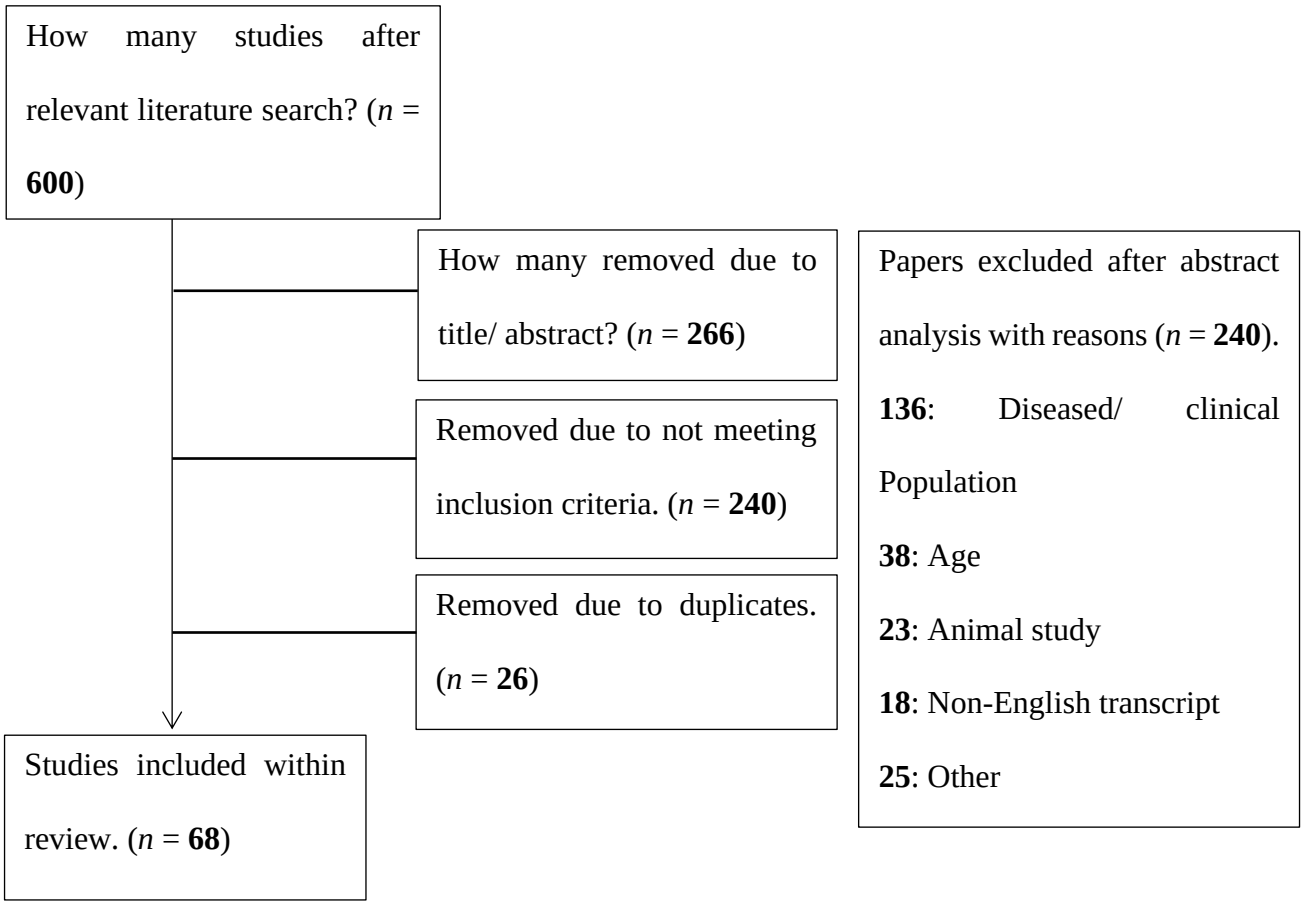

Figure 12-1. Illustrates the process of eliminating articles based on selection criteria.

Papers must meet both inclusion criteria's, these were; [(1)] participants had to be healthy in complete absence of any type of disease, [(2)] be within an age range of 16 and 50 years old. This ensures participants can partake off their own volition and are physically capable to participate in the study.

Studies were excluded if they included any of the subsequent terms; "children", "disease", "elderly adults", "animals". Articles were also excluded if they were not originally written in English. During the original literature searches papers were excluded if they were written prior to 1970, although multiple papers were used if they were cited by more than 10 of the

papers that were included in the final article list. Papers were also included if they were used for definitions or ground-breaking findings but fell outside the inclusion date range.