

The Influence of Respiratory Muscle Work on Locomotor and Respiratory Muscle Oxygenation Trends in Repeated-sprint Exercise

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ABSTRACT

This thesis investigated the role respiratory muscle work has on locomotor and respiratory muscle oxygen (O_2) utilisation during multiple sprint work. To measure O_2 delivery and uptake in real time, near-infrared spectroscopy (NIRS) can be used. However, there are inconsistent methods of smoothing and determining peaks and nadirs from the NIRS signal. Therefore, the aim of study 1 was to examine the effects of different methodologies commonly used in the literature on the determination of peaks and nadirs in the vastus lateralis deoxyhaemoglobin (HHb_{VL}) signal. Means derived from predetermined windows, irrespective of length and data smoothing, underestimated the magnitude of peak and nadir [HHb_{VL}] compared to a rolling mean approach. Based on the results, we suggest using a digital filter to smooth NIRS data, rather than an arithmetic mean, and a rolling approach to determine peaks and nadirs for accurate interpretation of muscle oxygenation trends.

In the second study, the effects of heightened inspiratory muscle work on [HHb_{VL}] and respiratory muscle deoxyhaemoglobin ([HHb_{RM}]) trends were examined. In response to the heightened inspiratory muscle work, HHb_{RM} was elevated across the sprint series. There were no clear differences in HHb_{VL} trends between exercise conditions. The lack of difference in HHb_{VL} between trials implies respiratory muscle O_2 uptake does not limit locomotor oxygenation trends.

Study 3 investigated the role of arterial hypoxemia on respiratory muscle oxygenation trends, and its implications on locomotor oxygenation. While exercising in hypoxia (14.5% O_2), HHb_{VL} was higher during the sprint and recovery phases of the

repeated-sprint protocol compared to normoxia (21% O₂). There were no clear differences in respiratory muscle oxygenation trends between conditions. The clear reduction in locomotor muscle O₂ delivery (inferred from HHb_{VL}) while respiratory muscle oxygenation was maintained, suggests preferential blood flow distribution to the respiratory muscle to compensate for arterial hypoxemia, which may explain in part compromise locomotor O₂ delivery.

The aim of the final study was to examine the role of respiratory muscle strength on locomotor and respiratory muscle oxygenation trends in repeated-sprint exercise. Inspiratory muscle training (IMT) was used to reduce the relative intensity of exercise hyperpnoea by strengthening the respiratory muscles. Repeat-sprint ability was again assessed in normoxia and hypoxia. After 4 weeks of training, there was a 35% increase of inspiratory muscle pressure in the IMT beyond the control group. Despite the substantial change in respiratory muscle strength, oxygenation trends were not affected in either normoxia or hypoxia.

The findings of this thesis do not support the work of breathing as being a limiting factor in locomotor muscle oxygenation in normoxia. The intermittent nature of repeated-sprint activity is likely a key mediating factor for which O₂ delivery can be maintained to both the locomotor and respiratory muscles. However, under conditions of arterial hypoxemia, locomotor muscle oxygenation may be compromised by preferential O₂ delivery to the respiratory muscles.

DOCTOR OF PHILOSOPHY DECLARATION

I, Ramón F. Rodríguez-Anderson, declare that the Ph.D. thesis entitled “The Influence of Respiratory Muscle Work on Locomotor and Respiratory Muscle Oxygenation Trends in Repeated-sprint Exercise” is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography and references. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature _____

Date _____

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LIST OF PUBLICATIONS

The Following work has been presented at scientific meetings or accepted for publication at peer-reviewed journals in support of this thesis:

1. **Ramón F. Rodriguez.**, Nathan E. Townsend., Robert J. Aughey., & François Billaut. (2018). Influence of Averaging Method on Muscle deoxygenation Interpretation in Repeated-Sprint Exercise. *Scandinavian Journal of Medicine and Science in Sports*, 28(11), 2263-2271. doi:10.1111/sms.13238 ([Chapter Three](#)).
2. **Ramón F. Rodriguez.**, Nathan E. Townsend., Robert J. Aughey., & François Billaut. (December 2016). Influence of Averaging Method on Muscle deoxygenation Interpretation in Repeated-Sprint Exercise. Presented at the *College of Sport and Exercise Science Higher Degree by Research conference*, Melbourne, Australia. ([Chapter Three](#)).
3. **Ramón F. Rodriguez.**, Nathan E. Townsend., François Billaut., & Robert J. Aughey. (July 2016). Inspiratory muscle loading during repeated-sprint exercise. Presented at the *21st Annual Congress of the European College of Sport Science ECSS*, Vienna, Austria ([Chapter Four](#)).
4. **Ramón F. Rodriguez.**, Nathan E. Townsend., François Billaut., & Robert J. Aughey. (December 2015). Inspiratory loading, muscle oxygenation and repeated-sprint exercise. Presented at the *College of Sport and Exercise Science Higher Degree by Research conference*, Melbourne, Australia. ([Chapter Four](#)).

The Following work is being prepared for publication at peer reviewed journals in support of this thesis:

1. **Ramón F. Rodriguez.,** Nathan E. Townsend., Robert J. Aughey., & François Billaut. Muscle oxygenation and performance maintained during repeated-sprints despite inspiratory muscle loading. *Currently under review at PLOS One.* ([Chapter Four](#)).
2. **Ramón F. Rodriguez.,** Nathan E. Townsend., & Robert J. Aughey., François Billaut. Respiratory muscle oxygenation is not impacted by hypoxia during repeated-sprint exercise. *Currently being prepared for submission to Respiratory Physiology and Neurobiology.* ([Chapter Five](#)).
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The following work has been published in a peer reviewed journal during candidature, but is outside the scope of this thesis:

1. Sweeting, A., Billaut, F., Varley, M. C., **Rodriguez, R. F.,** Hopkins, W., & Aughey, R. J. (2017). Variations in Hypoxia Impairs Muscle Oxygenation and Performance During Simulated Team-Sport Running. *Frontiers in Physiology*, 8(80). doi: 10.3389/fphys.2017.00080

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LIST OF ABBREVIATIONS

%	Percent
%·s ⁻¹	Percent per second
[H ⁺]	Concentration of hydrogen ions
[HHb]	Concentration of deoxyhaemoglobin
[O ₂ Hb]	Concentration of oxyhaemoglobin
~	Approximately
<	Less than
>	Greater than
Δ%[HHb]	Percent change in the concentration of deoxyhaemoglobin
ΔReoxy	Reoxygenation
∫P _m × f _b	Inspiratory muscle force development
≤	Less than or equal to
↑	Increase
→	No change
↓	Decrease
μm	Micrometre
2 _{MA}	2 s moving average
2 _{PD}	2 s predetermined average
5 _{MA}	5 s moving average
5 _{PD}	5 s predetermined average
A-aO ₂ diff	Alveolar to arterial O ₂ pressure difference
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate

ATP·kg ⁻¹	Adenosine triphosphate per kilogram
AU	Arbitrary units
b·min ⁻¹	Beats per minute
BL	Baseline
BWF _{MA}	Value obtained from a predetermined time point after the data was smoothed with the Butterworth filter
BWF _{PD}	Single peak/nadir value within each 40 s sprint/recovery cycle.
CL	Confidence limit
cm	Centimetre
cmH ₂ O	Centimetre of water
CO ₂	Carbon dioxide
Cr	Creatine
CTRL	Control
EMT	Expiratory muscle training
ES	Effect size
ET	Endurance training
f_b	breathing frequency
f_c	cut-off frequency
FEV ₁	Forced expiratory volume in 1 s
F _I CO ₂	fraction of inspired carbon dioxide
F _I O ₂	fraction of inspired oxygen
FVC	forced vital capacity
GET	Gas exchange threshold
H ⁺	Hydrogen ion
H ₂ CO ₃	Carbonic acid

H ₂ O	Water
Hb	Haemoglobin
HCO ₃ ⁻	Bicarbonate
HHb _{RM}	Respiratory muscle deoxyhaemoglobin
HHb _{VL}	Vastus lateralis deoxyhaemoglobin
HR	Heart rate
Hz	Hertz
IMT	Inspiratory muscle training
INSP	Inspiratory loading repeated-sprint exercise
IV	Inspiratory volume
J·L ⁻¹	Joules per litre
J·min ⁻¹	Joules per minute
kg	Kilogram
kJ	Kilojoule
km	Kilometre
Kp	Kilopond
L	Litre
L·min ⁻¹	Litters per min
m	Meter
m·s ⁻¹	Meters per second
MATCH	Work matched exercise
MEP	Maximal expiratory pressure
min	Minute
MIP	Maximal inspiratory mouth pressure
mL·min ⁻¹ ·kg ⁻¹	Millilitres per minute per kilogram
mm	Millimetre

mmHg	Millimetre of mercury
mmol	Millimole
MVC	Maximal voluntary contraction
MVV	Maximal voluntary ventilation
n	Sample size
N·kg ⁻¹	Newton per kilogram
NIRS	Near-infrared spectroscopy
O ₂	Oxygen
O ₂ Hb _{RM}	Respiratory muscle oxyhaemoglobin
P ₀	Maximal inspiratory pressure at zero flow
P ₁	pressure of first gas
P ₂	pressure of second gas
PaCO ₂	Partial pressure of arterial carbon dioxide
P _A CO ₂	Partial pressure of alveolar CO ₂
P _a O ₂	Partial pressure of arterial oxygen
P _A O ₂	Partial pressure of alveolar oxygen
P _a O ₂	Partial pressure arterial oxygen
PAV	Proportional assist ventilation
P _B	Barometric pressure
P _c O ₂	Partial pressure capillary oxygen
PCr	Phosphocreatine
PEF	Peak expiratory flow
P _{ET} CO ₂	End-tidal carbon dioxide
P _{ET} O ₂	End-tidal oxygen
P _{FV} O ₂	Partial pressure femoral vein oxygen
P _{H2O}	Pressure of inspired water vapour

PIF	Peak inspiratory flow
P_{iO_2}	Partial pressure of inspired oxygen
P_m	Mouth pressure
P_{OPT}	Optimal pressure for maximal flow production
PPO	Peak power output
\dot{Q}	Blood flow
R	Respiratory exchange ratio quotient
r	Pearson's product-moment correlation
r^2	Coefficient of determination
Reoxy rate	Vastus lateralis reoxygenation rate
RET	Respiratory endurance time
RMET	Respiratory muscle endurance training
RMS	Root-mean squared
RPE	Rating of perceived exertion
RPE_{Breath}	Rating of perceived exertion for breathing
$RPE_{Exercise}$	Rating of perceived exertion for exercise
rpm	Revolutions per minute
RS	Repeat-sprint
s	Second
SD	Standard deviation
SpO_2	Arterial oxygen saturation by pulse oximetry
tHb _{RM}	Respiratory muscle total haemoglobin
TSI _{RM}	Respiratory muscle tissue saturation index
TSI _{VL}	Vastus lateralis tissue saturation index
TT	Time trial
TTP_{HHb}	Time to peak vastus lateralis deoxyhaemoglobin

V_1	Volume of first gas
V_2	Volume of second gas
$\dot{V}CO_2$	Rate of carbon dioxide elimination
\dot{V}_E	Ventilation rate
\dot{V}_{Epeak}	Peak ventilation rate
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_{2max}$	Maximal rate of oxygen uptake
$\dot{V}O_{2peak}$	Peak rate of oxygen uptake
$\dot{V}O_{2RM}$	Respiratory muscle oxygen uptake
\dot{V}_{OPT}	Optimal flow
V_T	Tidal volume
W	Watt
$W \cdot \text{min}^{-1}$	Watts per minute
$W_{I_{max}}$	Maximal inspiratory power
wk	week

CHAPTER ONE: INTRODUCTION

The respiratory system is primarily responsible for regulating arterial blood gasses through pulmonary ventilation (\dot{V}_E). The degree of hyperpnoea is controlled by the integration of multiple factors in order to prevent hypocapnia and hypoxia from occurring (Forster, Haouzi, & Dempsey, 2012). At rest, and up to moderate intensity exercise (<80% of maximal oxygen uptake), the energy requirements of the respiratory muscle necessary to generate airflow is relatively low. However, the ventilation demands of high-intensity exercise (>80% of maximal oxygen uptake) require considerable blood flow and oxygen (O_2) supply to support the muscular work of breathing. It is estimated that the O_2 cost of exercise hyperpnoea accounts for 10-15% of the total whole body O_2 uptake ($\dot{V}O_2$) (Aaron, Seow, Johnson, & Dempsey, 1992; Harms et al., 1998; Turner et al., 2012). As both locomotor and respiratory muscle demands for O_2 rich blood flow begin to encroach on the maximal transport capacity of the cardiovascular system, competition for available cardiac output can arise. By elevating inspiratory muscle work during sustained high-intensity exercise, limb blood flow is attenuated (Harms et al., 1997), peripheral fatigue hastened (Romer, Lovering, Haverkamp, Pegelow, & Dempsey, 2006), and exercise performance impaired (Harms, Wetter, St Croix, Pegelow, & Dempsey, 2000). Conversely, the opposite effects have been observed when the work of breathing incurred during exercise has been lowered with assisted ventilation technology.

Most of the research in this area has focused on prolonged bouts of exercise, with very little on intermittent high-intensity exercise. One such model is repeated-sprint exercise, which is characterised by brief periods of maximal exertion, separated by short rest periods. Underpinning the capacity to maintain sprint performance over multiple efforts is the ability to resynthesise phosphocreatine (PCr), the primary metabolite in

sprint exercise (Dawson et al., 1997; Gaitanos, Williams, Boobis, & Brooks, 1993). Even though ATP generation from PCr is entirely an anaerobic process, PCr resynthesis is derived solely from aerobic metabolism, and is highly sensitive to muscle O₂ availability (Haseler, Hogan, & Richardson, 1999; Sahlin, Harris, & Hultman, 1979). Therefore, the ability to deliver O₂ to the locomotor muscles during rest periods between sprints is critical to maintaining maximal sprint performance (Billaut & Buchheit, 2013; Kime et al., 2003). It is currently unclear, however, if respiratory muscle work has any influence on muscle O₂ delivery during repeated-sprint exercise. There is some evidence that training targeted specifically at the respiratory muscles improves repeat-sprint performance (Archiza et al., 2017; Romer, McConnell, & Jones, 2002b). In fact, reducing the relative intensity of exercise hyperpnoea through training is reported to lessen the O₂ cost of breathing. However, there has been no investigation into the muscle oxygenation trends following respiratory muscle training.

Near-infrared spectroscopy (NIRS) is used to evaluate tissue oxygenation during exercise. This technology relies on the light absorbing characteristics of oxy- and deoxy-haemoglobin, and reflects the balance between O₂ delivery and utilisation (Ferrari, Mottola, & Quaresima, 2004). Before investigating the locomotor muscle oxygenation trends in repeated-sprint exercise, understanding the how varying methodology of NIRS analysis influences the reported outcomes was needed. Therefore, the aim of the first study ([Chapter Three](#)) was to compare and evaluate the effect of different NIRS signal analysis methods on vastus lateralis oxygenation trends during repeated-sprint exercise.

The next aim ([Chapter Four](#)) was to identify the consequences of heightened inspiratory muscle work during repeated-sprint exercise. Vastus lateralis and intercostal

muscle NIRS responses were examined as an index for locomotor and respiratory muscle O₂ delivery and uptake. The balance of O₂ delivery between the locomotor and respiratory muscle was assessed in relation to pulmonary $\dot{V}O_2$.

The aim of the third study ([Chapter Five](#)) was to examine the effects of acute arterial hypoxemia on intercostal muscle oxygenation relative to normoxia. It has been demonstrated that vastus lateralis reoxygenation kinetics between sprints is impaired in environmental hypoxia (Billaut & Buchheit, 2013). But how the respiratory muscles responded was unclear. Intercostal muscle oxygenation was assessed to determine if the respiratory muscles are equally affected by hypoxia, or if intercostal muscles “steal” O₂ from the locomotor muscles to maintain hyperpnoea.

The final research chapter ([Chapter Six](#)) explored respiratory muscle training as a potential pathway to enhance locomotor muscle reoxygenation, and repeated-sprint performance. Once again repeated-sprint ability was assessed in normoxia and hypoxia, and muscle oxygenation was assessed with NIRS. The training design used in this study was well established for enhancing inspiratory muscle strength and exercise performance (McConnell & Romer, 2004b).

Commencing with a literature review ([Chapter Two](#)), this thesis further comprises four experimental chapters:

- I. [Chapter Three](#) (Study 1): Influence of averaging method on muscle deoxygenation interpretation during repeated-sprint exercise.
- II. [Chapter Four](#) (Study 2): Muscle oxygenation and performance maintained during repeated sprints despite inspiratory muscle loading.

- III. [Chapter Five](#) (Study 3): Acute hypoxia and respiratory muscle oxygenation.
- IV. [Chapter Six](#) (Study 4): The Effects of inspiratory muscle training on muscle oxygenation trends.

The main findings of this thesis are summarised with a general discussion of the results ([Chapter Seven](#)), including limitations of the research presented, and suggestions for future research.

CHAPTER TWO: LITERATURE REVIEW

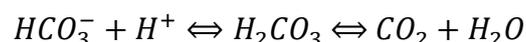
2.1 CHAPTER OUTLINE

This review of literature begins with an introduction to the control of breathing, with focus on exercise hyperpnoea. Ventilation will also be discussed in the context of high-intensity exercise and the response to hypoxia. After this, there will be a brief overview of the oxygen cascade and the regulation of blood flow during exercise. The next section details the interplay between respiratory muscle work, and the development of locomotor muscle fatigue. This is then followed by a review of the current literature surrounding respiratory muscle training. The review ends with a detailed overview of repeated-sprint exercise, integrating the previously discussed topics.

2.2 CONTROL OF BREATHING DURING EXERCISE

Ensuring homeostasis during exercise requires that the O₂ extracted from arterial blood by the muscles is replenished, and carbon dioxide (CO₂) produced by the muscles is eliminated. The primary challenge of the respiratory system is to regulate these gasses, to ensure hypocapnia and hypoxia do not develop (Casaburi, Whipp, Wasserman, Beaver, & Koyal, 1977; Douglas & Haldane, 1909; Forster et al., 1993; Forster, Pan, & Funahashi, 1986; Somers, Mark, Zavala, & Abboud, 1989; Weil et al., 1972). Pulmonary ventilation can also assist with buffering hydrogen ions (H⁺) during exercise. Bicarbonate (HCO₃⁻) will combine with H⁺ to form carbonic acid (H₂CO₃), which is then converted to CO₂ and water (H₂O).

Equation 2.1: Bicarbonate buffer system (Hultman & Sahlin, 1980).



At the onset of exercise, total pulmonary ventilation (\dot{V}_E) increases abruptly (Krogh & Lindhard, 1913). When the metabolic rate rises, \dot{V}_E proportionally increases to

prevent hypercapnia. This prevention of hypercapnia is achieved through a combination of elevated breathing frequency (f_b) and tidal volume (V_T) (Forster et al., 2012; Sheel & Romer, 2012). During light exercise (V_T below $\sim 50\%$ of vital capacity), an increase in \dot{V}_E is predominantly achieved by an elevation in V_T (Hey, Lloyd, Cunningham, Jukes, & Bolton, 1966). During more strenuous exercise (V_T between 50-60% of vital capacity,) there is no further rise in V_T . The continual increase in \dot{V}_E experienced during incremental exercise is therefore achieved by more rapid f_b (Hey et al., 1966; Younes & Kivinen, 1984). These changes in breathing pattern are closely tied to metabolic activity. However, above the ventilatory threshold, \dot{V}_E increases disproportionately to the metabolic rate (Figure 2.1) (Wasserman, Whipp, Koysl, & Beaver, 1973).

Exercise hyperpnoea and hyperventilation constrain the development of arterial hypoxemia as the alveolar-arterial O_2 gradient widens (Harms & Stager, 1995); provides some compensation for progressive metabolic acidosis (Forster et al., 2012); hyperventilation induced hypocapnia results in cerebral vasoconstriction (Raichle & Plum, 1972); and respiratory muscle work is supported by a large portion of cardiac output during high-intensity exercise (Harms et al., 1998). The degree of exercise hyperpnoea is regulated by the integration of multiple factors with built-in redundancy so that no one factor regulates ventilation.

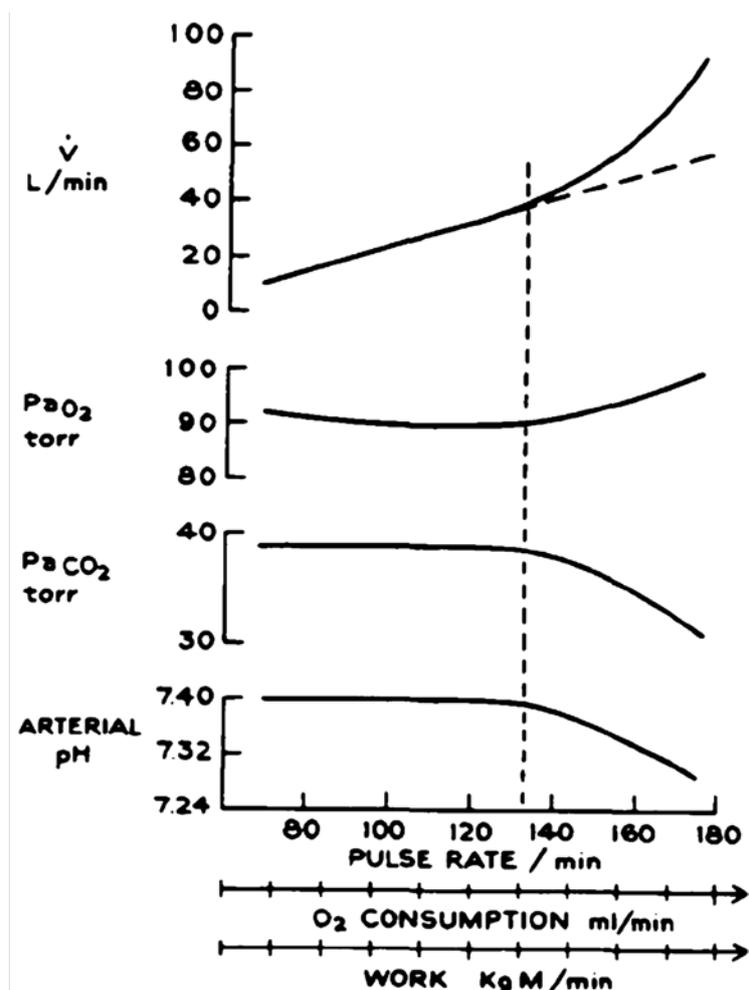


Figure 2.1: An illustration of the typical ventilation responses to exercise at different exercise intensities. Ventilation rate (in this case \dot{V} , but typically abbreviated to \dot{V}_E) rises proportionally to the work rate (O_2 consumption, and pulse rate) during rhythmic exercise, such that the partial pressure of arterial oxygen (P_{aO_2}) and carbon dioxide (P_{aCO_2}), and pH are held constant during mild to moderate exercise. Above an individual threshold, indicated by the vertical dashed line, ventilation rises exponentially to an increasing work rate, causing a fall in P_{aCO_2} and pH, and a concurrent rise in P_{aO_2} . Reproduced from Waldrop (1989).

2.2.1 Metabolic and Locomotor Feedback

The production of a respiratory motor pattern to drive the respiratory muscles involves the integration of multiple sensory inputs of both chemical and mechanical nature (Forster et al., 2012; Lahiri & Forster, 2003; Sheel & Romer, 2012). Afferent sensory input originating peripherally and centrally is responsible for generating an appropriate respiratory motor pattern to match metabolic demands. Peripheral chemoreceptors are sensitive to chemical changes of the circulating arterial blood, while

nerve endings in skeletal muscle provide feedback on the chemical and structural changes in response to muscle contraction.

2.2.1.1 Chemoreceptor Feedback

Peripheral arterial chemoreception is important for the reflex control of respiration, and the chemoreceptors are located in the carotid and aortic bodies (Haymans & Neil, 1959; Schmidt & Comroe, 1940). Carotid and aortic bodies are small clusters of cells within the arteries sensitive to hypocapnia, hypoxia, and acidosis (O'Regan & Majcherczyk, 1982). The carotid bodies are located at the carotid bifurcations. The advantageous location of the carotid bodies provide early feedback on the status of arterial blood prior to the blood entering the brain's circulation (Parkes, 2013). Secondary peripheral chemoreceptors exist at the aortic arch, but are less chemically sensitive (Comroe, 1939; Lahiri, Mokashi, Mulligan, & Nishino, 1981).

Additional chemoreceptors are in the medulla region of the brain stem (Mitchell, Loeschcke, Severinghaus, Richardson, & Massion, 1963; Nattie & Li, 2012). The central chemoreceptors are immersed in the brain's interstitial fluid, and are highly sensitive to changes in interstitial pH (Nattie & Li, 2012). However, the cerebral spinal fluid has a closely regulated environment partially enforced by the selective permeability of the blood-brain barrier. Arterial acids and bases diffuse slowly across the blood-brain barrier, whereas CO₂ permeates radially and changes the pH of the medullary interstitial fluid quickly and substantially (Hladky & Barrand, 2016; Paulson, 2002).

2.2.1.2 Feedback from Locomotor Muscles

Mechanical and biochemical stimuli provide feedback on contraction-induced perturbation of skeletal muscle, contributing to the generation of a respiratory motor pattern via group III (myelinated) and IV (unmyelinated) muscle afferent fibres (Amann,

2012; Haouzi, Chenuel, & Huszczuk, 2004). A sudden rise in ventilation occurs at the transition from rest to exercise (Krogh & Lindhard, 1913). The abrupt change in ventilation is too rapid for chemical feedback to be the initial stimuli for hyperpnoea (Torelli & Brandi, 1961; Whipp, 1977). Therefore, it may be the limb activity itself that is responsible for the initial increase in ventilation at exercise onset. Through passive limb movement an abrupt change in ventilation can be induced, which has been demonstrated to be persistent for up to 5 min (Waisbren, Whiting, & Nadel, 1990). Since there is an inherent delay in chemoreceptor mediated exercise hyperpnoea (Torelli & Brandi, 1961), immediate feedback is important for pre-emptive adjustments of ventilation in anticipation of metabolic disturbances (Forster et al., 2012).

Feedback during prolonged exercise is also important for fine tuning the degree of exercise hyperpnoea. When a neural blockade is used to inhibit sensory feedback (1 mL of fentanyl, injected into the L₃-L₄ intervertebral space), ventilatory responses to cycling exercise are attenuated in moderately trained males (Amann et al., 2010). These cyclists exercised for 3 min at 50, 100, and 150 W, followed by 4 min at 80% of peak power output (325 ± 19 W). At rest and 50 W cycling, the blockade had no discernible effects on \dot{V}_E . However, at the higher work rates \dot{V}_E was decreased ~8–10 L·min⁻¹ (8-17%) primarily by reduction in f_b . At the highest work rate, the blunted hyperpnoea resulted in arterial hypoxemia (monitored via pulse oximetry), accelerated the development of peripheral fatigue (quadriceps twitch interpolation), and reduced the time to exhaustion (Amann et al., 2010, 2011).

The combined data from both animal and human models reveal that at least a small degree of exercise hyperpnoea is mediated by contracting muscles. Group III and IV fibres within the muscle provide the important immediate feedback on length, tension,

and chemical status of contracting musculature, prior to metabolic by-products entering systemic circulation.

2.2.2 Central Command

Feedforward mechanisms are theorised to mediate hyperpnoea, and may in part be responsible for some of the increases in ventilation during exercise (Waldrop, Eldridge, Iwamoto, & Mitchell, 2010). The sudden rise in ventilation at exercise onset that was introduced in section 2.2.1.2 may have central origins. Specifically, co-activation of locomotor and respiratory areas of the brain serve as the feedforward control mechanism (Waldrop et al., 2010).

Electrical stimulation of the hypothalamus to produce locomotion in decorticated cats, results in a proportionate increase in respiration (Eldridge, Millhorn, & Waldrop, 1981). In this instance, respiration was quantified by the electrical activity of the phrenic nerve. The cats also had their carotid bodies and baroreceptors denervated. To further eliminate feedback as the source of hyperpnoea, four cats were paralysed using the neuromuscular blockade gallamine triethiodide delivered intravenously. The same relative increase in respiration to fictive locomotion (measured as a change in nerve activity of the hind limb) was observed in response to hypothalamus stimulation. The authors concluded by proposing that activation of the locomotor areas of the hypothalamus are primarily responsible for the proportional drive for locomotion and respiration (Eldridge et al., 1981).

In humans, the role of central command has been assessed in subjects with unilateral leg weakness (Innes, De Cort, Evans, & Guz, 1992). Three groups were studied: 1) six patients recovering from orthopaedic disorders, 2) two patients with neurological disorders, and 3) eight healthy subjects with temporary weakness induced by local

antistatic. Subjects performed single leg cycling for 4 min at an intensity which resulted in a similar $\dot{V}O_2$ when exercising each leg. Ventilation increased more when exercise was performed with the weakened leg in all subjects, and independently in metabolic rate. Since greater muscle activation to the weakened leg was likely necessary, the magnitude of central activation was believed to have influenced the heightened ventilatory responses (Innes et al., 1992).

2.2.3 Hyperventilation during Heavy Exercise

At work rates below the ventilatory threshold, there is a linear increase in \dot{V}_E proportional to the metabolic rate (Figure 2.1). However, above the ventilation threshold \dot{V}_E rises disproportionately to the metabolic rate, initially causing the pressure of alveolar O_2 to increase, then CO_2 to fall (Forster et al., 2012; Waldrop, 1989; Wasserman et al., 1973). It is likely that arterial acidosis is a major contributor to the hyperventilation. However, other feedback/feedforward mechanisms also appear to be influential to the excess ventilation.

2.2.3.1 Arterial Acidosis

Rapid elevation of H^+ concentration ($[H^+]$) in the blood and tissues occurs during high-intensity exercise, and accumulates when production exceeds the rate which CO_2 is eliminated from the body through ventilation (A. V. Hill, Long, & Lupton, 1924). A strong relationship exists between the onset of blood lactate accumulation and ventilation threshold (Loat & Rhodes, 1993). Therefore, it may be assumed that chemoreceptor exposure to CO_2/H^+ incurred with high-intensity exercise is the cause of the “extra” drive to breathe (Figure 2.1). However, an exercise and dietary intervention that promotes glycogen depletion and therefore reduced carbohydrate metabolism, resulted in an uncoupling of ventilation and lactate thresholds (Hughes, Turner, & Brooks, 1982). Since

ventilation and lactate thresholds can be manipulated independently of each other, lactic-acid accumulation is unlikely to be responsible for exercise-induced hyperventilation. Additionally, patients with McArdle's syndrome who are incapable of producing lactic-acid due to the lack on the enzyme glycogen phosphorylase, hyperventilation should not occur if acidosis was responsible for the "extra" ventilation during high-intensity exercise. However, these patients still display a normal hyperventilation response, despite no increase in blood lactate or H^+ concentration (Hagberg et al., 1982).

2.2.3.2 Locomotor Muscle Fatigue

Locomotor muscle fatigue rapidly develops during high-intensity exercise, and may be more influential than acidosis alone towards "excess" ventilation. Peripheral fatigue resulting in a decrease in muscle force-generating capacity, is associated with an increase in central command to maintain force production and subsequent co-activation respiratory muscles (Forster et al., 2012). Indirectly, increased motor drive is supported by an elevation in electromyography of the locomotor muscles coinciding with the ventilation threshold (Lucia, Sanchez, Carvajal, & Chicharro, 1999; Mateika & Duffin, 1994). To mimic fatigue, subjects can be given either a muscle relaxant or local anaesthetics to cause chemically induced muscle weakness. In these subjects, \dot{V}_E is higher during the "fatiguing" exercise compared to their fatigue free state (Asmussen, Johansen, Jørgensen, & Nielsen, 1965; Galbo, Kjaer, & Secher, 1987; Innes et al., 1992). For example, a neuromuscular blockade can be given as a method of inducing muscular weakness. At a given cycling work rate and at a similar $\dot{V}O_2$, \dot{V}_E was as least 37% higher when performed under the influence of the blockade (tubocurarine chloride) compared to control. It was speculated that in order to overcome the muscle weakness, there was compensatory

recruitment of additional accessory muscles, and therefore the requirement of greater central command (Galbo et al., 1987).

2.2.4 Acute Environmental Hypoxia

Ventilation in acute hypoxia is higher to mitigate the reduction in partial pressure of O₂ in the environment (Figure 2.2; Forster et al., 2012). Environmental hypoxia naturally occurs when the barometric pressure falls on the ascent of a mountain. Altitude can also be simulated in two ways 1) reducing the barometric pressure within an airtight chamber (hypobaric hypoxia), and 2) reducing the O₂ in the inspired gas mixture (normobaric hypoxia). Both these altitude simulation methodologies reduce the O₂ diffusion capacity between alveoli and pulmonary capillaries to promote arterial hypoxemia. The partial pressure of alveolar O₂ (P_AO₂) available for gas exchange is calculated as:

Equation 2.2: Calculation of the partial pressure of alveolar oxygen (Biro, 2013).

$$P_{AO_2} = F_{IO_2}(P_b - P_{H_2O}) - (P_{ACO_2} \div R)$$

where F_IO₂ is the fraction of inspired O₂; P_b is barometric pressure; P_{H₂O} is the pressure of inspired water vapour; P_ACO₂ is the pressure of alveolar CO₂; and R is respiratory exchange ratio quotient $\dot{V}CO_2/\dot{V}O_2$.

When cycling at the same absolute work rate and duration (82.1 ± 0.5% of the peak power output obtained during a graded exercise test in hypoxia) in normoxia and hypoxia (F_IO₂ = 0.15), \dot{V}_E is elevated by 53 ± 7% over the final minute of exercise (Amann, Pegelow, Jacques, & Dempsey, 2007). The elevation in \dot{V}_E was achieved through an increase in f_b (from 40.3 ± 2.8, to 59.5 ± 2.7 breaths·min⁻¹). However, when exercising at altitude, there is a linear decrease in maximal work rate and the maximal rate of O₂ uptake

($\dot{V}O_{2\max}$) (Martin & O'Kroy, 1993; Wehrlin & Hallén, 2006). Since the subjects in the previously mentioned study were exercised at the same absolute work rate (Amann, Pegelow, et al., 2007), it is likely that the relative intensity was much greater in hypoxia. To account for the decrease in exercise capacity at altitude, a lower work rate can be selected which would represent the same relative exercise intensity. Even after adjusting for the relative intensity of exercise, \dot{V}_E is still higher at altitude. When subjects performed submaximal exercise to exhaustion (75% $\dot{V}O_{2\max}$ in the respective environment), \dot{V}_E was elevated by 47% in the final moments of exercise at high altitude (5050 m, ~410 mmHg; equivalent to a F_{iO_2} of 0.11), even though the work rate was 23% lower (Cibella et al., 1996). The increase in ventilation was achieved through a 40.7% increase in f_b , which is comparable to what was described previously (Amann, Pegelow, et al., 2007).

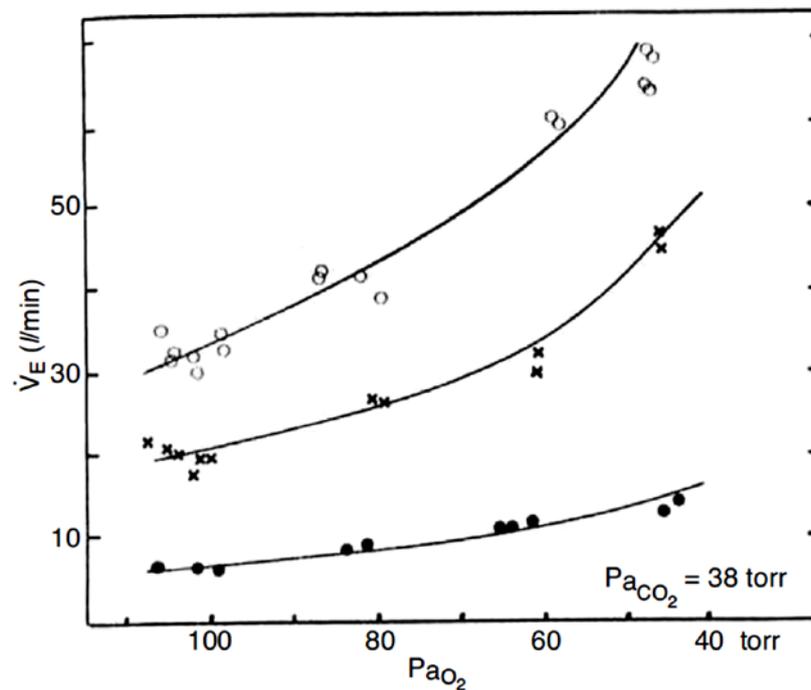


Figure 2.2: Effects of the partial pressure of arterial oxygen (P_{aO_2}) on pulmonary ventilation (\dot{V}_E) during rest and exercise. The partial pressure of arterial carbon dioxide (P_{aCO_2}) was held constant throughout the trials. Data represented by the closed symbols (\bullet) were obtained during rest, and the open symbols (\times and \circ) were obtained during two levels of submaximal exercise ($n = 3$). Reproduced from Forster et al. (2012) with data from Asmussen and Nielsen (1957).

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Aside from the direct mediating effects of arterial hypoxemia on ventilation via chemoreceptor activation (O'Regan & Majcherczyk, 1982), there are secondary effects of hypoxia influencing ventilation. Exposure to hypoxia causes the chemoreceptors to become more sensitive to changes in arterial blood gasses and pH (Eyzaguirre & Koyano, 1965; Lahiri & DeLaney, 1975). Second, to compensate for the reduced O₂ availability, there is a shift in metabolism towards greater reliance on anaerobic pathways for ATP formation (Ibañez, Rama, Riera, Prats, & Palacios, 1993; Morales-Alamo et al., 2012). Therefore, greater circulating by-products of anaerobic metabolism (CO₂/H⁺) are available to activate the respiratory chemoreceptors (Asmussen & Nielsen, 1957). Lastly, the hastened development of peripheral muscle fatigue associated with arterial hypoxemia (Amann & Calbet, 2008), contributes to exercise hyperpnoea. To maintain force production, greater central drive (central command) of the active muscle is necessary to overcome fatigue (Amann, Romer, Subudhi, Pegelow, & Dempsey, 2007; Moritani, Muro, & Nagata, 1986). As introduced earlier (section 2.2.3.2), it is likely that there is co-activation of locomotor and respiratory centres (Mateika & Duffin, 1994). Therefore, heightened central motor command associated with the progression of locomotor muscle fatigue is another likely source contributing of exercise hyperpnoea in hypoxia.

2.3 OXYGEN TRANSPORT

Human skeletal muscle has limited O₂ storage capability (Millikan, 1939). Because of this limited O₂ storage, a constant blood supply rich with O₂ is necessary to support aerobic metabolism. To meet the metabolic demands of exercise, cardiac output can increase from ~5 L·min⁻¹ at rest, to 20 L·min⁻¹ during maximal exercise in untrained subjects and up to 40 L·min⁻¹ in elite endurance athletes (Joyner & Casey, 2015). The regional distribution of blood flow is closely related to the metabolic rate of the exercising muscle (Andersen & Saltin, 1985; Hamann, Kluess, Buckwalter, & Clifford, 2005; Knight et al., 1992; Rowell, Saltin, Kiens, & Christensen, 1986). However, during high-intensity exercise competition can arise for available cardiac output between muscle groups (e.g. legs vs. arms) (Calbet et al., 2004; Harms et al., 1997; Secher, Clausen, Klausen, Noer, & Trap-Jensen, 1977; Volianitis, Krstrup, Dawson, & Secher, 2003; Volianitis & Secher, 2002). Along with blood flow, the passive movement of O₂ down its concentration gradient from environmental air to the mitochondria is fundamental for sustained aerobic metabolism.

2.3.1 Oxygen Cascade

Moving O₂ from the environment to the tissue involves a complex series of steps. Breakdown at any point along the pathway can result in inadequate muscle O₂ supply, and impair exercise performance. Though exposure to (simulated) altitude can impair O₂ transport at every step in the O₂ cascade, and attenuate muscle O₂ extraction (Figure 2.3).

Respiratory Muscle Work and Tissue Oxygenation Trends During Repeated-sprint Exercise

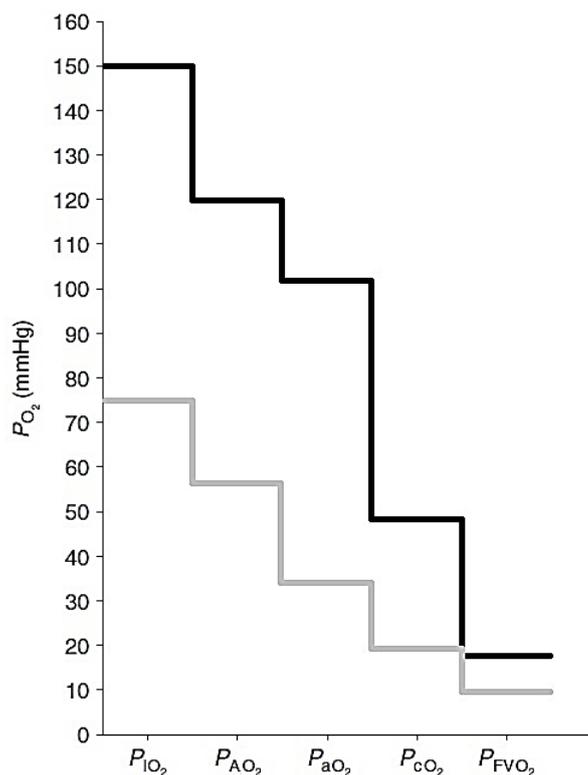


Figure 2.3: The partial pressure of oxygen along the oxygen cascade at sea-level, and simulated altitude. The black line represents subjects breathing room air ($F_{I}O_2 = 0.21$), and the grey line a hypoxic gas mixture ($F_{I}O_2 = 0.105$) during cycling exercise. Moving from left to right, values are for the partial pressure of inspired oxygen ($P_{I}O_2$); alveolar oxygen ($P_{A}O_2$); arterial oxygen ($P_{a}O_2$); estimated mean capillary oxygen ($P_{c}O_2$); and femoral vein oxygen ($P_{FV}O_2$). Adapted from Calbet, Rådegran, Boushel, and Saltin (2009).

2.3.1.1 Pulmonary ventilation

Pulmonary ventilation is responsible for O₂ and CO₂ gas exchange between the lungs and external environment. The respiratory muscles, primarily the diaphragm, act as a two-way flow generator, moving air in and out of the lungs (Aliverti et al., 1997). Ventilation is closely tied to metabolism, and regulated by continual feedback on the internal environment (Forster et al., 2012; Sheel & Romer, 2012). Through exposure to hypoxia and during high-intensity exercise, ventilation rises disproportionately to the metabolic rate (Amann, Pegelow, et al., 2007; Cibella et al., 1996; Waldrop, 1989; Wasserman et al., 1973). Hyperventilation can have a protective mechanism against hypoxemia, as a reduction in CO₂/H⁺ causes a leftward shift of the oxygen-haemoglobin

dissociation curve, increasing the O₂ binding affinity for haemoglobin (Figure 2.4) (Bohr, Hasselbalch, & Krogh, 1904; Jensen, 2004).

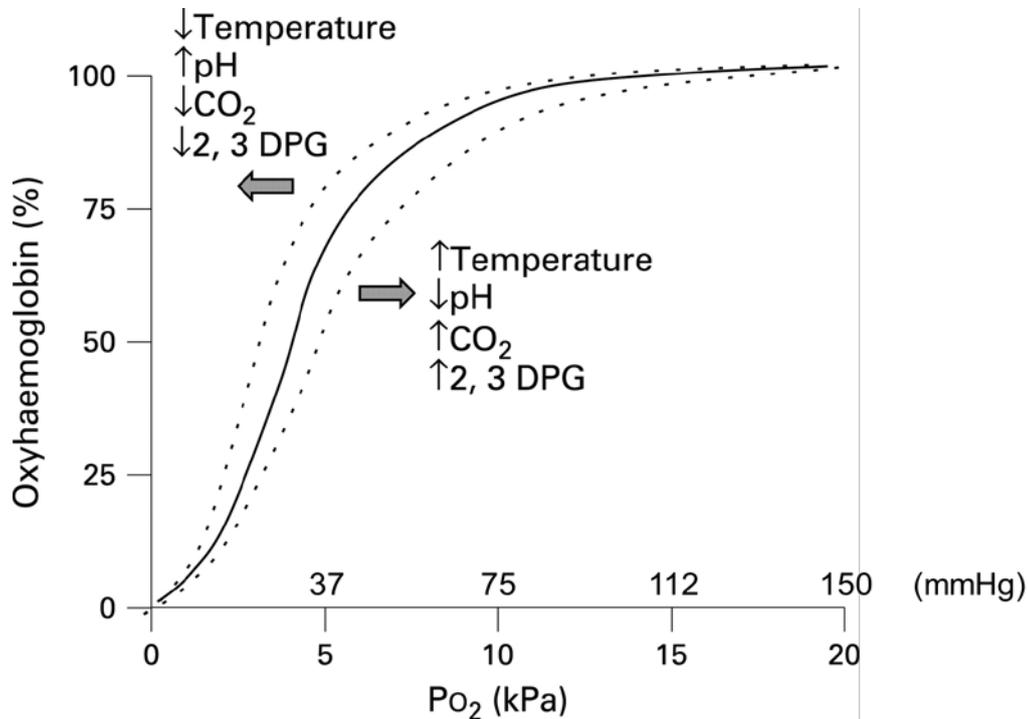


Figure 2.4: Oxygen-haemoglobin dissociation curve and factors affecting oxygen's binding affinity to haemoglobin. A leftward shift in the oxygen-haemoglobin dissociation curve results in an increased oxygen binding affinity. Whereas the consequence of a rightward shift is a decrease in binding affinity. Abbreviations are: CO₂, carbon dioxide; 2, 3 DPG, 2, 3-diphosphoglycerate acid. Reproduced from O'Driscoll, Howard, and Davison (2008).

2.3.1.2 Pulmonary gas exchange

Once inhaled air reaches the lung, gas exchange can occur between the thin walled alveoli and pulmonary capillaries. This gas exchange is achieved through the passive movement of O₂ (from air to blood) and CO₂ (from blood to air) down their respective pressure gradients (Wagner, 2015). In healthy young subjects, the alveolar to arterial O₂ pressure difference (A-aO₂diff) averages 5-10 mmHg at rest (Mellemaard, 1966; Raine & Bishop, 1963). This pressure difference facilitates the movement of O₂ to move down the concentration gradient and diffuse across the alveolar-capillary membrane. During exercise, there is a progressive widening of A-aO₂diff, which during maximal exercise can exceed 25-30 mmHg (Dempsey & Wagner, 1999). Once past the

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alveolar-capillary membrane, O₂ enters the blood stream (oxygenation). Because O₂ has such low solubility in plasma (Christoforides, Laasberg, & Hedley-Whyte, 1969), the majority of O₂, approximately 98%, is transported via a reversible bond to haemoglobin (Hb) (Collins, Rudenski, Gibson, Howard, & O'Driscoll, 2015).

2.3.1.3 Oxygen transport from the lungs to the tissue

The Hb protein can bind up to four molecules of O₂, and is densely packed within red blood cells (Jensen, 2009; Mairbäurl, 2013; Storz, 2016). The now oxygenated blood returning from the lungs is pumped into the aorta via the left ventricle. To meet the blood flow demands of exercise, cardiac output increases via a combination of increased heart rate (HR), and stroke volume (Joyner & Casey, 2015; Siebenmann & Lundby, 2015). For an increase in cardiac output to be effective at supplying O₂ for exercise metabolism, blood flow is directed away for regions of low metabolic activity, and towards regions of high activity (Hellsten, Nyberg, Jensen, & Mortensen, 2012; Joyner & Casey, 2014; Reglin & Pries, 2014). The release of vasoactive substance increases with tissue metabolism (i.e. muscle contraction), so that any increase in metabolism will result in a proportional rise in blood flow to that region (Joyner & Wilkins, 2007).

2.3.1.4 Tissue gas exchange

When a red blood cell passes through capillary beds of muscle tissue, it enters an environment of low O₂ and the steep portion of the oxygen dissociation curve (Figure 2.4) (Mairbäurl, 2013). The change in O₂-Hb binding affinity causes O₂ to be released into the plasma, and then diffuse into the tissue (Jensen, 2004). By-products of metabolism, which are especially prominent during exercise, also have a negative allosteric effect on O₂-Hb binding affinity. The major effectors are, 2,3-diphosphoglycerate acid (by-product of glucose metabolism in red blood cells), temperature, H⁺ and CO₂ (Astrup, Engel,

Severinghaus, & Munson, 1965; Benesch & Benesch, 1967; Bohr et al., 1904; Dill & Forbes, 1941; Næraa, Petersen, Boye, & Severinghaus, 1966). Therefore, when a red blood cell passes through tissues with high metabolic demands, O₂ will be readily unbound from Hb (Mairbäurl, 1994, 2013). As exercise intensity increases, the amount of O₂ extracted by the contracting muscles increase, which results in a reduction in O₂ returning to the alveoli and widening of A-aO₂diff (Dempsey, Johnson, & Saupe, 1990). The rate of muscle oxygen uptake determined based on the Fick principal (Fick, 1870), and is calculated as:

Equation 2.3: Fick equation (Albouaini, Egred, Alahmar, & Wright, 2007).

$$\dot{V}O_2 = \dot{Q}(a - \bar{v}O_2diff)$$

where \dot{Q} is blood flow, and $a - \bar{v}O_2diff$ is arterial venous oxygen difference.

2.3.2 Blood Flow Redirection and Competition during Exercise

Blood flow to contracting muscles closely matches the metabolic rate (Andersen & Saltin, 1985; Hamann et al., 2005; Harms et al., 1998; Knight et al., 1992; Rowell et al., 1986). It has been robustly demonstrated that there is a positive linear relationship between the rate of O₂ uptake ($\dot{V}O_2$) in the quadriceps muscles and blood flow through the femoral artery (Andersen & Saltin, 1985; Richardson et al., 1993), which ensures that there is a match between O₂ supply and demand for the exercising muscles. Blood flow is directed to areas of need by vasoconstriction in the relatively inactive regions, and vasodilatation in the active locomotor muscles (Harms et al., 1997; Harms et al., 1998; Hellsten et al., 2012; McAllister, 1998; Secher & Volianitis, 2006). During high-intensity and maximal exercise, the accompanying increase in cardiac output is almost exclusively devoted to the working skeletal muscle (Joyner & Casey, 2015), whereas blood flow to the splanchnic, renal and inactive skeletal muscle tissue beds can fall by ~70% from resting values during maximal exercise (Poortmans, 1984; Rowell, Blackmon, Kenny, &

Escourrou, 1984). It is likely that multiple biological factors contribute to biological redundancy in the system (Joyner & Wilkins, 2007). However, there does appear to be a limit to systemic vasodilation, a procreative mechanism to maintain arterial blood pressure and ensure adequate O₂ supply to vital organs (Calbet & Lundby, 2012; Dempsey, Romer, Rodman, Miller, & Smith, 2006; Saltin, 1985; Secher & Volianitis, 2006). Additionally, when the metabolic demands of multiple muscle groups are high, and cardiac output is nearing maximal flow rates, competition for available blood flow can arise between exercise muscle groups. However, there is some conflicting evidence.

When arm exercise is superimposed on ongoing leg exercise, $\dot{V}O_2$ is lower than the sum of the arm and leg exercise alone, which is suggestive of compromised O₂ delivery (Secher et al., 1977; Volianitis & Secher, 2002). For example, when subjects performed upright cycle ergometer and arm crank exercise in isolation, a $\dot{V}O_2$ of 67% and 44% of $\dot{V}O_{2max}$ respectively was induced (Secher et al., 1977). However, when performed in combination, a $\dot{V}O_2$ of only 77% $\dot{V}O_{2max}$ was observed during exercise. The mismatch required O₂ uptake and actual O₂ uptake was likely caused by an increased leg vascular resistance (1.8 [S.E. 0.51] mmHg·min·1⁻¹), which resulted in a decrease in leg blood flow by 1.9 L·min⁻¹ (S.E. 0.72). Not only is O₂ uptake compromised in the lower limbs, but in the upper body too. While performing the combined exercise of arm crank and cycle ergometry, a $\dot{V}O_2$ of ~95% of $\dot{V}O_{2max}$ was elicited (Volianitis & Secher, 2002), lower than what would have been predicted by combining the arm and leg exercise (arm $\dot{V}O_2$: 58% $\dot{V}O_{2max}$; leg $\dot{V}O_2$: 60% $\dot{V}O_{2max}$). In this instance arm blood flow and O₂ uptake was attenuated by 0.58 L·min⁻¹ and 0.40 ± 0.06 L·min⁻¹ during the combined exercise (Volianitis & Secher, 2002). In both these examples, blood flow, and consequently muscle

O₂ uptake, was compromised when combined arm and leg exercise was performed compared to when the muscle groups are solicited in isolation.

To gain insights on the effects of combined upper and lower body exercise on arm blood flow and muscle tissue oxygenation, thermodilution has been used in conjunction with near-infrared spectroscopy (NIRS) of the biceps brachii (Volianitis et al., 2003). Arm blood flow was $\sim 0.35 \text{ L}\cdot\text{min}^{-1}$ lower when combined arm and leg exercise was performed compared to arm exercise alone. The concentration of oxyhaemoglobin ([O₂Hb]) and total haemoglobin ([tHb]) biceps brachii was also lower during the combined exercise compared to arm exercise alone. The reduction in muscle tissue oxygenation during the combined exercise was likely due to the attenuation of arm blood flow caused by competition for available cardiac output with the lower limbs (Volianitis et al., 2003).

There is also some evidence that single leg blood flow can be maintained during whole body maximal exercise. Leg blood flow was measured in five healthy male competitive cyclists during incremental single leg knee extensor exercise, and during incremental double-legged knee extensor with superimposed incremental arm crank exercise (Richardson, Kennedy, Knight, & Wagner, 1995). Data presented in this study did not support blood flow competition since leg blood flow was not compromised during the combined arm and leg exercise. However, leg blood flow was $\sim 1.0 \text{ L}\cdot\text{min}^{-1}$ lower while exercising at 90% and 100% of max work rate, but the difference was not statically significant. Differences in relative work rate subjects exercise at, and low statistical power due to the small sample size ($n = 5$), may have caused this discrepancy between studies (Secher et al., 1977; Volianitis & Secher, 2002). In a meta-analysis included within the Volianitis and Secher (2002) study which took into account the negative findings

(Richardson et al., 1995), revealed that the combination of arm and leg exercise limits lower limb blood flow by $11.0 \pm 3.7\%$ (Glass's effect size: 0.732 [95% confidence limits, 0.328-1.137]).

While the exercise model of superimposed arm exercise lacks ecological validity, it does highlight how cardiac output is distributed between exercising muscle groups competing for O_2 . Perhaps more relevant to the exercising human is the interaction between locomotor and respiratory muscles during high-intensity exercise. There is evidence that the O_2 cost of exercise hyperpnoea, and associated blood flow requirements, can compromise the proportion of cardiac output devoted to the locomotor muscles (Aaron, Seow, et al., 1992; Harms et al., 1997; Harms et al., 1998). Though the mechanisms responsible for the change in blood flow distribution are likely similar, the relevance to exercise is far more persistent. The work and O_2 cost of exercise hyperpnoea rises exponentially with \dot{V}_E (Aaron, Johnson, Seow, & Dempsey, 1992; Turner et al., 2012). Therefore during high-intensity exercise when ventilation demands are higher, an O_2 competitive environment can arise between locomotor and respiratory muscles for available cardiac output (Harms et al., 1998).

2.4 RESPIRATORY MUSCLE WORK DURING EXERCISE

The increased \dot{V}_E required for effective CO_2/H^+ elimination is achieved by the respiratory pump muscles (Sheel & Romer, 2012). From rest to moderate exercise, the energy requirements of exercise hyperpnoea can be readily met by utilising only a small fraction of the respiratory system capacity (Margaria, Milic-Emili, Petit, & Cavagna, 1960). Whereas during high-intensity exercise when ventilation requirements are great, and the O_2 cost of exercise hyperpnoea can pose limitations to exercise capacity (Dempsey et al., 2006; Harms et al., 2000).

2.4.1 Mechanics of Pulmonary Ventilation

Pulmonary ventilation is the process by which air flow is generated by the respiratory muscles to change the pressure within the lungs by acting on the thoracic cavity to change their volume (Wilson, 2016). The relationship between pressure and volume is described by Boyle's Law:

Equation 2.4: Boyle's law (Boyle, 1662).

$$P_1 \times V_1 = P_2 \times V_2$$

where P_1 and V_1 represent the pressure and volume of the original gas, and, P_2 and V_2 are the second pressure and volume. Inhalation commences when the diaphragm contracts, moving downwards to increase the space in the thoracic cavity for the lungs to expand (Aliverti et al., 1997). The intercostal muscles aid in increasing the space by pulling the ribs upward and outward (Aliverti, 2016; Ratnovsky, Elad, & Halpern, 2008). As the lungs expand air is drawn in via the mouth or nose, down the trachea, through the bronchial tubes and into the pulmonary alveoli (Ratnovsky et al., 2008; Strohl, Butler, & Malhotra, 2012; Wilson, 2016). Once inspiration is complete, respiratory muscles relax and elastic recoil compress the thoracic cavity to reduce the size of the lungs. The positive pressure created by decreasing lung volume forces air out of the lungs and trachea through the mouth/nose (Strohl et al., 2012). When breathing demands are high, such as during exercise, exhalation becomes a more active process to reduce expiratory time and the overall duty cycle of each breath (Aliverti et al., 1997; Henke, Sharratt, Pegelow, & Dempsey, 1988; Strohl et al., 2012; Younes & Kivinen, 1984). The work done by the respiratory muscles increases from rest to maximal exercise, along with the O_2 cost of breathing. (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992). That is, as hyperpnoea

risers, O_2 is consumed at an increasing rate by the respiratory muscles to move air in and out of the lungs for gas exchange.

2.4.2 Respiratory Muscle Work and the Oxygen Cost of Breathing

When breathing, the respiratory muscles perform work to overcome the elastic recoil of the lungs and chest, resistance from turbulent and viscous air flow through the respiratory tract and tissue deformation (Otis, Fenn, & Rahn, 1950). In healthy subjects, at rest, the work of breathing is approximately $0.25-1.5 \text{ J}\cdot\text{L}^{-1}$ (Dellweg, Haidl, Siemon, Appelhans, & Kohler, 2008). As \dot{V}_E rises, there is an exponential increase in the work being performed by the respiratory muscles (Figure 2.4) (Aaron, Johnson, et al., 1992; Margaria et al., 1960; Otis, 1954; Otis et al., 1950). The rise in work of breathing in this way is caused by two factors, 1) dynamic hyperinflation to accommodate greater expiratory flow rates (Pellegrino et al., 1993), and 2) progressive increase in the contribution of the expiratory muscles to breathing (Aliverti et al., 1997). As the lungs and chest are progressively stretched to accommodate the increasing volume of inhaled air and end-expiratory lung volume is reduced, the contribution of elasticity in these tissues to the work of breathing increases (Guenette, Witt, McKenzie, Road, & Sheel, 2007; B. D. Johnson, Babcock, Suman, & Dempsey, 1993).

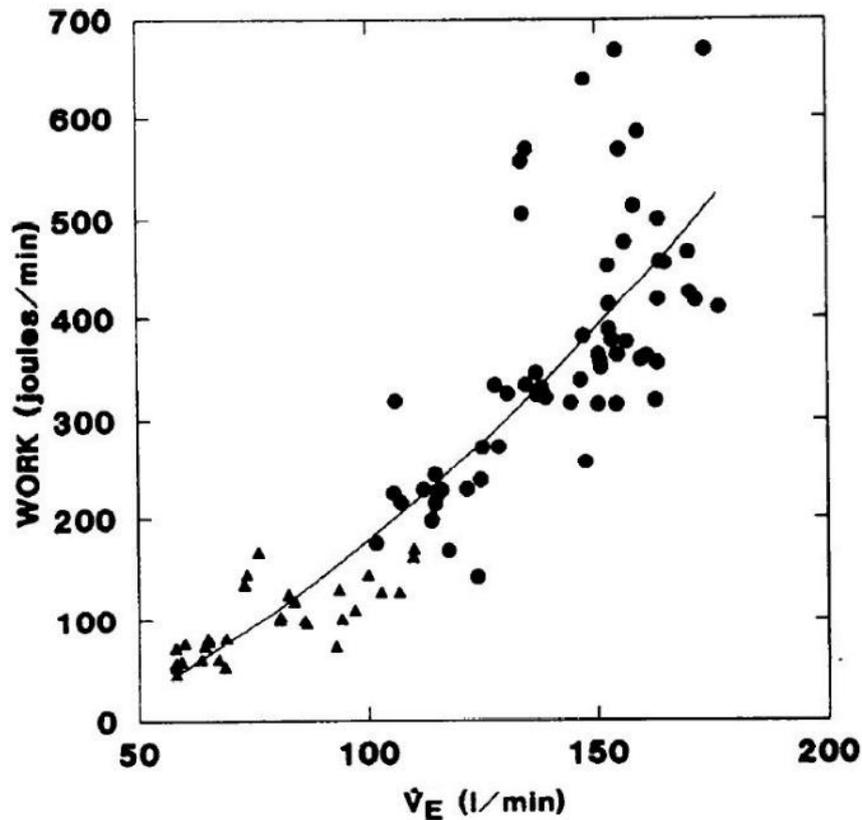


Figure 2.5: Mechanical work of breathing relative to pulmonary minute ventilation. The work performed by the respiratory muscles during exercise is expressed as joules per minute (joules/min; $1 \text{ J}\cdot\text{min}^{-1} = 0.0167 \text{ W}$), and pulmonary ventilation (\dot{V}_E) is expressed as liters per minute (l/min). Subjects ($n=8$) mimicked their \dot{V}_E from an incremental exercise test at two work rates corresponding to the attainment of 70% (64-78%) and 100% of $\dot{V}O_{2\text{max}}$. Each symbol represents a single mimicking trial in an individual subject with a fitted regression line ($r=0.88$). The symbols \blacktriangle and \bullet represent data from the 70% and 100% $\dot{V}O_{2\text{max}}$ mimicking trials. Reproduced from Aaron, Johnson, et al. (1992).

Accompanying the changes in work of breathing with \dot{V}_E , there is a certain O_2 cost of exercise hyperpnoea which increases from rest to maximal exercise (Figure 2.5). (Aaron, Johnson, et al., 1992; Dominelli et al., 2015). In exercise, respiratory muscle oxygenation progressively declines with increasing intensity (Legrand et al., 2007; Mancini, Ferraro, Nazzaro, Chance, & Wilson, 1991; Moalla, Dupont, Berthoin, & Ahmaidi, 2005; Terakado et al., 1999). Using NIRS to interrogate respiratory muscle oxygenation, there is a gradual increase in [HHb] and decrease in [O₂Hb]. At the respiratory compensatory point, the rate of [HHb] and [O₂Hb] change is greatly accelerated (Legrand et al., 2007; Terakado et al., 1999).

By mimicking the ventilation pattern obtained during exercise while at rest, it is possible to estimate the proportion of whole body $\dot{V}O_2$ that is devoted to the respiratory muscles. To determine the O_2 cost of exercise hyperpnoea, a target \dot{V}_E is maintained for 4-6 min by replicating the exercise f_b and V_T . Eucapnia is maintained by inspiring a gas mixture consisting of 4-5% CO_2 and 21% O_2 . To calculate the O_2 cost of hyperpnoea, $\dot{V}O_2$ determined during quiet rest is subtracted from the values obtained from the mimicking trials. During moderate exercise, it is estimated that the O_2 cost of breathing accounts for 3-6% of the total whole body $\dot{V}O_2$. During high-intensity exercise, the relative contribution of exercise hyperpnoea to whole body $\dot{V}O_2$ increases to 10-15% (Aaron, Seow, et al., 1992; Harms et al., 1998; Turner et al., 2012). Though, there is a broad range which the O_2 cost of hyperpnoea can represent as a percentage of total $\dot{V}O_2$ during maximal exercise. Estimates have ranged between 5.0% and 17.6% (mean = $8.8 \pm 3.3\%$) with only six of the twenty one subjects examined obtaining results greater than 10% (Vella, Marks, & Robergs, 2006). Similarly broad results of the O_2 cost of breathing have been published by others (Dominelli et al., 2015). The O_2 cost of breathing was estimated to range between ~8-24% (mean = 13.8%) in females, and ~6-18% (mean = 9.4%) in males during maximal exercise (Dominelli et al., 2015). Aside from the structural characteristics of the pulmonary system, the relative strength of the respiratory muscles is likely to have a key role in the O_2 cost of exercise hyperpnoea. After 6 weeks of inspiratory muscle strength training, ventilation O_2 efficiency was improved compared with sham training (training at an intensity shown to exhibit no changes in inspiratory muscle function) (Turner et al., 2012). Specific training targeting the respiratory muscles reduces the relative intensity of hyperpnoea, and therefore, the O_2 cost of breathing at any given \dot{V}_E (Witt, Guenette, Rupert, McKenzie, & Sheel, 2007).

The work of breathing associated with high-intensity and maximal exercise requires a considerable portion of whole-body O_2 uptake, which creates an environment where the locomotor and respiratory muscles compete for O_2 delivery (Harms et al., 1997; Harms et al., 1998). Therefore, respiratory muscle work likely contributes to the development of locomotor muscle fatigue, and reduces the capacity to sustain high-intensity exercise (Dempsey et al., 2006; Romer & Polkey, 2008).

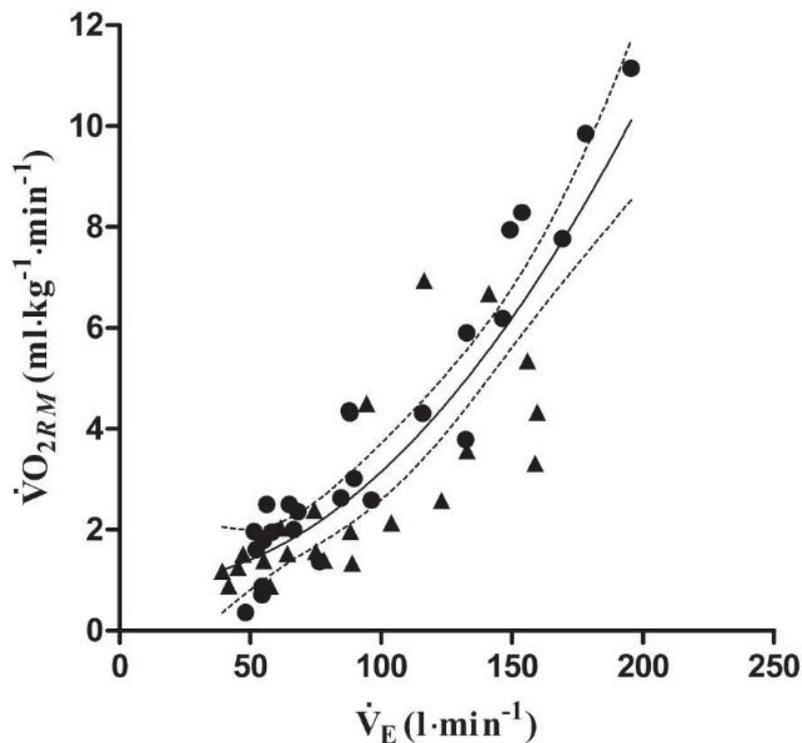


Figure 2.6: Relationship of exercise pulmonary ventilation to respiratory muscle oxygen uptake. Subjects ($n=16$; $\dot{V}O_{2max}$: $61.8 \text{ mL}\cdot\text{Kg}^{-1}\cdot\text{min}^{-1}$) mimicked their exercise ventilation rate (\dot{V}_E) at rest which corresponded to the attainment of 50, 75, and 100% $\dot{V}O_{2max}$. Each symbol represents a single mimicking trial in an individual subject with a fitted regression line (solid) and 95% confidence intervals. A significant correlation between respiratory muscle oxygen uptake ($\dot{V}O_{2RM}$) and \dot{V}_E was found ($P>0.05$, $r=0.88$). The symbols \blacktriangle and \bullet represent pre-training data from control and inspiratory muscle training groups respectively. Reproduced from Turner et al. (2012).

2.4.3 Consequences of Sustained Respiratory Muscle Work

In a comparable way that the lower and upper limbs compete for cardiac output during strenuous exercise, the respiratory and lower limb locomotor muscles also compete. The relationship between respiratory and lower limb locomotor muscle groups is particularly challenged during sustained high-intensity exercise ($>80\% \dot{V}O_{2mx}$). The degree of hyperaemia necessary to support the substantial demands of the respiratory muscles can limit the proportion of cardiac output available for the locomotor muscles to perform high-intensity work.

2.4.3.1 Locomotor Muscle Fatigue

An inverse relationship exists between the work of breathing and leg O_2 uptake during maximal exercise (Harms et al., 1997). To reduce the work of breathing, proportional assist ventilation (PAV) can be used to generate inspiratory pressure proportional to the effort of the patient/subject (Younes et al., 1992). Conversely, to elevate inspiratory muscle work, a mesh screen can be placed over the inspiratory line, or the aperture of an inspiratory port can be reduced. In one such study employing these techniques, subjects exercised at a workload sustainable for 2.5-3 min at, or near a work rate corresponding to the attainment of $\dot{V}O_{2max}$. The work of breathing was attenuated by 60% with PAV, and increased by 95% with inspiratory loading compared to control during the exercise bout. Elevating the work of breathing had negligible effect on whole body $\dot{V}O_2$. Moreover, both leg blood flow and $\dot{V}O_2$ fell compared to control exercise which coincided with an increase in leg vascular resistance, which suggests that cardiac output did not increase to accommodate the additional muscular work (Harms et al., 1998). It is likely that blood flow was redistributed to the respiratory muscles to support the heightened metabolic activity at the expense of the locomotor muscles (Harms et al.,

1997). When the respiratory muscles were unloaded with PAV, there was a slight increase in limb blood flow which corresponded with an increase in leg $\dot{V}O_2$. By reducing the metabolic demands of the respiratory muscles, O_2 delivery to the lower limbs can be improved. The “normal” work of breathing incurred during high-intensity exercise may actually be a limiting factor of O_2 transport to the locomotor muscle during high-intensity exercise (Harms et al., 1997).

When inspiratory loading has been added to continuous locomotor exercise to increase the work of breathing, locomotor muscle oxygenation trends are negatively impacted (Turner et al., 2013). While exercising at a work rate corresponding to 80% of $\dot{V}O_{2max}$, locomotor and respiratory muscle oxygenation was monitored with NIRS. Before inspiratory loading was added, there was a plateau of $[O_2Hb]$ and $[HHb]$ in the different muscle groups, indicative of achieving a steady state (Grassi et al., 2003). Exercise was continued for a further 3 min with the addition of an inspiratory load, which was achieved by reducing the aperture of the inspiratory line to 10 mm, and 8 mm. In response to the elevated work of breathing, there was a proportional increase in respiratory muscle $[HHb]$ (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992; Wetter, Harms, Nelson, Pegelow, & Dempsey, 1999). There was also an increase in limb $[HHb]$ from $12.2 \pm 9.0 \mu\text{m}$, to $15.3 \pm 11.7 \mu\text{m}$, but only during exercise with the smallest inspiratory aperture (Turner et al., 2013). The increase in limb $[HHb]$ in the absence of increased O_2 uptake, reflects a decrease in O_2 delivery to the locomotor muscles (Kowalchuk, Rossiter, Ward, & Whipp, 2002).

Exercise intensity also plays an important role in the competition between the locomotor and respiratory muscles for available O_2 . While exercising at submaximal work rates (50-75 % $\dot{V}O_{2max}$), there was a small but significant increase in whole body $\dot{V}O_2$ in

response to an elevated work of breathing (Wetter et al., 1999). Since $\dot{V}O_2$ responded proportionally to the changes in inspiratory muscle work, suggests that there is enough capacity in cardiac output to increase to meet the demands of additional muscular work during submaximal exercise (Harms et al., 1997). It is only during high-intensity exercise when cardiac output begins to approach maximal flow rates that competition for available blood flow begins to develop (Harms et al., 1997).

Since the high work of breathing during high-intensity exercise ($>80\% \dot{V}O_{2\max}$) seems to have a limiting effect on locomotor muscle blood flow, the rate development of peripheral fatigue is likely affected too. To examine exercise-induced quadriceps muscle fatigue, supra-maximal femoral nerve stimulation was used, which provided an objective measure of muscle force generation capacity (Polkey et al., 1996). Peripheral muscle fatigue was assessed after four bouts of exercise at a work rate corresponding to the attainment of 92% of $\dot{V}O_{2\max}$ (Romer, Lovering, et al., 2006). On one occasion, subjects exercised to volitional exhaustion (13.2 min). On a separate visit, exercise of the same work rate and duration was repeated while the respiratory muscle were unloaded using PAV (56% reduction of inspiratory muscle work). Following the completion of exercise, peripheral muscle fatigue was 8% greater when subjects were not using the breathing assistance. To examine how a heightened work of breathing affects peripheral fatigue, exercise was repeated with inspiratory loading (80% increase of inspiratory muscle work) to exhaustion (7.9 min). Following the termination of exercise, the force generating capacity of the quadriceps was 8% lower when performed with inspiratory loading compared to control (Romer, Lovering, et al., 2006). These data show that peripheral fatigue can be manipulated by altering the work for breathing, which suggests that respiratory muscle work is a limiting factor of high-intensity exercise. It is likely that

blood flow competition between the locomotor and respiratory muscles contribute to exercise-induced fatigue (Harms et al., 1997; Harms et al., 1998).

It is unlikely for healthy humans to undergo inspiratory loading during exercise. However, exposure to (simulated) altitude is a more common environmental condition that will increase the work of breathing compared to normoxia via a stimulation of \dot{V}_E . To examine the relationship between hypoxia-induced elevated work of breathing and peripheral fatigue, subjects were exercised at a constant work rate (~ 273 W) corresponding to 82% of $\dot{V}O_{2\max}$ in simulated altitude ($F_{I}O_2 = 0.15$) to exhaustion (Amann, Pegelow, et al., 2007). Exercise was then repeated at the same work rate for an identical duration in normoxia (~ 273 W for 8.6 min). Compared to hypoxia, subjects performed 36% less inspiratory muscle work while exercising in normoxia, and incurred a lesser reduction in quadriceps force generation (normoxia -16%, hypoxia -30 %). To isolate the effects of the work of breathing on peripheral fatigue, subjects repeated both exercise trials (normoxia and hypoxia) using PAV. Inspiratory muscle work was nearly identical during exercise and the reduction in hypoxia induced peripheral fatigue was slightly attenuated compared to normoxia (normoxia -15%, hypoxia -22%). Exercising at a work rate where the work of breathing has no discernible effect of quadriceps fatigue in normoxia, fatigue development is accelerated in hypoxia when matched for inspiratory muscle work. (Amann, Pegelow, et al., 2007; Romer, Lovering, et al., 2006). By alleviation respiratory muscle work in hypoxia, the development of peripheral fatigue can be lessened during high-intensity exercise.

2.4.3.2 Respiratory Muscle Fatigue

Diaphragm fatigue can develop concomitantly with locomotor fatigue during high-intensity exercise, primarily due to the respiratory muscle work necessary to sustain the required \dot{V}_E (Amann, Pegelow, et al., 2007; B. D. Johnson et al., 1993; Vogiatzis et al., 2008). Using supra-maximal stimulation of the phrenic nerve, the pressure generation capacity of the diaphragm can be assessed. Phrenic nerve stimulation (PNS) depolarises nerves at the base of the neck responsible for diaphragm contractions. When exercising above 80% $\dot{V}O_{2max}$ until volitional exhaustion, pressure generation capacity can decrease by 40% from pre-exercise levels, and take over an hour to recover (B. D. Johnson et al., 1993). However, if the same work of breathing is mimicked at rest, diaphragm fatigue does not develop (Babcock, Pegelow, McClaran, Suman, & Dempsey, 1995). Blood flow competition may explain the diaphragm fatigue that occurs during high-intensity exercise which is not present when a similar diaphragm work is performed at rest (Secher & Volianitis, 2006).

To explore the role hypoxia plays in the development of diaphragm fatigue, a group of subjects exercised at 85% of $\dot{V}O_{2max}$ to exhaustion while breathing either room air ($F_{iO_2} = 0.21$) or a hypoxic gas mixture ($F_{iO_2} = 0.15$) (Babcock, Johnson, et al., 1995). There was a decrease in exercise time by 9 min breathing the hypoxic gas mixture (normoxia: 24.9 min; hypoxia: 15.8 min), and the work of breathing was 24% higher at the start and middle portions of the exercise bout. However, the degree of diaphragm fatigue incurred at the end of exercise was similar after exercise in both conditions. The hypoxic effects on exercise-induced diaphragm fatigue is that the rate of fatigue development was accelerated so that the same reduction of pressure generating capacity was reached in a shorter time. It has been proposed that fatigue can be “centrally”

regulated to ensure that a critical threshold of fatigue is not exceeded, to prevent excessive homeostasis disruption (Noakes, 2011). Once the critical fatigue threshold is reached, central motor drive is reduced so that exercise can no longer continue at the same intensity (Amann & Dempsey, 2008; Amann, Eldridge, et al., 2006; Billaut et al., 2013). When exercise is performed at the same absolute work rate between normoxic and hypoxic (Babcock, Johnson, et al., 1995), \dot{V}_E and the work of breathing is likely to be higher to offset the reduced O_2 availability (Amann, Pegelow, et al., 2007; Forster et al., 2012). Therefore the development of diaphragm fatigue may not solely be affected by hypoxemia per se, but simply the elevated work being performed by the diaphragm.

To explore the relationship between respiratory muscle work and O_2 availability, locomotor work can be manipulated to produce a similar work of breathing in varying environmental conditions. To replicate the work of breathing at varying inspired O_2 gas concentration, seven trained cyclists were exercised at work rates corresponding to 90%, 80% and 65% of $\dot{V}O_{2max}$, while breathing air consisting an $F_{I}O_2$ of 1.00, 0.21 and 0.13 (Vogiatzis et al., 2008). At the conclusion of exercise (5 min), diaphragm fatigue was greater in hypoxia despite less leg locomotor work being performed (Vogiatzis et al., 2008; Vogiatzis et al., 2007a). There was also no compensatory increase in respiratory muscle blood flow (measured with NIRS and an indocyanine green tracer) in response to the altered O_2 availability (Vogiatzis et al., 2008).

In theory, reduced leg work should increase the proportion of cardiac output available to the respiratory muscles. Since this is not the case, perhaps the respiratory muscle work associated with high-intensity exercise is sufficiently strenuous to induce maximal vasodilatation. Though hypoxia is a potent vasodilator (Joyner & Casey, 2014),

there is no additive effect on respiratory muscle blood flow when exercising at near maximal work rates (Koskolou, Calbet, Radegran, & Roach, 1997).

2.4.3.3 Respiratory Muscle Metaboreflex

Despite exercise hyperpnoea being a protective mechanism against hypercapnia and hypoxia, the work of breathing is associated with increased locomotor and diaphragm fatigue, and is accelerated by hypoxia (Amann, Pegelow, et al., 2007; Babcock, Johnson, et al., 1995; Gudjonsdottir et al., 2001; Verges, Bachasson, & Wuyam, 2010). A respiratory muscle metaboreflex (Figure 2.6) is the likely cause of limited blood flow during a sustained high work of breathing (Dempsey et al., 2006). In resting and submaximal exercising dogs, it has been demonstrated that infusing lactate acid into the diaphragm via the phrenic artery causes limb vasoconstriction and reduced blood flow, and increases mean arterial pressure (Rodman, Henderson, Smith, & Dempsey, 2003). An accumulation of reflex-activating by-products of muscle contraction (lactate, potassium, deprotonated phosphate, adenosine and CO₂) stimulates the discharge of chemically sensitive group III/IV afferent nerve fibres in the fatiguing diaphragm (Amann, 2012; J. M. Hill, 2000). Reflexively, discharge of group III/IV afferent nerve fibres causes an increased lower limb sympathetic nerve discharge and vasoconstriction (Harms et al., 1997; Sheel et al., 2001; St Croix, Morgan, Wetter, & Dempsey, 2000). Changes in lower-limb vascular conductance serve to support blood flow demands of the fatiguing respiratory muscles to ensure critical failure does not occur (Joyner & Casey, 2015). As a consequence of increased blood flow to the respiratory muscles, locomotor O₂ transport is attenuated which accelerated development of limb fatigue during high-intensity exercise (Dempsey et al., 2006).

RESPIRATORY MUSCLE METABOREFLEX

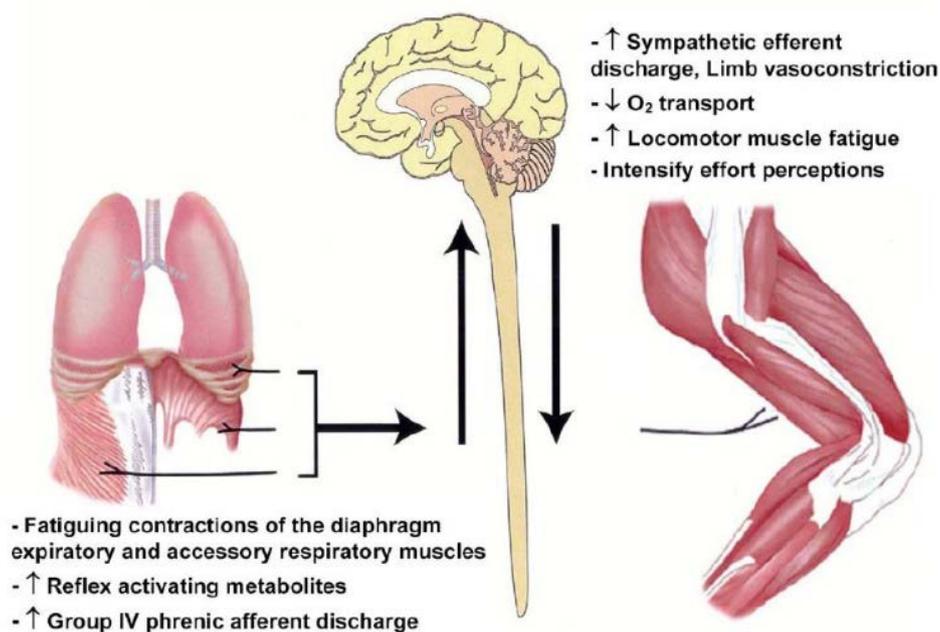


Figure 2.7: Proposed respiratory muscle metaboreflex and its effects. Reproduced from Dempsey et al. (2006).

2.5 RESPIRATORY MUSCLE TRAINING

Since the work of breathing necessary to sustain high-intensity exercise can be a limiting factor of maximal exercise capacity (Dempsey et al., 2006), targeted training of the respiratory muscles can delay exercise induced fatigue and improve exercise performance (Romer & Polkey, 2008). Like other training regimes, respiratory muscle training is designed to improve the strength and endurance capacity of the respiratory muscles to reduce the relative intensity of breathing at any given work rate (Sheel, 2002). Training programs will either focus on enhancing the endurance or strength capacity of the respiratory muscles. Typically, two models of respiratory muscle training are used (McConnell & Romer, 2004b; Sheel, 2002): 1) voluntary normocapnic hyperpnoea for endurance adaptations; and 2) inspiratory loading, either flow resistance or pressure threshold loading, to improve strength (Table 2.1).

2.5.1 Respiratory Muscle Endurance Training

Voluntary normocapnic hyperpnoea requires individuals to sustain a high target \dot{V}_E (60-90% maximal voluntary ventilation) for up to 30 min, 5 days per week (Boutellier, Büchel, Kundert, & Spengler, 1992; McMahon, Boutellier, Smith, & Spengler, 2002; Sonetti, Wetter, Pegelow, & Dempsey, 2001; Wylegala, Pendergast, Gosselin, Warkander, & Lundgren, 2007). Multiple studies have demonstrated positive functional respiratory adaptations after voluntary normocapnic hyperpnoea training, which have translated to enhanced exercise capacity (see Table 2.1). For example, a group of fifteen subjects completed 40 sessions of voluntary hyperpnoea over a 15-week period, consisting of 30 min breathing at 60% of maximal voluntary ventilation (Markov, Spengler, Knöpfli-Lenzin, Stuessi, & Boutellier, 2001). After the training intervention, respiratory muscle time to exhaustion was extended from 4.6 min (range: 2.0-10.2 min) to 40 min (15.4-40.0 min; tests were terminated after 40 min if no sign of exhaustion was present). Constant-load cycling time to exhaustion (cycling at 70% of $\dot{V}O_{2max}$; same absolute work rate was used in post testing) was extended by 24% (35.6 ± 11.9 min vs. 44.2 ± 17.6 min). Time to exhaustion was improved without the cardiovascular adaptation ($\dot{V}O_{2max}$, cardiac stroke volume, substrate utilisation) that are traditionally seen with whole body training. Seeing as cycling time to exhaustion was extended without changes in cardiovascular function, exercise was likely extended through localised adaptation of the respiratory muscles (Markov et al., 2001). Following training, the respiratory muscles were more fatigue resistant, and accumulated less metabolic by-products (Verges, Renggli, Notter, & Spengler, 2009). As introduced earlier (section 2.4.3), diaphragm fatigue is known to be a potent stimulant of sympathetically mediated vasoconstriction (Dempsey et al., 2006). Therefore, delaying the development of diaphragm fatigue would suspend a respiratory

muscle metaboreflex having a subsequent significant effect on locomotor blood flow (McConnell & Lomax, 2006; Witt et al., 2007).

There are a few drawbacks from respiratory muscle endurance training. First, it is relatively time consuming compared to other forms of respiratory muscle training, and requires a high degree of motivation from the participants to maintain their target \dot{V}_E . Second, preventing hypocapnia during voluntary hyperpnoea has traditionally been constrained to the laboratory setting. Commercially available rebreathing equipment (SpiroTiger®) have made training more accessible, but the devices are considerably more complicated and expensive than pressure threshold training devices.

2.5.2 Respiratory Muscle Strength Training

Another training method to improve the functional capacity of the respiratory muscle is inspiratory pressure threshold training (inspiratory muscle training, IMT). Commercially available training devices (POWERbreathe®) that are relatively cheap and simple to use have made this form of training easily accessible to large groups of people. Training typically involves inspiring against a closed valve set to open at ~50% of an individual's maximal inspiratory mouth pressure (MIP), repeated 30 times twice per day. Pressure threshold training is associated with diaphragm hypertrophy (Downey et al., 2007) and an increased in inspiratory pressure generation of ~20-40% (see Table 2.1). Strengthening the inspiratory muscles has translated to reduced O₂ cost of voluntary hyperpnoea, attenuated exercise-induced respiratory muscle fatigue, and improved exercise capacity. (Downey et al., 2007; Illi, Held, Frank, & Spengler, 2012; Turner et al., 2012).

Enhanced exercise performance following training likely results from an enhanced functional capacity of the respiratory muscles. Therefore, for any given \dot{V}_E

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during exercise, a smaller fraction of the maximal pressure generating capacity is required to maintain hyperpnoea. The O_2 cost of exercise hyperpnoea has been estimated to account for 10-15% of whole body $\dot{V}O_2$ during high-intensity exercise (Aaron, Johnson, et al., 1992; Dominelli et al., 2015). However, training the respiratory muscles can increase their pumping efficiency. Following 6 weeks of IMT, a group of trained cyclists improved their MIP by 22% (Turner et al., 2012). As a result of increasing inspiratory muscle strength, the contribution of exercise hyperpnoea to whole body $\dot{V}O_2$ during maximal exercise decreased from 11% to 8% (Turner et al., 2012). Presumably lowering the O_2 cost of exercise hyperpnoea increased the proportion of cardiac output available for locomotor exercise (Harms et al., 1998). Additionally, the magnitude of the respiratory muscle metaboreflex can be weakened by attenuating respiratory muscle fatigue. Following 6 weeks of IMT, a decrease in respiratory muscle function (mean maximal inspiratory mouth pressure at zero flow rate, and inspiratory flow rate at 30% of maximal inspiratory pressure) was lessened after 20-km and 40-km trials (Romer, McConnell, & Jones, 2002a, 2002c). Exercise time to completion was also improved by 3.8% and 4.6% during the 20-km and 40-km time trials, respectively. Since exercise induced respiratory muscle fatigue is lower after training, improvements in performance are likely derived from a blunted respiratory muscle metaboreflex and locomotor O_2 availability (McConnell & Lomax, 2006). Increasing respiratory muscle strength may even be transferable to hypoxic environments where there is greater strain on the respiratory musculature and connective O_2 transport.

At terrestrial altitude, or when breathing a hypoxic gas mixture, the work of breathing can be 20-30% higher and diaphragm fatigue is accelerated (Babcock, Johnson, et al., 1995). Following a constant speed treadmill running test in hypoxia ($F_{I}O_2 = 0.14$)

at 85% of $\dot{V}O_{2\max}$, four weeks of IMT blunted exercise-induced inspiratory muscle fatigue (Downey et al., 2007). Attenuating a fall in MIP following exercise is likely achieved through a reduction in the fraction of maximal pressure generated with each breath during exercise. Following IMT, \dot{V}_E can also be lower during exercise which would lower the work of breathing (Downey et al., 2007; Lomax, Massey, & House, 2017). It is unclear why \dot{V}_E would be lower following training, but could be partly attributed to a reduction in ventilatory drive achieved by a 5-6% increase arterial O_2 saturation (Downey et al., 2007; Lomax, 2010). Despite improvements in inspiratory muscle strength being a potential mechanism enhancing exercise performance, the effectiveness of IMT in improving exercise performance in hypoxia has been mixed (Downey et al., 2007; Salazar-Martínez, Gatterer, Burtscher, Naranjo Orellana, & Santalla, 2017). After 4 weeks of IMT and a $\sim 24\%$ increase in inspiratory muscle strength, no change in time to exhaustion (cycling at 85% of $\dot{V}O_{2\max}$) occurred while exercising in hypoxia ($F_{I}O_2 = 0.14$) (Downey et al., 2007). Whereas others have shown an increase in mean power output by 7% during a 10-min time trial in hypoxia ($F_{I}O_2 = 0.165$) (Salazar-Martínez et al., 2017). Exercise protocols where subjects can “self-pace” their efforts in response to sensory information may yield the greatest ergogenic effects of IMT (Amann & Calbet, 2008; Noakes, St Clair Gibson, & Lambert, 2005).

Research so far on work of breathing and respiratory muscle training has predominantly used time-to-exhaustion or a time trial as a performance measure (Table 2.1). Time-to-exhaustion tests are a good measure for adaptation because external work can be controlled, and physiological responses compared. However, exercise intensity cannot be regulated by subjects in response to metabolic disturbances (Amann, Eldridge, et al., 2006). Moreover, constant load exercise tests are a poor representation of athletic

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Trends During Repeated-sprint Exercise*

performance. Self-paced exercise protocols are a better representation of athletic performance, because they allow subjects to alter their power/velocity in response to feelings of fatigue and distance/time remaining in the test. One such exercise model which has had little work investigating the influence of breathing demands on performance is repeated-sprint exercise.

Table 2.1: Effects of respiratory muscle training on respiratory function, physiological responses to exercise, and performance in healthy individuals.

Author	Subject characteristics	Respiratory Muscle Training	Change in Respiratory Function	Exercise Testing	Change in Exercise Responses and Performance
Archiza et al. (2017)	Professional female football (soccer) players IMT: n = 10 Sham: n = 8	IMT: Pressure threshold. 6 wk, 2 session/day, 30 breaths at ~50% MIP Sham: Pressure threshold. 6 wk, 2 session/day, 30 breaths at ~15% MIP	↑ MIP 22%	Time to exhaustion: Treadmill running at 100% of the speed reached during a graded exercise test. RS exercise: Six 40 m (20 m + 180° turn + 20 m) sprints, with 20s of passive rest between each sprint.	Time to exhaustion: ↑ Time to exhaustion by 42%. ↓ Δ[HHb] and ↑ Δ[tHb] of intercostal muscles ↑Δ[O ₂ Hb] and ↑Δ[tHb] of vastus lateralis RS: ↓ Mean RS time 6% ↓ % decrement 2.4%
Bailey et al. (2010)	Recreationally active IMT: n = 8 (6 male, 2 female) Sham: n = 8 (6 male, 2 female)	IMT: Pressure threshold. 4 wk, 2 sessions per day, 30 breaths at ~50% MIP Sham: 4 wk, 1 session per day, 60 breaths at ~15% MIP	↑ MIP 17%	Incremental test to exhaustion: 3 min of cycling at 0 W, after which the work rate was increased by 30 W·min ⁻¹ for males and 24 W·min ⁻¹ for females Step tests: Moderate, 80% GET; Severe, 60% the difference between GET and $\dot{V}O_{2max}$; “maximal”, 100% $\dot{V}O_{2max}$	Cycling incremental test to exhaustion: → $\dot{V}O_{2max}$ ↑ Max work rate Step tests: ↓ Respiratory muscle fatigue after severe and maximal exercise. ↓ $\dot{V}O_2$ slow component and exercise tolerance during severe and maximal exercise.
(Downey et al., 2007)	Moderately active (1 – 5 h/wk aerobic exercise).	IMT: Pressure threshold. 4 wk, 5 day/wk, 2 sessions/day, 30 breaths at ~50% MIP Control: no training	↑ MIP 25% ↑ Diaphragm thickness	Time to exhaustion: Cycling at 85% $\dot{V}O_{2max}$, normoxia F _I O ₂ 0.21, hypoxia F _I O ₂ 0.14	↓ post exercise respiratory muscle fatigue ~7.5% in normoxia and hypoxia ↓ RPE and dyspnoea ratings in hypoxia

		IMT: n = 7 (4 male, 3 female)				→ Time to exhaustion ↓ $\dot{V}O_2$ by 8 – 12% in hypoxia
		Control: n = 5 (2 male, 3 female)				↓ Cardiac output 14% in hypoxia ↓ \dot{V}_E 25% in hypoxia ↑ SpO_2 in hypoxia 5-6%
(Edwards, 2013)	Healthy	IMT: 4 wk, 7 day/wk, 1 sessions/day, 30 breaths at ~55% MIP Sham: 4 wk, 7 day/wk, 1 session per day, 60 breaths at ~10% MIP	↑ MIP 15%	Incremental test to exhaustion: Incremental treadmill running to volitional exhaustion	→ $\dot{V}O_{2max}$ ↑ Time to exhaustion 5% ↑ Peak running velocity ~2% ↓ Submaximal RPE 9%	
(Esposito, Limonta, Alberti, Veicsteinas, & Ferretti, 2010)	Moderately active. No specific aerobic training	Eucapnic voluntary hyperventilation. 8 wk, 5 sessions per week, 10 – 20 min including warmup RMET: n = 9	↑ MIP 75% ↑ FVC 9% ↑ FEV ₁ 9% ↑ PEF 8%	Incremental test to exhaustion: Cycling at 50 W and 100 W for the first two stages. Higher work were selected based on cardiorespiratory responses to reach maximum work rate within three additional work rates. Test were performed in normoxia (room air) and hypoxia ($F_{iO_2} = 0.11$)	→ $\dot{V}O_{2max}$	
(Gething, Williams, & Davies, 2004)	Healthy, regular exercise	10 wk, 3 days/wk IMT: 2 mm opening (270 cmH ₂ O/L/s) Sham: 30 mm opening (10 cmH ₂ O/L/s)	↑ MIP 34% ↑ respiratory endurance 38%	Time to exhaustion: Cycling 75% of $\dot{V}O_{2max}$	↑ Time to exhaustion 36%	
		IMT: n = 5 Sham: n = 5 Control: n = 5				

Control: no training

(Griffiths & McConnell, 2007)

Competitive male rowers

IMT: n = 10

EMT: n = 7

IMT: Pressure threshold training. 4 wk, 2 sessions per day, 30 breaths at ~50% MIP

EMT: 4 wk, 2 sessions per day, 30 breaths at ~50% MEP

IMT:
↑ MIP 26%
→ MEP

EMT:
→ MIP
↑ MEP 18%,

6-min all out rowing:
Drag factor set to 138 and damper setting ~4

IMT:
↑ Mean power output 2.7%

EMT:
→ Mean power output

(M. A. Johnson, Sharpe, & Brown, 2007)

Competitive male cyclists

IMT: n = 9

Sham: n = 9

IMT: 6 wk, 2 sessions per day, 30 breaths at ~50% MIP

Sham: breathing through the same trainer but filled with "oxygen absorbent" gravel, 15 min 5 days per week

↑ MIP 17%

Time trial: 25 km cycling time trial on own racing bicycle, mounted to an air-braked ergometer.

25 km time-trial:
↑ Mean power output 2.7%
↓ TT completion time 6%

Critical power:
→ Critical power
↑ Anaerobic work capacity 17%

Critical power test:
Square-wave constant power test. Power outputs were chosen to elicit volitional exhaustion within each of the following domains 3-10 min, 10-20 min, and 20-30 min.

(Leddy et al., 2007)

Experienced male distant runners

RMET: n = 15

Sham: n = 7

RMET: Eucapnic voluntary hyperventilation. 50% MVV, fR 30 min⁻¹ for 30 min, 4 wk, 5 day/wk.

Sham: 10 s breath holding repeated every 90 s for 30 min. rest was reduced to 80, 70 and 60 s during weeks 2, 3 and 4.

↑ MVV 10%
↑ RET 208%

Incremental test to exhaustion: Treadmill running for 2 min at 1.8 m·s⁻¹, then was increased to 2.9 m·s⁻¹ at a grade of 2.5% for 3 min. Thereafter, the grade was increased by

Maximal incremental exercise:
↑ $\dot{V}O_{2max}$ 2%

Time to exhaustion:
↓ $\dot{V}O_2$ 6%
↓ \dot{V}_E 7%
↓ Blood lactate 18%
↑ Treadmill run time 50%

				2,5% every 2 min until exhaustion.	Time trial (n = 7) ↓ Run time 4%
				Time to exhaustion: treadmill running at 80% of $\dot{V}O_{2max}$	
				Time trial: 4-mile run (6.44 km) on an indoor circular running track	
(Lomax, 2010)	Healthy service personal from British Armed Forces IMT n = 7 Control n = 7	IMT: 4 wk, 2 sessions per day, 30 breaths at ~50-60% MIP Control: No training	↑ MIP 15%	Resting: Resting at terrestrial altitude of 1400 m, 4880 m, and 5550 m	SpO_2 : → 1400 m ↑ 4880 m 6% ↑ 5550 m 6% → Dyspnoea
(Lomax et al., 2017)	Healthy males IMT n = 8 Sham n = 9	IMT: 4 wk, 7 day/wk, 1 sessions/day, 30 breaths at ~50% MIP Sham: 4 wk, 7 day/wk, 1 session per day, 30 breaths with no load	↑ MIP 15%	Submaximal exercise test: Cycling for 10 min and 100 W while in normoxia (room air), and hypoxia (F_{iO_2} 0.146)	Normoxia: no clear changes Hypoxia: ↓ $\dot{V}CO_2$ 12% ↓ \dot{V}_E 14% ↑ SpO_2 3% ↓ Dyspnoea 42%
(Markov et al., 2001)	Sedentary subjects RMET n = 13 (8 male, 5 female) Endurance training n = 9 (4 male, 5 female) Control n = 15 (8 male, 7 female)	RMET: Eucapnic voluntary hyperventilation at 60% MVV for 30 min. 40 sessions over 15 wk. ET: Cycling and/or running. 40 sessions over 15 wk, 30 min per session Control: No training	Breathing endurance at 65-70 % MVV. RMET: ↑ 600% ET: → Control: →	Incremental test to exhaustion: Cycling at an initial work rate of 60 W for females, and 80 W for males. Every 2 min the work rate was increased by 20 W until volitional exhaustion. Time to exhaustion: Cycling at 70% $\dot{V}O_{2max}$	Incremental test to exhaustion: VO_{2max} : → RMT ↑ ET 19% → Control Time to exhaustion: ↑ RMT 24%, ↑ ET 40% → Control

				until volitional exhaustion.	
(McConnell & Lomax, 2006)	IMT: n = 8 (1 male)	IMT: 4 wk, 2 sessions per day, 30 breaths at ~50% MIP	<p>↑ MIP 17%</p> <p>↑ Inspiratory muscle work to task failure (same relative intensity) 26%</p>	<p>Time to exhaustion: Planter flexion time to exhaustion at 85% of MVC after inspiratory muscle pre-fatigue</p>	<p>↓ Time to exhaustion 36% (3.6 min)</p>
McMahon et al. (2002)	<p>Healthy males, cycling trained</p> <p>RMET: n = 10</p> <p>Control: n = 10</p>	<p>RMET: Eucapnic voluntary hyperventilation. 20, 30 min session at 60% MVV over 4-6 wk</p> <p>Control: no training</p>	<p>↑ vital capacity 3%</p> <p>↑ MVV 10%</p> <p>↑ Time to fatigue 260%</p>	<p>Incremental test to exhaustion: Cycling at 100 W to begin with, and increased by 30 W every 2 min there after until volitional exhaustion</p> <p>Time to exhaustion: cycling at 85% of $\dot{V}O_{2max}$</p>	<p>Incremental test to exhaustion: → VO_{2max}</p> <p>Time to exhaustion: ↑ Time to exhaustion ~27%</p>
Mickleborough, Nichols, Lindley, Chatham, and Ionescu (2010)	<p>Recreational active road runners</p> <p>IMT: n = 8</p> <p>Sham: n = 8</p> <p>Control: n = 8</p>	<p>IMT: Repeated sets of 6 inspirations at 80% sustained MIP until task failure, 3 days/wk. rest between sets gradually reduced from 45 s to 5 s.</p> <p>Sham: Repeated sets of 6 inspirations at 30% sustained MIP until task failure, 3 days/wk. rest between sets gradually reduced from 45 s to 5 s.</p>	<p>↑ MIP 43%</p> <p>↑ sustained MIP 26%</p> <p>↑ MVV 9%</p>	<p>Time to exhaustion: Running at 80% of $\dot{V}O_{2max}$</p>	<p>↑ Time to exhaustion 16%</p> <p>↓ $\dot{V}O_2$ 13%</p> <p>↓ \dot{V}_E 26%</p> <p>↓ Blood lactate 39%</p> <p>↓ RPE 33%</p>

Control: no training

Mickleborough, Stager, Chatham, Lindley, and Ionescu (2008)

Competitively trained swimmers

IMT: n= 10

Sham: n = 10

Control: n = 10

All subjects maintain their swim training designed to enhance athletic performance. 10-12 supervised session per week.

IMT: Repeated sets of 6 inspirations at 80% sustained MIP until task failure, 3 days/wk. rest between sets gradually reduced from 45 s to 5 s.

Sham: Repeated sets of 6 inspirations at 30% sustained MIP until task failure, 3 days/wk. rest between sets gradually reduced from 45 s to 5 s.

Control: Swim training only

↑ MIP 46%
↑ Sustained MIP 49%
↑ RET 17%

* No clear differences from performing swim training only.

Morgan, Kohrt, Bates, and Skinner (1987)

Moderately trained male cyclists.

RMET: n = 4

Control n = 5

RMET: Eucapnic voluntary hyperventilation. 5 days per week for 3 weeks at 85% MVV

Control: No training

↑ MVV 14%
↑ Endurance breathing time 385%

Incremental test to exhaustion: Cycling at 60 rpm against a resistance of 0.5 kp, which was increased every 2 min by 0.5 kp until volitional exhaustion

Time to exhaustion: Cycling at 95% $\dot{V}O_{2max}$

Incremental test to exhaustion:

→ $\dot{V}O_{2max}$

Time to exhaustion:
→ time to exhaustion

Ozmen, Gunes, Ucar, Dogan, and Gafuroglu (2017)	Male soccer player RMET: n = 9 Control: n = 9	RMET: 15 min, twice per week, for 5 weeks Control: no training	↑ MIP 14% → FVC → FEV ₁ → MVV → MEP	Multistage shuttle run: 20 m multi-stage shuttle run test	→ Shuttle run
Romer et al. (2002b)	Male team sport athletes. IMT n = 12 Sham n = 12	IMT: Pressure threshold training. 6 wk, 2 sessions per day, 30 breaths per session at ~50% MIP Sham: 6 wk, 1 session per day, 60 breaths per session at ~15% MIP	↑ PIF 20% ↑ MIP 31%	Multistage shuttle run: 20 m multi-stage shuttle run test RS exercise: Fifteen 20 m sprints, maximum of 30 s passive recovery	Multistage shuttle run: → Maximal speed → Estimated $\dot{V}O_{2max}$ RS exercise: → Sprint time ↓ Self-selected recovery time 7%
Romer et al. (2002a) and Romer et al. (2002c)	Male trained road cyclists/triathletes IMT: n = 8 Sham: n = 8	IMT: Pressure threshold training. 6 wk, 2 sessions per day, 30 breaths per session at ~50% MIP Sham: 6 wk, 1 session per day, 60 breaths per session at ~15% MIP	↑ P ₀ 28% ↑ V _{max} 17% ↑ W _{I max} 49% ↑ P _{opt} 25% ↑ V _{opt} 17%	Incremental test to exhaustion: Cycling work rate increased by 35 W every 3-min starting from 95 W until volitional exhaustion Time trial: 20 km and 40 km cycling time trial	Maximal incremental exercise → W _{max} , ↓ dyspnoea 16% ↓ RPE 18% 20 km time trial ↓ Time to completion 3.8% ↑ MPO 4% ↓ Diaphragm fatigue ~7% ↓ RPE 16% 40 km time trial ↓ Time to completion 4.6% ↑ MPO 3%. ↓ Diaphragm fatigue ~6% ↓ RPE 16%
Salazar-Martínez et al. (2017)	Sixteen physically active males (n = 9) and females (n = 7) IMT: n = 9	IMT: Pressure threshold training. 6 wk, 5 d/wk, 2 sessions per day, 30 breaths per session at ~50% MIP	↑ MIP 28%	Maximal incremental exercise: Cycling work rate increased by 25 W every 1-min starting	Maximal incremental exercise: → $\dot{V}O_{2max}$ in normoxia and hypoxia.

	Control: n = 7	Control: no training		from 50 W until volitional exhaustion	↑ PPO in normoxia 5.26%, and hypoxia 2.51%.
				Time trial: 10 min time trial in normoxia (room air), and hypoxia ($F_{iO_2} = 0.165$).	Time trial ↑ MPO 11% in normoxia, and 7% in hypoxia. Linear relationship between MIP and MPO in normoxia ($R^2 = 0.69$) and hypoxia ($R^2 = 0.67$).
(Segizbaeva, Timofeev, Donina Zh, Kur'yanovich, & Aleksandrova, 2015)	Healthy, moderately trained males. IMT: n = 10 Control: n = 6	IMT: Pressure threshold training 3 wk, 7 day/wk, 1 session per day, 60 breaths per session. Pressure threshold was set to 60%, 70%, and 80% of MIP for weeks 1, 2, and 3 respectively. Control: 3 wk, 7 day/wk, 1 session per day, 30 breaths per session. Pressure threshold was set to the minimum setting.	↑ MIP 18%	Maximal incremental exercise: Cycling for 2 min at 1 W·kg ⁻¹ and 60-70 rpm. The work rate was increased thereafter by 0.5 W·kg ⁻¹ every 2 min until volitional exhaustion	↑ Maximal work rate 10% ↑ $\dot{V}O_{2max}$ 24% ↑ Anaerobic threshold 8% ↓ Respiratory muscle fatigue 6%
(Sonetti et al., 2001)	Competitive male cyclists REMT + IMT: n = 9 Sham n = 8	REMT + IMT: Endurance and strength training 5 wk, 1 session per day; Hyperpnoea endurance training, 50-60% MVV at f_R 50-60 min ⁻¹ for 30 min; Pressure threshold training, ~50% MIP until task failure (~40 breaths). Pressure threshold training. 6 wk, 5 d/wk, 2 sessions per day, until task failure at ~50% MIP.	↑ FVC 3% ↑ MIP 8%	Maximal incremental exercise: 10 min warm up on a cycle ergometer at 117 W. The test began at an initial work rate of 167 W and increased by 17 W every 1 min thereafter until volitional exhaustion Time to exhaustion: Cycling at 80-85% of	Maximal incremental exercise: ↑ maximal work rate 9% → $\dot{V}O_{2max}$ Time to exhaustion: ↑ 26%, Sham ↑16% 8 km TT ↓ Time to completion 1.8%

		Sham: breathing through the same trainer but filled with “oxygen absorbent” gravel, 30 min 5 days per week		W_{max} until volitional exhaustion Time trial: 8 km cycling time trial	No clear differences between groups
(Tong et al., 2008)	Healthy males, regularly engaged in either soccer or rugby training IMT: n = 10 Sham: n = 10 Control: n = 10	IMT: Pressure threshold training. 6 wk, 6 d/wk, 2 sessions per day, 30 breaths per session at ~50% MIP Sham: 6 wk, 6 d/wk, 2 sessions per day, 30 breaths per session at ~15% MIP Control: No training	↑ P0 32% → Vmax 3% ↑ $W_{I,max}$ 40% ↑ Popt 39% → Vopt 2%	Multistage shuttle run: Yo-Yo intermittent recovery tests (level 1)	↑ Shuttle run 16% ↓ Rate of increased breathlessness 11%
Turner et al. (2012)	Highly trained male cyclists IMT: n = 8 Control: n = 8	IMT: Pressure threshold training. 6 wk, 2 sessions per day, 30 breaths per session at ~50% MIP Sham: 6 wk, 1 session per day, 60 breaths per session at ~15% MIP	↓ O ₂ cost of voluntary hyperpnoea		
Turner et al. (2016)	Highly trained male cyclists IMT: n = 8 Control: n = 8	IMT: Pressure threshold training. 6 wk, 6 d/wk, 2 sessions per day, 30 breaths per session at ~50% MIP Sham: 6 wk, 6 d/wk, 2 sessions per day, 30 breaths per session at ~15% MIP	↑ MIP 26%	Constant load exercise. Cycling at 80% of $\dot{V}O_{2max}$ with “moderate” and “heavy” inspiratory loading. Cycling at 100% of $\dot{V}O_{2max}$	Heavy inspiratory loading: ↓ Locomotor muscle [HHb] 37% ↓ Respiratory muscle [HHb] 59%
Verges et al. (2009)	Moderately trained males REMT: n = 8	REMT: 60% MVV for 30 min. 20 training sessions, 1 day of rest following 2 consecutive days of training, for 4 wk.	REMT: ↑ respiratory endurance 62%		

IMT: n = 8
Sham: n = 8

IMT: area under the curve training equating to approximately 62% MIP. 2 sessions per day, 30 breaths per session, for 4 wk.

Sham: respiratory muscle “co-ordination training”. 1 session per day, 60 breaths per session, for 4 wk.

↓ MIP 4%;
 ↑ MEP 2%

IMT:
 ↑ respiratory endurance 38%
 ↑ MIP 31%;
 ↓ MEP 17 %

Sham:
 ↑ respiratory endurance 9%
 ↑ MIP 5%
 ↓ MEP 2%

(Volianitis et al., 2001)

Female competitive rowers

IMT: n = 7
Sham: n = 7

IMT: Pressure threshold training. 11 wk, 6 day/wk, 2 sessions per day, 30 breaths per session at ~50% MIP

Sham: 11 wk, 6 d/wk, 2 sessions per day, 30 breaths per session at ~15% MIP

IMT:
 ↑ MIP 38% at 4 wk, 42% at 11 wk

Sham:
 ↑ MIP 4% at 4 wk, 4% at 11 wk

6-min all out rowing: Rowing ergometer

Time trial: 5 km rowing ergometer time trial

6 min “all-out” rowing.
 ↑ Distance covered by 3% and 4% after wk 4 and 11.
 ↓ Post exercise respiratory muscle fatigue 8%

Time trial
 ↑ distance travelled 3% after 4 wk

(Witt et al., 2007)

Healthy males

IMT: n = 8
Sham: n = 8

IMT: Pressure threshold training. 5 wk, 6 day/wk, 1 sessions per day, 3 sets of 75 breaths, 5 min recovery between sets at ~50% MIP.

Sham: 5 wk, 6 day/wk, 1 sessions per day, 3 sets of 75 breaths, 5 min recovery between sets at ~10% MIP.

↑ MIP 17%

Resistive breathing task. Inspiring against a resistance of 60% MIP. $f_b = 15 \text{ b} \cdot \text{min}^{-1}$, duty cycle 0.70. Iso-time post training

↓ HR 27%
 ↓ Mean arterial blood pressure 4%

(Wylegala et al., 2007)

Male experienced swimmers

Respiratory muscle strength training (RMST): n = 10

RMET: n = 10

SHAM: n = 10

Training for all protocols 30 min/day, 5 days/wk, 4 wk.

RMST: Inspiratory and expiratory pressure threshold training. One breath every 30 s at an opening pressure of 50 cmH₂O.

RMET: Eucapnic voluntary hyperventilation at 60% MVV

SHAM: Breath holding

RMST:

↑ MIP 11%

↑ MEP 15%

↑ Respiratory muscle endurance 31%

RMET:

↑ 7% MVV

↑ FVC 3%

↑ FEV₁ 3%

↑ MVV 217%

Surface endurance swim.

Swimming at ~75% HR max heart rate until they could no longer maintain the pace.

Under water swim.

Swimming continued until tethered weight could not be kept off the bottom of swimming pool

Surface endurance swim.

↑ Time to exhaustion: RMST 66%; RMET 26%.

Under water swim.

↑ Time to exhaustion: RMST 33%; RMET 38%.

Abbreviations are: EMT, expiratory muscle training; FEV₁, forced expiratory volume in 1 s; F_IO₂, fraction of inspired oxygen; f_R, breathing frequency; FVC, forced vital capacity; GET, gas exchange threshold; HR, heart rate, IMT, inspiratory muscle training; MIP, maximal inspiratory mouth pressure; MVC, maximum voluntary contraction; MVV, maximum voluntary ventilation; P₀, maximal inspiratory pressure at zero flow; PEF, peak expiratory flow; PIF, peak inspiratory flow; P_{opt}, optimal pressure for maximal flow production; PPO, peak power output; RET, respiratory endurance time; RMET, respiratory muscle endurance training; RPE, rating of perceived exertion; RS, repeated-sprint; S_PO₂, arterial oxygen saturation; V̇CO₂, pulmonary carbon dioxide elimination; V̇_E, pulmonary ventilation; V_{max}, ; V̇O₂, pulmonary oxygen uptake; V̇O_{2max}, maximal pulmonary oxygen uptake; V_{opt}, optimal flow ; W_Imax, maximal inspiratory power; wk, week(s); W·min⁻¹, watts per minute. Arrows indicate the direction of change: ↑ increase, ↓ decrease, and → no change.

2.6 REPEATED SPRINT-EXERCISE

Typically, prolonged bouts of exercise have been used to examine how the work of breathing influences muscle O₂ delivery and exercise capacity with very little research on multiple sprint work. Repeated-sprint exercise is characterised by brief “all-out” exercise of 4-15 s, separated by incomplete recovery periods of 14-30 s (Billaut et al., 2013; Dupont, Moalla, Guinhouya, Ahmaidi, & Berthoin, 2004; Faiss et al., 2013; Sweeting et al., 2017). Performance in a repeat-sprint context is therefore represented as the ability to reproduce power output after a previous bout of maximal exercise (da Silva, Guglielmo, & Bishop, 2010; Mendez-Villanueva, Hamer, & Bishop, 2007). Over the course of a repeated-sprint series, there is a progressive decline in total mechanical work performed in each successive sprint (Figure 2.7). The rate of performance decline is also typically accelerated in low O₂ environments (Billaut et al., 2013; Bowtell, Cooke, Turner, Mileva, & Sumners, 2014; Goods, Dawson, Landers, Gore, & Peeling, 2014). Initial sprint performance is largely determined by muscular strength and power production (Morin et al., 2012; Newman, Tarpinning, & Marino, 2004; Young, McLean, & Ardagna, 1995), whereas the ability to resist fatigue and maintain performance is underpinned by aerobic capacity and the ability to deliver O₂ to the locomotor muscles in the between sprint recovery periods (Billaut & Buchheit, 2013; Gharbi, Dardouri, Haj-Sassi, Chamari, & Souissi, 2015).

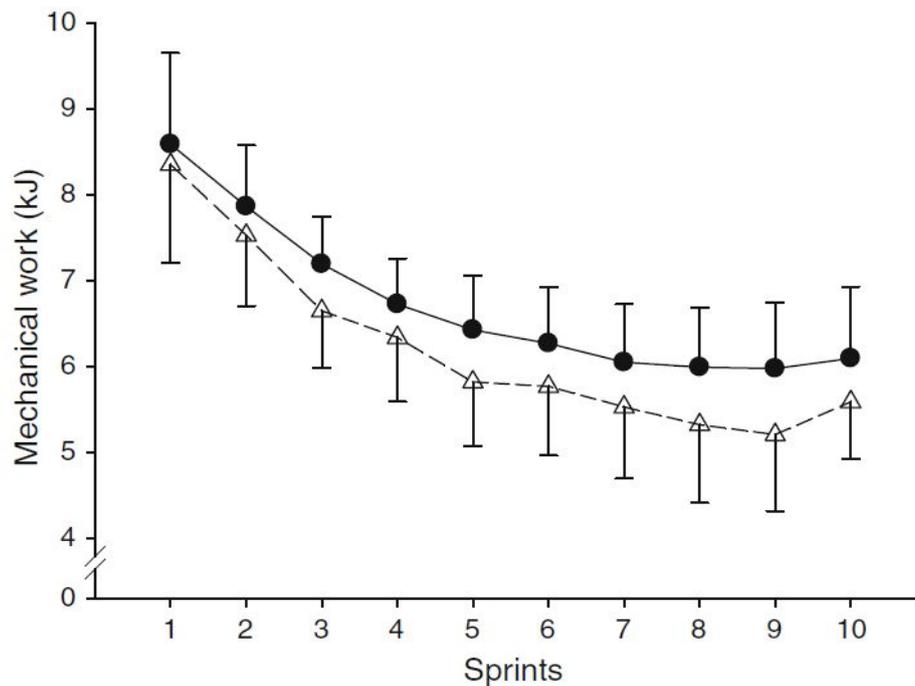


Figure 2.8: Mechanical work performed during repeated-sprint exercise. Ten 10 s sprints with 30 s passive rest were performed in normoxia (•) and hypoxia ($F_{iO_2} = 0.13$; Δ) on a friction-loaded cycle ergometer with a breaking force of $0.9 \text{ N}\cdot\text{Kg}^{-1}$ of body mass. Initial sprint performance was similar between conditions, but a significant main effect of condition on total work performed was observed (normoxia, $67.2 \pm 5.5 \text{ kJ}$ vs. hypoxia $62.1 \pm 5.4 \text{ kJ}$). Arterial O_2 saturation (estimated via pulse oximetry) was 12% lower in hypoxia. Reproduced from Smith and Billaut (2010).

2.6.1 Metabolic Determinates of Repeated-sprint Exercise

Muscle contractions rely on the release of energy through the hydrolysis of adenosine triphosphate (ATP) (Baker, McCormick, & Robergs, 2010). Resting intramuscular stores of ATP are limited to $\sim 20\text{-}25 \text{ mmol}\cdot\text{kg}^{-1}$ of dry muscle weight, which during a sprint, can only provide energy for 1-2 s (Bogdanis, Nevill, Lakomy, & Boobis, 1998; Gaitanos et al., 1993; Parolin et al., 1999). As resting ATP stores become depleted, three major energy systems are responsible for ATP resynthesis. Rapid ATP resynthesis is achieved through phosphocreatine (PCr) degradation (Gaitanos et al., 1993). Anaerobic glycolysis also has a large involvement in sprint metabolism (Gaitanos et al., 1993). Though as sprints are repeated, the relative contribution of anaerobic glycolysis towards ATP resynthesis declines (Gaitanos et al., 1993; Parolin et al., 1999). Conversely, aerobic metabolism has a very small role in isolated sprint performance ($\sim 10\%$ of total ATP

production), which increases as sprints are repeated (Bogdanis, Nevill, Boobis, & Lakomy, 1996; Parolin et al., 1999).

2.6.1.1 Phosphocreatine Degradation

Intramuscular PCr is especially important for the rapid resynthesis of ATP during explosive activities via the reversible PCr-creatine kinase pathway (Baker et al., 2010; Guimaraes-Ferreira, 2014; Schlattner, Tokarska-Schlattner, & Wallimann, 2006). In the presence of the enzyme creatine kinase, adenosine diphosphate (ADP) is converted to ATP through the dephosphorylation of PCr to form creatine (Cr).

Equation 2.5: Adenosine triphosphate resynthesis by phosphocreatine dephosphorylation reaction (Baker et al., 2010).



It is estimated that during a single 6-s sprint, 50% of anaerobic ATP production is derived predominantly through PCr degradation (Gaitanos et al., 1993). The remaining anaerobic energy contribution during an isolated sprint is supported mainly by glycolysis (44%), and in minority by intramuscular ATP stores (6%). When sprints are repeated, the relative contribution of PCr to anaerobic ATP resynthesis increases. By the tenth 6-s sprints, each separated by 30 s passive rest, PCr degradation is estimated to account for 80% of the total anaerobic energy contribution (Gaitanos et al., 1993). However, intramuscular PCr stores are limited to ~80 mmol·kg⁻¹ of dry muscle weight, and after only a single 6-s sprint, stores are reduced ~50% from baseline (Dawson et al., 1997; Gaitanos et al., 1993). When multiple sprints are performed, PCr depletion can be up to 75% after five repetitions (Dawson et al., 1997), and 84% after ten (Gaitanos et al., 1993). Since PCr degradation has such a large contribution to ATP resynthesis, the recovery of

intramuscular stores PCr are critically important to the restoration of power output (Sahlin et al., 1979).

The capacity to recover PCr is limited in a multiple sprint series, largely constrained by the short recovery periods between sprints. The rate of PCr resynthesis follows an initial fast phase, followed by a second longer slow component (Harris et al., 1976; Walter, Vandenborne, McCully, & Leigh, 1997). After a single 6-s sprint, approximately 70% of PCr replenishment is achieved in the first 30 s of passive rest (Dawson et al., 1997). But as sprints are repeated and muscle stores are further depleted, PCr can only recover to 50% of resting stores after just five repetitions. When rest is extended post a repeat-sprint series, only 80% of PCr is recovered after 3 min (Dawson et al., 1997), and 85% after 6 min of passive rest (Mendez-Villanueva, Edge, Suriano, Hamer, & Bishop, 2012). Though PCr degradation is an anaerobic process, PCr resynthesis is an aerobic process, and is sensitive to O₂ availability (Harris et al., 1976; Haseler et al., 1999; Kime et al., 2003; Sahlin et al., 1979). When breathing a hypoxic gas mixture (F_IO₂ = 0.10), the rate of PCr resynthesis has been demonstrated to be attenuated by 23% (Haseler et al., 1999). While breathing a hyperoxic gas (F_IO₂ = 1.00) enhances recovery by 20% compared with normoxia, which suggests that under normal exercise conditions PCr resynthesis is limited by O₂ availability (Haseler et al., 1999). Therefore, if the work of breathing is high enough to limit locomotor muscle O₂ delivery, PCr resynthesis in repeated-sprint exercise may be impaired.

2.6.1.2 Anaerobic Glycolysis

The energy debt created by the rapid decrease in muscle PCr during a single sprint is met by a sizable contribution of anaerobic glycolysis to ATP resynthesis.

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Approximately 44% of ATP resynthesis is derived from anaerobic glycolysis during a single 6 s sprint (Gaitanos et al., 1993). However, the relative contribution of anaerobic metabolism decreases as sprints are repeated (McGawley & Bishop, 2015). By the tenth sprint, Gaitanos et al. (1993) estimated that glycolysis was only responsible for 16% of total anaerobic ATP production. Moreover, in four of the seven subjects, it was estimated to be zero (range 0-23.1 mmol ATP·kg⁻¹ of dry muscle weight). Many mechanisms play a role in the relative decrease in anaerobic glycolysis during multiple-sprint work. The most likely being the progressive depletion of muscle glycogen that is associated with high-intensity activity (Balsom, Gaitanos, Soderlund, & Ekblom, 1999).

2.6.1.3 Aerobic Metabolism

The aerobic contribution to an isolated sprint is minimal since the maximal rate of ATP resynthesis is far below the requirements of maximal sprint work (Baker et al., 2010). In an isolated sprint, aerobic metabolism is responsible for ~10% of total energy production (McGawley & Bishop, 2015; Parolin et al., 1999). But as sprints are repeated, the relative increase in aerobic metabolism to total ATP turnover rate rises to compensate for reduced energy supply from anaerobic pathways (Bogdanis et al., 1996; Trump, Heigenhauser, Putman, & Spriet, 1996). Following five 6-s sprints, it is estimated the aerobic energy contribution rises to ~40% of total ATP production (McGawley & Bishop, 2015). The remaining 60% being derived from anaerobic pathways, predominantly PCr degradation (Dawson et al., 1997; Gaitanos et al., 1993). Pulmonary $\dot{V}O_2$ can fluctuate between 70-100% of $\dot{V}O_{2max}$ from sprint to recovery periods in the latter stages of a repeat-sprint series (Buchheit et al., 2009; Dupont, Millet, Guinhouya, & Berthoin, 2005). When no external work is being performed (i.e. passive rest) during the recovery period between sprints, the elevated $\dot{V}O_2$ above baseline is representative of

lactate metabolism, removal of inorganic phosphate, and most importantly PCr resynthesis (Bahr, Gronnerod, & Sejersted, 1992; Gaesser & Brooks, 1984; Guimaraes-Ferreira, 2014).

Aerobic metabolism may have a limited role in ATP formation during multiple sprint work (Bogdanis et al., 1996; McGawley & Bishop, 2015), but is fundamental to PCr resynthesis between sprints. Compartment specific creatine kinase isozymes are located in the cytosol and mitochondrial intermembrane space, and are associated with either the ATP-consuming or -delivering process, respectively (Guimaraes-Ferreira, 2014; Schlattner et al., 2006). In the PCr shuttle system (Figure 2.8), mitochondrial creatine kinase mediates the reaction between creatine and ATP formed by oxidative metabolism, to generate PCr and ADP (Bessman & Carpenter, 1985). Therefore, the rate at which the mitochondria can generate ATP through oxidative phosphorylation, will dictate PCr resynthesis. A positive correlation between aerobic fitness, and maintaining repeated-sprint performance exists (Bishop, Edge, & Goodman, 2004; da Silva et al., 2010; Gharbi et al., 2015; Tomlin & Wenger, 2001). It is likely that improvements in mitochondria function and content, that are associated with exercise training (Bishop, Granata, & Eynon, 2014), underpin the correlation between aerobic fitness and repeated-sprint ability. Additionally, muscle O₂ availability between sprint efforts likely affects mitochondrial oxidative phosphorylation, which would explain the connection between PCr resynthesis and O₂ availability (Haseler et al., 1999; Sahlin et al., 1979).

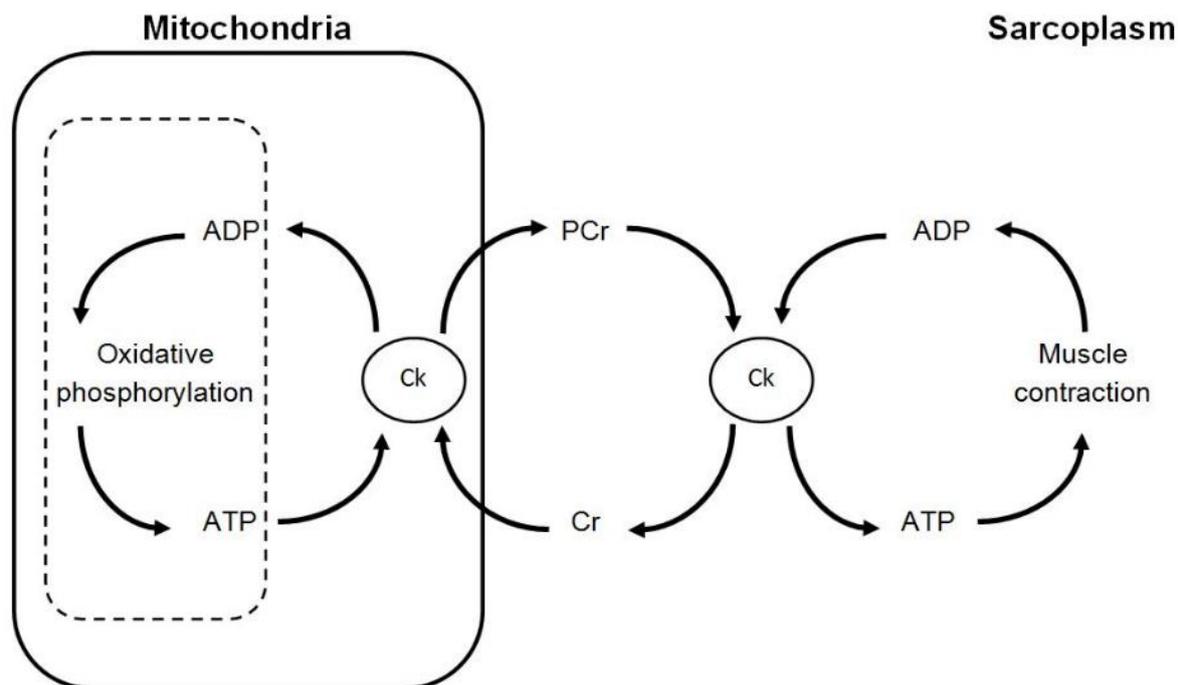


Figure 2.9: Phosphocreatine shuttle system. Abbreviations are: ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ck, creatine kinase; PCr, phosphocreatine, Cr, creatinine. Adapted from Guimaraes-Ferreira (2014).

2.6.2 Skeletal Muscle Tissue Oxygenation

Muscle O₂ availability during repeated-sprint exercise is critical for supporting PCr resynthesis, which underpins the capacity to maintain power out over a sprint series (Gaitanos et al., 1993; Haseler et al., 1999). Changes in local O₂ balance (delivery vs. consumption) can be measured in real time with NIRS (Boushel & Piantadosi, 2000; Ferrari, Muthalib, & Quaresima, 2011). The NIRS technology relies on the relative transparency of biological tissue to near-infrared light (650-950 nm), and light absorption of oxy-haemoglobin (O₂Hb) and deoxy- haemoglobin (Alhems, Zhiyun, & Deen, 2013). The concentration of [HHb] and [O₂Hb] rises and falls, respectively, proportional to an increase in metabolic activity in the underlying tissue and display similar kinetics to pulmonary $\dot{V}O_2$ (Grassi, Quaresima, Marconi, Ferrari, & Cerretelli, 1999; Subudhi, Dimmen, & Roach, 2007).

By employing NIRS in repeated-sprint exercise, muscle oxygenation kinetics can be studied in real time (Boushel & Piantadosi, 2000; Ferrari et al., 2011). Analysis typically focuses on [HHb] due to [O₂Hb] being influenced by rapid blood volume and perfusion variations caused by forceful muscle contractions (De Blasi, Cope, Elwell, Safoue, & Ferrari, 1993; Takaishi et al., 2002). Changes in muscle [HHb] during exercise is relatively independent of blood volume compared to [O₂Hb], (De Blasi et al., 1993; Grassi et al., 2003), and reflect venous [HHb] to provide an estimate of muscular O₂ extraction (DeLorey, Kowalchuk, & Paterson, 2003; Grassi et al., 2003). At the onset of a sprint, there is a rapid increase in vastus lateralis [HHb] (deoxygenation), which during the rest period subsequent to the sprint trends back towards baseline (reoxygenation) (Figure 2.9).

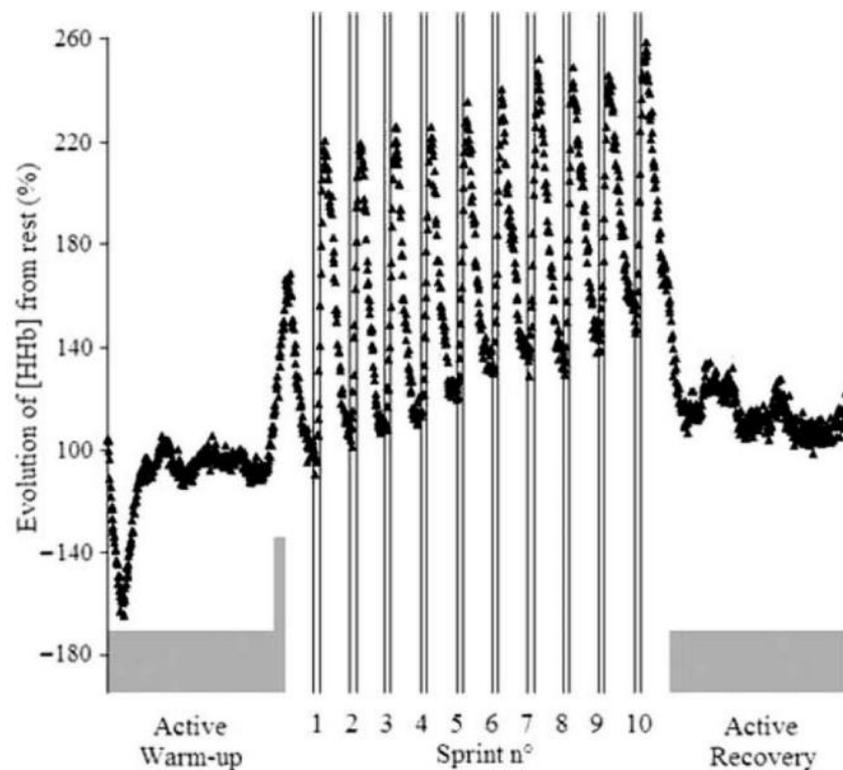


Figure 2.10: Evolution of vastus lateralis deoxyhaemoglobin during repeated-sprint exercise. The concentration of vastus lateralis deoxyhaemoglobin ([HHb]) is expressed as a percentage relative to resting baseline. Subjects ($n = 9$) performed ten 6 s sprints (represented by the vertical bars) on a friction braked cycle ergometer against a resistive force of $0.9 \text{ N}\cdot\text{kg}^{-1}$ of body mass. Sprints were interspersed by 30 s of passive rest. Data was continuously sampled at 2 Hz. Reproduced from Racinais et al. (2007).

2.6.2.1 Problems with Analysis Methods of Near-infrared Spectroscopy

To obtain a single sprint and recovery value for each sprint and recovery phase, a mean is typically calculated over a predetermined duration within the closing seconds of each sprint and recovery periods, which serves to smooth the large fluctuations in NIRS variables (as seen in Figure 2.10). Predetermined analysis windows have been used in acute settings (Racinais et al., 2007; Sandbakk et al., 2015), varying environmental O₂ availability (Billaut & Buchheit, 2013; Billaut et al., 2013; Smith & Billaut, 2010, 2012), active vs. passive rest (Buchheit et al., 2009; Dupont et al., 2004), after respiratory muscle warm-up (Cheng et al., 2013), and in response to training (Buchheit, Hader, & Mendez-Villanueva, 2012; Buchheit & Ufland, 2011; Galvin, Cooke, Sumners, Mileva, & Bowtell, 2013). A drawback of predefined analysis windows is that the true, physiological peak and/or nadir of the [HHb] signal may occur outside the predefined analysis windows (Figure 2.10). It may be that [HHb] continues to rise if tissue O₂ consumption remains elevated post sprint (Figure 2.9 and Figure 2.10). The nadir of [HHb] may also not occur within a predefined window of analysis, especially since [HHb] will be affected by limb activity when the athlete prepares for the next sprint (i.e., leg movement to place the pedal in the right position and static contraction of the quadriceps (Gotshall, Bauer, & Fahrner, 1996). To overcome this, a rolling mean approach may be applied to smooth the data to determine the true peak and nadir of the NIRS signal (Bowtell et al., 2014; Faiss et al., 2013; Ihsan, Abbiss, Lipski, Buchheit, & Watson, 2013; Jones, Hamilton, & Cooper, 2015; Ohya, Aramaki, & Kitagawa, 2013; Ohya, Hagiwara, & Suzuki, 2015). It is currently unclear if there are any clear differences in the reported vastus lateralis [HHb] means calculated from predetermined time periods or a rolling mean approach. A digital filter is another typical technique used to attenuate noise and smooth raw data (Elmer & Martin,

2009). Such filters have been used to smooth the NIRS signal, removing the necessity to calculate an arithmetic mean (Faiss et al., 2013; Sandbakk et al., 2015; Willis, Alvarez, Millet, & Borrani, 2017). There is currently no consistency among researchers for analysing NIRS data collected during repeated-sprint exercise (Table 2.2). Furthermore, the effect that different analysis methods may have on NIRS derived variables it is currently unclear.

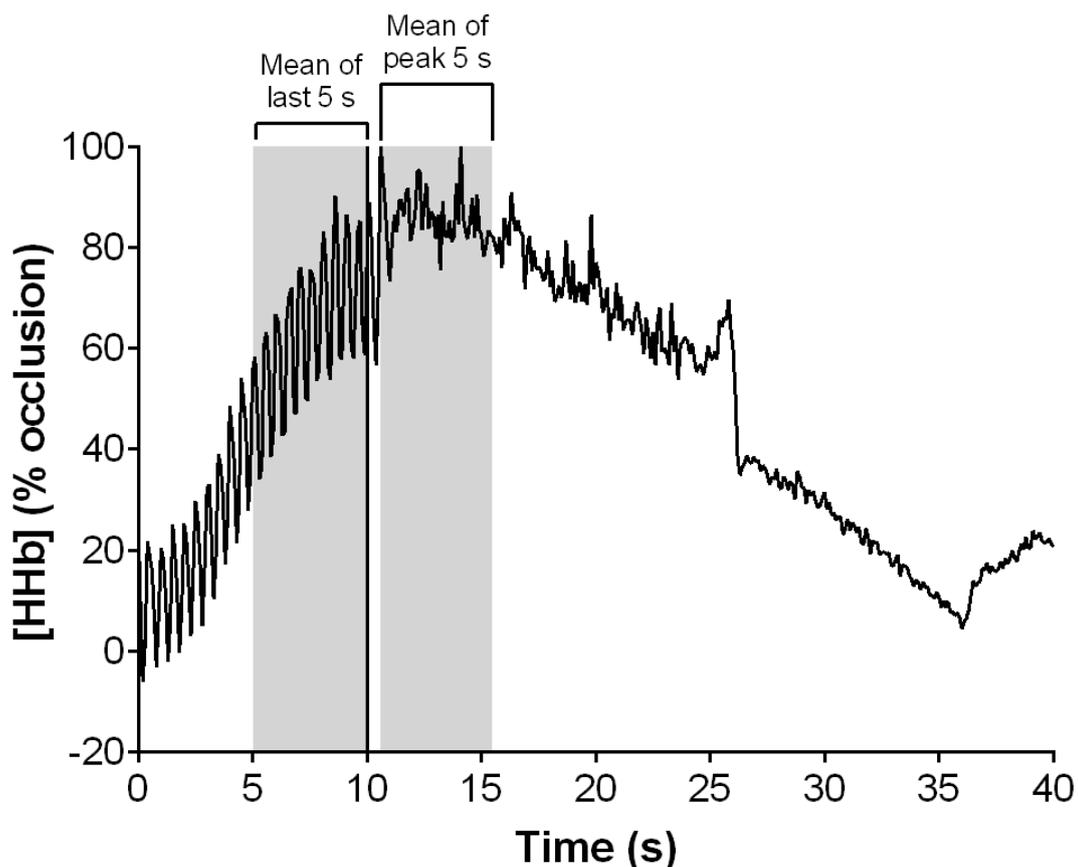


Figure 2.11: An example of averaging windows used to determine vastus lateralis deoxyhaemoglobin during repeated sprint exercise. Representative data from an individual subject during a single sprint/recovery cycle of repeated-sprint exercise. The exercise protocol was ten 10 s sprints, separated by 30 s of passive rest. The grey shaded areas represent examples of averaging windows used to determine the change in vastus lateralis deoxyhaemoglobin ([HHb]) induced by a sprint bout. Change in [HHb] is expressed relative to the maximal 5 s average obtained during femoral arterial occlusion (100%).

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Even though there is no consensus on how best to approach to identifying a single value to represent each sprint and recovery phase during multiple sprint work, lessons on muscle oxygenation during repeated-sprint exercise can still be drawn. Peak sprint [HHb] will reflect muscle O₂ uptake during exercise (Smith & Billaut, 2010; Tran et al., 1999). The [HHb] recovery value, and the difference between peak and nadir [HHb] values (reoxygenation), will provide information on the quality of metabolic recovery between sprint efforts (Billaut & Buchheit, 2013; Buchheit & Ufland, 2011; Kime et al., 2003).

Table 2.2: Overview of the methodology used to analyse near-infrared spectroscopy data collected during repeated-sprint exercise

Author	Protocol	Sample Rate	Analysis	
			Sprint	Recovery
Billaut and Buchheit (2013)	10 x 10 s cycling sprints, 30 s rest	10 Hz	Mean of the last 5 s	Mean of the last 5 s
Billaut et al. (2013)	Three sets of 5 x 5 s cycling sprints, 25 s rest between sprints, and 120 s between sets	10 Hz	Mean of the last 2.5 s	
Buchheit et al. (2009)	6 x 4 s sprints on a non-motorised treadmill, 21 s of passive or active ($2 \text{ m}\cdot\text{s}^{-1}$) rest	6 Hz (averaged to give 1 s value)	Maximum 5 s average	Minimum 5 s average
Buchheit and Ufland (2011)	2 x 15 s all-out shuttle runs, 15 s rest	10 Hz (averaged to give 1 s value and 3 s moving average applied)	End of both sprints	Immediately before second sprint
Cheng et al. (2013)	6 X 10 cycling sprints, 60 s active recovery	10 Hz	Average	
Dupont et al. (2004)	15 s high-intensity cycling, 15 s passive recovery until task failure	1 Hz	Lowest value	
Faiss et al. (2013)	10 s cycling sprints, 20 recovery until task failure	10 Hz (10 th order Butterworth filter)	Maximum value	Minimum Value
Galvin et al. (2013)	10 x 20 m running sprints, 30 s rest	Not provided		Prior to each sprint
Jones et al. (2015)	5 x 30 s cycling sprints, 4 min rest	10 Hz	Maximum 3 s average	Minimum 3 s average
Ohya et al. (2013)	10 x 5 s cycling sprints, active recovery (40% $\dot{V}O_{2\text{max}}$) and passive recovery of either 25, 50 or 100 s	10 Hz	Maximum 1 s average	Minimum 1 s average
Ohya et al. (2015)	10 x 5 s cycling sprints, 25 s of active recovery (40% $\dot{V}O_{2\text{max}}$)	5 Hz	Maximum 1 s average	Minimum 1 s average

Racinais et al. (2007)	10 x 6 s cycling sprints, 30 s rest	2 Hz	End value	End value
Sandbakk et al. (2015)	8 x 8 s poling sprints (skiing), 22 s rest	10 Hz (8 th order Butterworth filter)	Mean of the last 2 s	
Smith and Billaut (2010)	10 x 10 s cycling sprints, 30 s rest	10 Hz	Mean of the last 5 s	
Smith and Billaut (2012)	10 x 10 s cycling sprints, 30 s rest	10 Hz	Mean of the last 5 s	
Willis et al. (2017)	10 s cycling sprints, 20 s of active rest at 20 W. Sprints were repeated until exhaustion (cadence <70 rpm)	10 Hz (4 th order Butterworth filter)	Maximum value	Minimum value

Abbreviations are: rpm, revolutions per minute; $\dot{V}O_{2max}$, maximal rate of pulmonary oxygen uptake.

2.6.2.2 *Vastus Lateralis Deoxygenation*

Elevated [HHb] during exercise reflects an increased muscle O₂ uptake. But muscle deoxygenation may also be caused by an increase of muscle CO₂/H⁺, which reduces the hemoglobin-O₂ binding affinity (Astrup et al., 1965; Næraa et al., 1966). There is a tendency for peak [HHb] to plateau over a repeat-sprint series (Buchheit et al., 2009; Smith & Billaut, 2010), whereas others have shown a slight increase with each sprint repetition (Racinais et al., 2007). A plateau in [HHb] is taken as evidence of maximal muscle O₂ extraction, which is typically observed during arterial occlusion (Esaki et al., 2005; Tran et al., 1999). Since the average increase in [HHb] during a sprint remains fairly consistent across repetition, an increase in peak [HHb] as sprints are repeated seems to result from a limited ability of the locomotor muscles to reoxygenate between sprint bouts (Racinais et al., 2007). Using hypoxia to examine the role of O₂ availability on vastus lateralis deoxygenation has returned mixed results. While performing ten 10-s sprints with 30 s of passive rest, vastus lateralis deoxygenation can be up to 12% greater when breathing a hypoxic gas mixture (F_IO₂ = 0.13) compared to normoxia (Billaut & Buchheit, 2013). Though using the same experimental protocol, peak deoxygenation can be no higher in hypoxia compared to normoxia when examined in a similar group of subjects (Smith & Billaut, 2010, 2012). Individual variability in muscle O₂ extraction is the likely cause for this discrepancy vastus lateralis deoxygenation trends. Some subjects may be able to better compensate for reduced O₂ availability through greater muscle O₂ extraction as sprints are repeated.

2.6.2.3 *Vastus Lateralis Reoxygenation*

Because PCr resynthesis is achieved through oxidative processes (Haseler et al., 1999; Hogan, Richardson, & Haseler, 1999), the availability of muscle O₂ during rest periods is critically important for metabolic recovery. In maximal voluntary isometric

handgrip exercise, reoxygenation rate measured as the rate change of [O₂Hb] during recovery was strongly correlated with the recovery of muscle PCr ($r^2 = 0.939$) (Kime et al., 2003). Therefore, factors affecting muscle reoxygenation between sprint efforts will likely affect PCr resynthesis and repeated-sprint performance.

Vastus lateralis reoxygenation capacity can be attenuated by performing low intensity activity (jogging/cycling) between sprint efforts (Buchheit et al., 2009; Ohya et al., 2013). By reducing the O₂ availability, the restoration of peak cycling power and peak running speed following periods of “active” recovery is 3-7% lower compared to passive rest. The time to exhaustion is also lowered by performing “active” recovery when performing 15-s sprints, repeated every 15 s (745 ± 171 s vs. 445 ± 79 s; -60%) (Dupont et al., 2004). Performing active recovery between sprints, muscle tissue reoxygenation is impaired through the constant O₂ uptake supporting the metabolic requirements of the active recovery. Therefore, PCr resynthesis is likely blunted because ATP from oxidative phosphorylation is devoted directly to maintain muscle contractions, rather than towards PCr resyntheses (Ohya et al., 2013; Schlattner et al., 2006).

The influence of limited reoxygenation on repeated-sprint ability has also been highlighted by manipulating the F_IO₂. When performing ten 10-s sprints with 30 s of passive rest and inspiring a hypoxic gas mixture (F_IO₂ = 0.13), reoxygenation was attenuated by 11% (Billaut & Buchheit, 2013). There was a ~8% reduction in total mechanical work in hypoxia compared to normoxia, and the reduction in work was strongly correlated with the attenuated muscle reoxygenation ($r = 0.78$; 90% confidence interval: 0.49, 0.91). Since PCr resynthesis has similar recovery kinetics to reoxygenation (Kime et al., 2003), it is likely that muscle PCr recovery was hindered by limited O₂ availability.

Reoxygenation capacity is improved after endurance training, which may explain in part the positive relationship between aerobic fitness and repeat-sprint ability (Bishop et al., 2004; da Silva et al., 2010; Gharbi et al., 2015; Tomlin & Wenger, 2001). After eight weeks of endurance training, repeated-sprint running (Two 15 s 20 m shuttle sprints, with 15 s of passive rest), and muscle oxygenation were evaluated (Buchheit & Ufland, 2011). Initial-sprint performance was unaffected, presumably because improvements in aerobic function do not support the anaerobic nature of an isolated sprint. However, prior to the commencement of the second sprint, muscle oxygenation was 152% higher following training, and the decrement in subsequent sprint performance was attenuated by 26% (Buchheit & Ufland, 2011). It is likely that by improving O₂ delivery to the locomotor muscle, O₂ availability for oxidative phosphorylation was enhanced, and in turn, the phosphocreatine shuttle system (Baker et al., 2010; Guimaraes-Ferreira, 2014).

2.6.3 Ventilation in Repeated-sprint Exercise

There has been limited research on the role respiratory muscle work plays in repeated-sprint exercise, and specifically reoxygenation capacity between sprint efforts. Unlike sustained constant load exercise that induces respiratory muscle fatigue (B. D. Johnson et al., 1993), the same has not been demonstrated for multiple-sprint work (Minahan et al., 2015). Following four sets of 4 x 6-s sprints, with 24-s rest between sprints, and 2 min between sets, no reduction in respiratory muscle strength was reported in a group recreational active individual (Minahan et al., 2015). The intermittent nature of repeated-sprint exercise may be sufficient to mitigate the fatiguing effects on the diaphragm that is associated with high-intensity exercise. Therefore, activation of the respiratory muscle metaboreflex (Figure 2.6) may not necessarily occur (Dempsey et al., 2006). However, there is evidence that respiratory muscle training does provide some

benefit towards maintaining repeated-sprint performance, though the mechanisms are unclear (Archiza et al., 2017; Romer et al., 2002b).

After a six-week period of IMT, repeated-sprint ability was reported in a group of recreational sprint sport players (soccer, rugby, field hockey and basketball) (Romer et al., 2002b). Performance was assessed during fifteen 20-m sprints, which they were allowed a maximum of 30 s rest. Following the IMT intervention, there were no clear changes in sprint times. However, self-selected recovery time was attenuated by 6.9% (range: -0.9-14.5%). Strengthening the inspiratory muscles presumably reduced the O₂ cost of exercise hyperpnoea and blunted the respiratory muscle metaboreflex, which would in turn reduce O₂ competition between locomotor and respiratory muscles (Turner et al., 2012; Witt et al., 2007). Through attenuating a respiratory muscle metaboreflex, it is likely that the quality of metabolic recovery was enhanced with IMT, so that subjects could maintain performance with less rest between sprints. But since there were no measurements of muscle oxygenation (Romer et al., 2002b), it is difficult to separate potential changes in O₂ delivery from reduced feelings of dyspnoea that is associated with respiratory muscle training (McConnell & Romer, 2004a).

The effectiveness of IMT on repeat-sprint ability and time to exhaustion in a constant speed running test has also been assessed in a group of professional female soccer players (Archiza et al., 2017). Repeated-sprint ability was assessed with six 40 m sprints (20 m + 180° turn + 20 m) with 20 s passive rest between each sprint. Muscle oxygenation was only examined during the time to exhaustion trials (100% of the speed obtained during a maximal incremental exercise test). Both placebo and experimental groups had improvements in time to exhaustion, but the effect size in the IMT group was larger (Sham: 0.46; IMT: 0.74). Specific training of the respiratory muscles therefore

provided additional performance benefits beyond professional soccer training. Performance benefits were partly attributed to a blunted increase in respiratory muscle [HHb], with a concurrent increase in vastus lateralis [O₂Hb] (Archiza et al., 2017). In terms of the athlete's ability to preserve repeat-sprint performance, the IMT group showed the greatest improvement in the capacity to maintain sprint time over multiple sprints. The blunted respiratory muscle metaboreflex in the exhaustion test may have also occurred during the repeated-sprint test. However, without muscle oxygenation measurements during the sprint trials, it is unclear if there were any changes to O₂ availability after training. The few studies demonstrating enhanced repeated-sprint performance following IMT (Archiza et al., 2017; Romer et al., 2002b) support the notion that respiratory muscle work plays a negative effect on high-intensity exercise.

In conclusion, a high work of breathing limits locomotor O₂ muscle delivery during sustained high-intensity exercise, and can limit maximal exercise capacity (Amann, Pegelow, et al., 2007; Harms et al., 1997; Harms et al., 2000). Training the respiratory muscles can reduce the O₂ cost of exercise hyperpnoea (Turner et al., 2012), and attenuate blood flow competition between the locomotor and respiratory muscles (McConnell & Lomax, 2006). However, there remains very limited understanding of the role exercise hyperpnoea plays during repeated-sprint exercise. It remains to be answered if respiratory muscle work influences vastus lateralis reoxygenation during repeated-sprint exercise. Also, it is unclear if the enhanced repeated-sprint ability following respiratory muscle training (Archiza et al., 2017; Romer et al., 2002b) is derived from improved muscle oxygenation kinetics.

2.7 STUDY AIMS

The general aim of this thesis is therefore to investigate the influence of respiratory muscle work on skeletal muscle tissue oxygenation during repeated-sprint exercise. This thesis will build upon previous work examining repeated-sprint exercise and vastus lateralis tissue oxygenation (Billaut & Buchheit, 2013; Billaut et al., 2013; Faiss et al., 2013; Galvin et al., 2013; Smith & Billaut, 2010, 2012; Sweeting et al., 2017). Respiratory muscle oxygenation was also to be assessed which to date has not been examined in the context of repeated-sprint exercise. This thesis will use similar study designs as previous work in this area, which will allow for direct comparisons between studies. The specific aims of the research chapters were as follows.

2.7.1 Study 1 ([Chapter Three](#))

Currently, there is no consistency for smoothing and determining peaks and nadirs from a NIRS signal, which can make interpretation and comparisons between studies difficult. Therefore, the aim of study 1 was to evaluate the current methodologies employed to evaluate NIRS responses of repeated-sprint exercise.

2.7.2 Study 2 ([Chapter Four](#))

A high work of breathing has been demonstrated to compromise limb O₂ delivery during sustained high-intensity exercise. But it is unclear if intermittent exercise can induce a respiratory muscle metaboreflex. Therefore, the aim of study 2 was to explore the influence of elevated inspiratory muscle work on locomotor and respiratory muscle oxygenation.

2.7.3 Study 3 ([Chapter Five](#))

Following study 2, the capacity to increase $\dot{V}O_2$ to meet the demands of heightened respiratory muscle work appears to be a crucial factor in the maintenance of O_2 delivery to both the locomotor and respiratory muscles. Therefore, the aim of study 3 was to evaluate the respiratory muscle oxygenation responses to arterial hypoxemia during repeated-sprint exercise, and contrast them against the oxygenation trends of the locomotor muscles.

2.7.4 Study 4 ([Chapter Six](#))

Specific training targeting the respiratory muscles has been demonstrated to attenuate the activation of the respiratory muscle metaboreflex and improve exercise performance. The aim of study 4 was therefore to examine the effects of respiratory muscle training on muscle oxygenation trends and repeated-sprint performance. Secondly, the training effects were also examined in response to arterial hypoxemia.

**CHAPTER THREE: INFLUENCE OF AVERAGING
METHOD ON MUSCLE DEOXYGENATION
INTERPRETATION IN REPEATED-SPRINT
EXERCISE**

3.1 INTRODUCTION

Near-infrared spectroscopy (NIRS) is a common tool to indirectly measure muscular oxygen availability and microvascular reactivity non-invasively (De Blasi et al., 1993; DeLorey et al., 2003; Ferrari et al., 2011; Grassi et al., 2003; McLay et al., 2016). Implementation of NIRS relies on the transparency of human tissue, and the light absorbing characteristics of oxy- (O_2Hb) and deoxy-haemoglobin (HHb) chromophores for the determination of their concentration ($[\text{O}_2\text{Hb}]$ and $[\text{HHb}]$ respectively) in a localised tissue bed (Ferrari & Quaresima, 2012). Changes in $[\text{O}_2\text{Hb}]$ and $[\text{HHb}]$ reflect the dynamic balance between muscle O_2 delivery and extraction in the underlying tissue (Ferrari et al., 2004). In continuous exercise where NIRS responses are relatively stable, averages can be calculated over discrete and pre-determined time points for identification of overall trends within the exercise bout (DeLorey et al., 2003; Grassi et al., 2003). When maximal sprint efforts are repeated, however, there is a rapid deoxygenation at exercise onset that slowly recovers at sprint cessation. The evolution of peaks and nadirs across the NIRS signal is often used to describe the quality of metabolic recovery between sprint bouts (Billaut & Buchheit, 2013; Ohya et al., 2015; Sandbakk et al., 2015). Because of the rapid oxygenation adjustments and short duty cycle of repeated-sprint exercise (Ohya et al., 2013; Ohya et al., 2015; Racinais et al., 2007), accurate identification of peaks and nadirs in the NIRS signal is critical.

Analysis of NIRS data obtained during repeated-sprint exercise is often constrained to $[\text{HHb}]$ (Billaut & Buchheit, 2013; Bowtell et al., 2014; Buchheit et al., 2009; Galvin et al., 2013; Racinais et al., 2007) due to $\Delta[\text{O}_2\text{Hb}]$ being influenced by rapid blood volume and perfusion variations caused by forceful muscle contractions (De Blasi et al., 1993; Takaishi et al., 2002). Additionally, the HHb signal is considered to be relatively

independent of blood volume (De Blasi et al., 1993; Grassi et al., 2003), and taken to reflect venous [HHb] which provides an estimate of muscular oxygen extraction (DeLorey et al., 2003; Grassi et al., 2003). However, across studies there are differing methods used to smooth the NIRS signal and determine peak and nadir [HHb], which can potentially affect comparisons between studies and, therefore, interpretation.

To analyse a NIRS signal, single values for each sprint and recovery are typically determined for each peak and nadir (Buchheit et al., 2009; Buchheit & Ufland, 2011; Faiss et al., 2013; Ohya et al., 2013; Racinais et al., 2007). A mean is calculated over a predetermined duration within the closing seconds of each sprint and recovery periods in order to smooth fluctuations in raw NIRS data during sprint exercise (Billaut et al., 2013; Buchheit et al., 2009; Jones et al., 2015; Ohya et al., 2013; Sandbakk et al., 2015; Smith & Billaut, 2010, 2012). This method has been used on numerous occasions in acute settings (Racinais et al., 2007; Sandbakk et al., 2015), varying inspired O₂ fraction (Billaut & Buchheit, 2013; Billaut et al., 2013; Smith & Billaut, 2010, 2012), active vs passive rest (Buchheit et al., 2009; Dupont et al., 2004), after respiratory muscle warm-up (Cheng et al., 2013), and in response to training (Buchheit et al., 2012; Buchheit & Ufland, 2011; Galvin et al., 2013). However, a possible drawback is that the true, physiological peak and/or nadir [HHb] may not fall within the predefined analysis window. It may be that [HHb] continues to rise if tissue O₂ consumption remains elevated post sprint, and/or if O₂ delivery decreases. Additionally, the recovery nadir may be affected by limb activity when the athlete prepares for the next sprint (i.e., leg movement to place the pedal in the right position and static contraction of the quadriceps). To overcome this, a rolling mean approach may be applied to smooth the data in order to determine the true peak and nadir of the NIRS signal (Bowtell et al., 2014; Faiss et al., 2013; Ihsan et al., 2013; Jones et

al., 2015; Ohya et al., 2013; Ohya et al., 2015). But currently, there is no comparison of means calculated from predetermined time periods or a rolling mean approach. Additionally, there is no consistency of the moving average window duration, which may be constrained to sprint duration (Billaut & Buchheit, 2013; Ohya et al., 2015). A digital filter is another typical technique used to attenuate noise and smooth raw data (Elmer & Martin, 2009). For example, when a low-pass filter is used, a cut-off frequency is chosen so that lower signal frequencies remain and higher frequencies are attenuated (Yu, Gabriel, Noble, & An, 1999). Such filters have been employed to smooth the NIRS signal during repeated-sprint exercise (Faiss et al., 2013; Sandbakk et al., 2015; Willis et al., 2017), but again, the relevance of such technique has yet to be confirmed compared to more widely used averaging methods.

Therefore, the purpose of this study was to compare and evaluate the effect of different NIRS signal analysis methods (predetermined temporal window, rolling mean, and Butterworth filter) on muscle tissue oxygenation trends during a repeated-sprint protocol. We propose that the combination of a digital filter to smooth the NIRS signal, and the identification of a local maximum and minimum for each sprint/recovery phase will improve our ability to detect changes in the signal.

3.2 METHODS

3.2.1 Subjects

Nine males accustomed to high-intensity activity were recruited for this study (mean \pm SD: Age 25 ± 3 years; Height, 183.2 ± 7.7 cm; Body mass, 81.0 ± 8.7 kg; $\dot{V}O_{2peak}$, 54.6 ± 6.2 mL \cdot min $^{-1}\cdot$ Kg $^{-1}$). All participants reported to be healthy and with no known neurological, cardiovascular or respiratory diseases. After being fully informed of the requirements, benefits, and risks associated with participation, each participant gave written consent. Ethical approval

for the study was obtained from the institutional Human Research Ethics Committee and conformed to the declaration of Helsinki.

3.2.2 Experimental Design

Participants were part of a larger project that required six separate laboratory visits. Data presented here were taken from the control trial that took place after familiarisation. Testing was performed on an electronically braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands), set to “isokinetic” mode. In this mode, a variable resistance is applied to the flywheel proportional to the torque produced by the subjects to constrain their pedalling rate to 120 rpm. Below 120 rpm, no resistance is applied to the flywheel. This mode was chosen to avoid cadence-induced changes in mechanical power production (van Soest & Casius, 2000), and haemodynamics (Gotshall et al., 1996), within and between sprints. After a 7-min warm-up consisting of 5 min of unloaded cycling at 60-70 rpm and two 4 s sprints (separated by 1 min each), participants rested for another 2.5 min before the repeated-sprint protocol was initiated. The repeated-sprint protocol consisted of ten 10 s self-paced sprints separated by 30 s of passive rest. Participants were instructed to give an “all-out” effort for every sprint and verbally encouraged throughout to promote a maximal effort. Each sprint was performed in the seated position and initiated with the crank arm of the dominant leg at 45°. Before sprint one, subjects were instructed to accelerate the flywheel to 95 rpm over a 15-s period and assume the ready position 5 s before the commencement of the test. This ensured that each sprint was initiated with the flywheel rotating at ~90 rpm so that subjects could quickly reach 120 rpm. Five seconds prior to the initiation of each sprint, participants were asked to assume the ready position, followed by a verbal 3-2-1 countdown.

3.2.3 Near-infrared Spectroscopy

Participants were instrumented with NIRS probes (Oxymon MKIII, Artinis, the Netherlands) fixed over the distal part of the vastus lateralis muscle belly of their dominant leg, approximately 10-15 cm above the proximal border of the patella and held in place with plastic spacers with an optode distance of 4 cm. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpندن Ltd.) to account for skin and adipose tissue thickness covering the muscle. The skinfold thickness (12.4 ± 6.9 mm) was less than half the distance between the emitter and the detector in each case. Probes were attached using double-sided stick disks, secured with tape, and shielded from light with black elastic bandages. Between sprints, participants were asked to minimise leg movement by remaining seated and relax their dominant leg in the extended position. A modified form of the Beer-Lambert law was used to calculate micromolar changes in tissue [HHb] across time using received optical density from one continuous wavelength of NIR light (763 nm). A differential pathlength factor of 4.95 was used (Billaut et al., 2013; Smith & Billaut, 2010). Data was acquired at 10 Hz and exported to Excel for analysis. These data were expressed as a percentage so that resting baseline represented 0% and maximal [HHb] represented 100% ($\Delta\%[\text{HHb}]$). Maximal [HHb] was obtained with femoral arterial occlusion using a pneumatic tourniquet (inflated to 300-350 mm Hg) around the root of the thigh for 3-5 min until the [HHb] increase reached a plateau. Arterial occlusion was performed after the completion of the repeated-sprint protocol (within 10 min), while the subjects lay on an examination bed with the leg under examination at 90° knee flexion, and foot on the bed. During the trials, markers were placed in the NIRS software at sprint onset to demarcate the 40-s sprint/recovery windows for analysis.

The application of the 10th order zero-lag low-pass Butterworth filter was conducted in the *R* environment (R Core Team, 2016) using the *signal* package (Signal developers, 2013). The filter order was determined based on previous research (Faiss et al., 2013; Sandbakk et al., 2015), and the effects of filter order on the sharpness of filter response. The filters cut-off frequency (f_c) was determined based on a combination of previous research (Faiss et al., 2013), residual analysis (of data from three subjects) of the effects of a range of different normalised f_c on HHb (Figure 3.1), and visual inspection with attention paid to local maxima and minima of filtered data compared to the raw signal (Winter, 2009). Based on these, it was concluded that 0.1 was suitable f_c to be applied to the data for the remaining subjects. After the filter passed through the data, the resulting output was exported to Excel for standardisation to occlusion values and determination of peaks and nadirs.

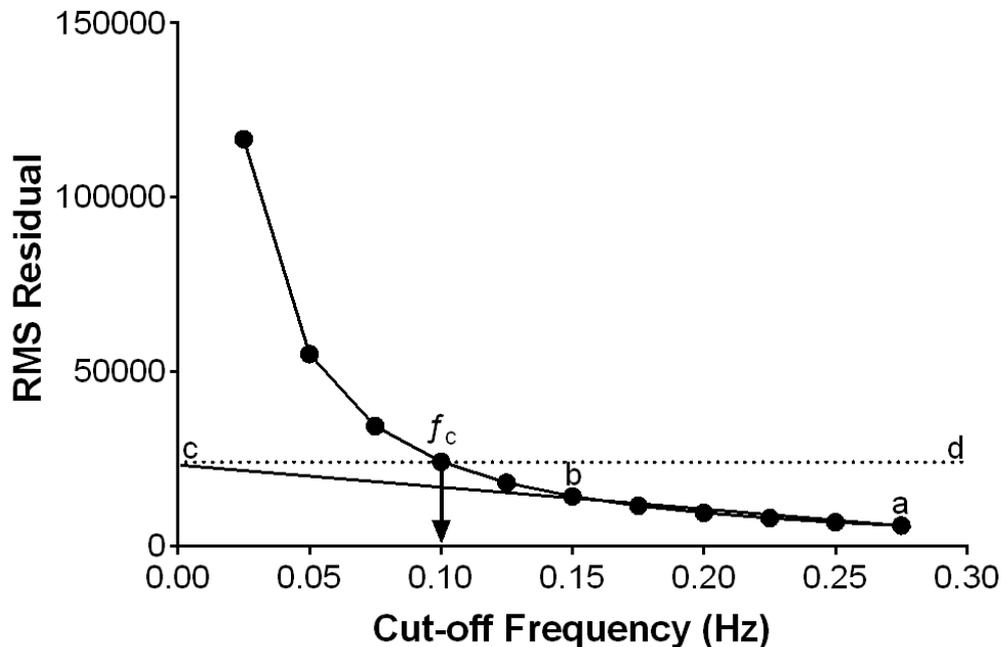


Figure 3.1: A plot of the root-mean-square (RMS) residuals between filtered and unfiltered signals as a function of the filter cut-off frequency from the data of a representative subject. A line of best fit (*ab*) is projected to the Y-axis. At the intercept *c*, the horizontal line *cd* is drawn to intersect with the residuals. The chosen cut-off frequency f_c is at this point of intersection.

3.2.4 Data Analysis

Six methods were used to obtain a single peak and nadir $\% \Delta[\text{HHb}]$ for every sprint and recovery period based on the methods outlined in previous research (Billaut & Buchheit, 2013; Billaut et al., 2013; Buchheit et al., 2009; Faiss et al., 2013; Jones et al., 2015; Ohya et al., 2013; Ohya et al., 2015; Sandbakk et al., 2015; Smith & Billaut, 2010, 2012; Willis et al., 2017).

1. Averages calculated from a predetermined range over the final 2 s of exercise (peak) and recovery (nadir): 2_{PD} .
2. Averages calculated from a predetermined range over the final 5 s of exercise (peak) and recovery (nadir): 5_{PD} .
3. Moving average with a window of 2 s applied to the data, followed by the identification peaks and nadirs within each 40-s exercise-recovery cycle: 2_{MA} .
4. Moving average with a window of 5 s applied to the data, followed by the identification peaks and nadirs within each 40-s exercise-recovery cycle: 5_{MA} .
5. Application of a Butterworth filter smooth the raw NIRS data, followed by the identification of peaks and nadirs from predetermined time points. A single value prior to each phase change (i.e. end of exercise and end of recovery, 0.1 s): BWF_{PD} .
6. The application of a Butterworth filter smooth the data, followed by the identification of a peak and nadir using a rolling approach within each 40-s exercise-recovery cycle (0.1 s): BWF_{MA} .

Tissue reoxygenation (ΔReoxy) was calculated as the difference between the peak and nadir for each analysis method.

3.2.5 Statistical Analysis

Data in text and figures are presented as mean \pm SD. Relative changes (%) are expressed with 95% confidence limits (95% CL). Effects of the Butterworth filter on the NIRS signal was assessed by calculating Pearson's product-moment correlation (r), and standardised residuals of the raw vs filtered data in the *R* environment using the *stats* package (R Core Team, 2016). The correlation between the raw and filtered NIRS signal was assessed by fitting a linear regression model to the pooled subject data. The following criteria were adopted to interpret the magnitude of the correlation between variables: ≥ 0.1 , trivial; $>0.1-0.3$, small; $>0.3-0.5$, moderate; $>0.5-0.7$, large; $>0.7-0.9$, very large; and $>0.9-1.0$, almost perfect (Hopkins, Marshall, Batterham, & Hanin, 2009). To determine the effects of analysis method, practical significance was also assessed by standardised effects and presented with 95% CL (Cohen, 1988). Effect sizes (ES) between $0- <0.2$, $>0.2-0.5$, $>0.5-0.8$, and >0.8 were considered to as trivial, small, moderate and large respectively. Probabilities were also calculated to establish if the chance the true (unknown) differences were lower, similar or higher than the smallest worthwhile change (0.2 multiplied by the between-subject SD, based on Cohen's effect size principle). Quantitative probability of lower, similar, or higher differences were assessed qualitatively as follows: $<1\%$, *almost certainly not*; $>1\%-5\%$, *very unlikely*; $>5\%-25\%$, *unlikely*; $>25\%-75\%$, *possible*; $>75\%-95\%$, *likely*; $>95\%-99\%$, *very likely*; $>99\%$, *almost certainly*. If the probability of having higher/lower values than the smallest worthwhile difference was both $>5\%$, the true difference was assessed as unclear (Batterham & Hopkins, 2006; Hopkins et al., 2009). Data analysis was performed using a modified

statistical Excel spreadsheet (Hopkins, 2006b). To examine the interaction effects between the method for identifying $\Delta\%[\text{HHb}]$ peak, nadir and ΔReoxy (predetermined and moving), and the size of the analysis window (0.1 s, 2 s and 5 s), two-way repeated measure ANOVA's were performed. Post hoc analysis was conducted using the Holm-Šídák method and adjusted for multiple comparisons. A threshold for significance was set at the of $P < 0.05$ level. Analysis was performed GraphPad Prism 6.

3.3 RESULTS

3.3.1 Application of the Butterworth Filter

An example of the raw compared to the filtered data of a representative subject is presented in Figure 3.2. There was an *almost perfect* Pearson's correlation between the raw NIRS data and that after the Butterworth filter (Figure 3.3 A). The mean standardized residual of the raw data compared to the filtered was $-9.71 \times 10^3 \pm 3.80$ (Figure 3.3 B). When rectified, the mean residual was 2.51 ± 2.86 with a relative difference of 2.5% [CL: 1.7, 3.4].

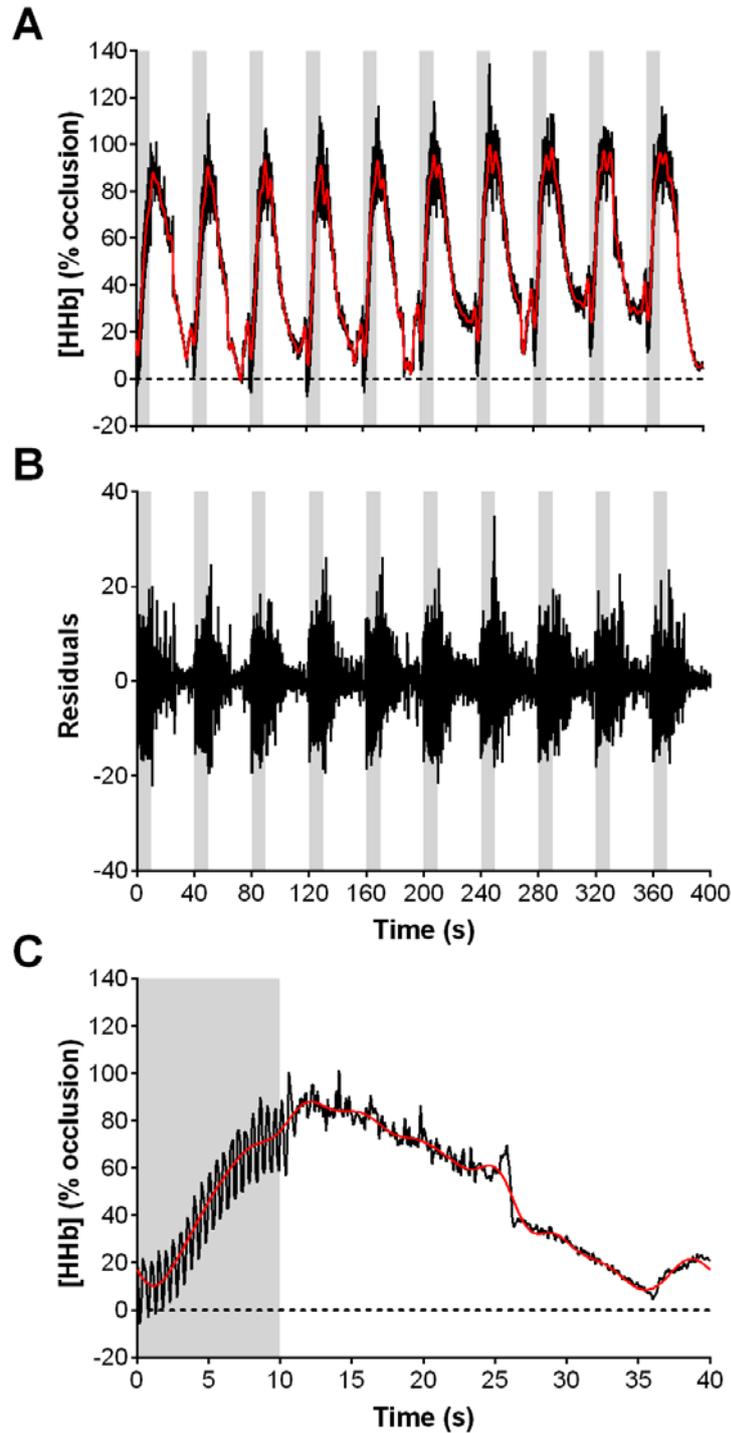


Figure 3.2: Representative data from a single subject illustrating the effects of a 10th order zero-lag low-pass Butterworth filter compared with raw data. (A) Deoxy-haemoglobin concentration changes ([HHb]) over the entire repeated-sprint protocol. (B) Residuals between raw and filtered data shown in panel A. (C) Change of [HHb] during sprint one and subsequent recovery period. Grey shaded areas represent the 10-s sprint periods. The black lines represent raw HHb data. Red lines are the resulting data after the application of the Butterworth filter.

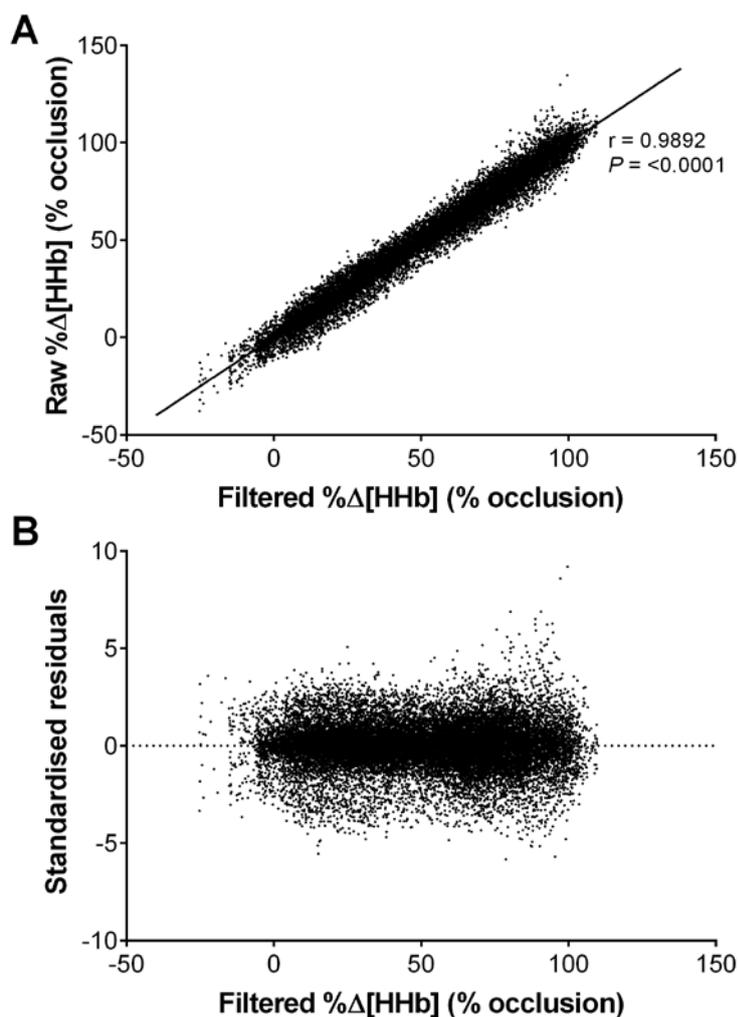


Figure 3.3: Correlation and residual analysis of the pooled subject data comparing the output from the Butterworth filter to the raw deoxyhaemoglobin (HHb) data. (A) Pearson's product-moment correlation (r) with associated P value of a linear regression model fitted to the filtered and raw HHb data. (B) Standardized residuals from the raw vs. filtered data of the pooled subject data set.

3.3.2 Peak Muscle Deoxyhaemoglobin

Mean results of the different analysis methods are presented in Figure 3.4 A. Comparisons of analysis methods are shown in Figure 3.1. There was a significant effect of the method for identify peaks on peak muscle $\Delta\%[\text{HHb}]$ at the $P < 0.05$ level [$F(1, 8) = 5.346, P = 0.0495$]. The size of the analysis window also had a significant effect on peak muscle $[\text{HHb}]$ [$F(2, 16) = 29.68, P < 0.0001$]. There was also a significant interaction effect [$F(2, 16) = 6.445, P = 0.0089$]. Changes in $\Delta\%[\text{HHb}]$ across all sprints were *almost certainly* higher when calculated from 5_{MA} compared to 5_{PD} with a small effect (15.3%

[11.7, 19.1]; $P < 0.0001$). There was also a *likely* small difference between 2_{MA} and 2_{PD} (8.2% [5.4, 11.0]; $P < 0.0001$). An *almost certainly* small effect was also observed when 2_{PD} was compared to 5_{PD} . Differences between 2_{MA} and 5_{MA} were *almost certainly* trivial. Means determined from BWF_{PD} was *almost certainly* higher than 5_{PD} , and *almost certainly* trivial compared to 2_{PD} . When the results from BWF_{MA} were compared to other moving averages, BWF_{MA} was *almost certainly* higher than 5_{MA} (19.2% [15.4, 23.1]; $P < 0.0001$), but there was an *almost certainly* trivial difference when compared to 2_{MA} (0.4% [0.1, 0.7]; $P = 0.4348$). There was a *likely* trivial difference between BWF_{MA} and BWF_{PD} .

Table 3.1: Comparison of smoothing method responses on peak [HHb]. Standardised effects relative differences are presented as change score [95% confidence limits].

Variable	Analysis method comparison	Standardised effect	Relative difference (%)
Peak [HHb] (%)	$2_{PD} - 5_{PD}$	0.30 [0.34, 0.25]	9.8 [8.2, 11.4]
	$2_{MA} - 5_{MA}$	0.09 [0.12, 0.06]	2.9 [3.9, 2.0]
	$5_{MA} - 5_{PD}$	0.47 [0.57, 0.36]	15.3 [11.7, 19.1]
	$2_{MA} - 2_{PD}$	0.25 [0.33, 0.17]	8.2 [5.4, 11.0]
	$BWF_{PD} - 5_{PD}$	0.40 [0.47, 0.32]	12.9 [10.4, 15.5]
	$BWF_{PD} - 2_{PD}$	0.09 [0.13, 0.06]	2.9 [1.7, 4.0]
	$BWF_{MA} - 5_{MA}$	0.56 [0.66, 0.46]	19.2 [15.4, 23.1]
	$BWF_{MA} - 2_{MA}$	0.01 [0.02, 0.00]	0.4 [0.1, 0.7]
	$BWF_{MA} - BWF_{PD}$	0.17 [0.25, 0.10]	5.6 [3.0, 8.1]

Abbreviations are: 2_{PD} , 2 s predetermined average; 5_{PD} , 5 s predetermined average; 2_{MA} , 2 s moving average; 5_{MA} , 5 s moving average; BWF_{PD} , value obtained from a predetermined time point after the data was smoothed with the Butterworth filter; BWF_{MA} , single peak/nadir value within each 40-s sprint/recovery cycle.

3.3.3 Nadir Muscle Deoxyhaemoglobin

Mean results of the different analysis methods are presented in Figure 3.4 A. Comparisons of analysis methods are shown in Figure 3.1. There was a significant effect of the method for identify peaks on peak muscle $\Delta\%[\text{HHb}]$ at the $P < 0.05$ level [$F(1, 8) = 5.346, P = 0.0495$]. The size of the analysis window also had a significant effect on peak muscle $[\text{HHb}]$ [$F(2, 16) = 29.68, P < 0.0001$]. There was also a significant interaction effect [$F(2, 16) = 6.445, P = 0.0089$]. Changes in $\Delta\%[\text{HHb}]$ across all sprints were *almost certainly* higher when calculated from 5_{MA} compared to 5_{PD} with a small effect (15.3% [11.7, 19.1]; $P < 0.0001$). There was also a *likely* small difference between 2_{MA} and 2_{PD} (8.2% [5.4, 11.0]; $P < 0.0001$). An *almost certainly* small effect was also observed when 2_{PD} was compared to 5_{PD} . Differences between 2_{MA} and 5_{MA} were *almost certainly* trivial. Means determined from BWF_{PD} was *almost certainly* higher than 5_{PD} , and *almost certainly* trivial compared to 2_{PD} . When the results from BWF_{MA} were compared to other moving averages, BWF_{MA} was *almost certainly* higher than 5_{MA} (19.2% [15.4, 23.1]; $P < 0.0001$), but there was an *almost certainly* trivial difference when compared to 2_{MA} (0.4% [0.1, 0.7]; $P = 0.4348$). There was a *likely* trivial difference between BWF_{MA} and BWF_{PD} .

Table 3.2: Comparison of smoothing method responses on nadir [HHb]. Standardised effects relative differences are presented as change score [95% confidence limits].

Variable	Analysis method comparison	Standardised effect	Relative difference (%)
Nadir [HHb] (%)	2 _{PD} – 5 _{PD}	-0.20 [0.18, -0.59]	-8.9 [-23.5, 8.6]
	2 _{MA} – 5 _{MA}	-0.38 [-0.13, -0.64]	-20.3 [-31.4, -7.3]
	5 _{MA} – 5 _{PD}	-0.19 [0.04, -0.42]	-8.3 [-17.6, 1.8]
	2 _{MA} – 2 _{PD}	-0.37 [-0.05, -0.70]	-19.7 [-33.8, -2.7]
	BWF _{PD} – 5 _{PD}	-0.47 [-0.10, -0.84]	-31.0 [-48.5, -7.5]
	BWF _{PD} – 2 _{PD}	-0.35 [-0.15, -0.55]	-24.3 [-35.3, -11.5]
	BWF _{MA} – 5 _{MA}	-0.61 [-0.19, -1.02]	-40.4 [-58.1, -15.2]
	BWF _{MA} – 2 _{MA}	-0.34 [-0.10, -0.59]	-25.3 [-39.5, -7.8]
	BWF _{MA} – BWF _{PD}	-0.27 [-0.09, -0.46]	-20.8 [-32.2, -7.4]

Abbreviations are: 2_{PD}, 2 s predetermined average; 5_{PD}, 5 s predetermined average; 2_{MA}, 2 s moving average; 5_{MA}, 5 s moving average; BWF_{PD}, value obtained from a predetermined ed time point after the data was smoothed with the Butterworth filter; BWF_{MA}, single peak/nadir value within each 40-s sprint/recovery cycle.

3.3.4 Muscle Reoxygenation

Mean results of the different analysis methods are presented in Figure 3.4 C. Comparisons of analysis methods are shown in Table 3.3. There was a significant effect of the method for identify peaks and nadirs on ΔReoxy [$F(1, 8) = 40.00, P = 0.0002$]. A significant effect of the window size was also detected [$F(2, 16) = 108.9, P < 0.0001$]. There was also a significant interaction effect [$F(2, 16) = 13.31, P = 0.0004$]. Using the 5_{MA} method to calculate ΔReoxy yielded *almost certainly* higher results than 5_{PD} (28.2% [17.7, 39.7]; $P < 0.0001$). Similarly, 2_{MA} was *very likely* to be greater than 2_{PD} (19.4 [11.2, 28.3]; $P < 0.0001$). Comparing the predetermined mean approaches, 2_{PD} was *very likely* greater than 5_{PD}. Rolling means was *possibly* higher when the 2_{MA} approach was used compared to 5_{MA}. When the Butterworth filter was used, values from predetermined time points were (BWF_{PD}) *almost certainly* greater than 5_{PD} (31.0% [22.4, 40.3]; $P < 0.0001$),

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and *possibly* greater than 2_{PD} (10.9% [8.3, 13.5]; $P < 0.0001$). Means calculated from the BWF_{MA} approach were *almost certainly* higher than 5_{MA} , *almost certainly* trivial compared to 2_{MA} , and *likely* greater than BWF_{PD} (13.2% [7.9, 18.7]; $P < 0.0001$).

Table 3.3: Comparison of smoothing method responses on $\Delta Reoxy$ [HHb]. Standardised effects relative differences are presented as change score [95% confidence limits].

Variable	Analysis method comparison	Standardised effect	Relative difference (%)
$\Delta Reoxy$ [HHb] (%)	$2_{PD} - 5_{PD}$	0.34 [0.48, 0.20]	18.2 [10.1, 26.9]
	$2_{MA} - 5_{MA}$	0.21 [0.26, 0.17]	10.1 [7.9, 12.3]
	$5_{MA} - 5_{PD}$	0.52 [0.71, 0.34]	28.2 [17.7, 39.7]
	$2_{MA} - 2_{PD}$	0.39 [0.55, 0.23]	19.4 [11.2, 28.3]
	$BWF_{PD} - 5_{PD}$	0.56 [0.70, 0.42]	31.0 [22.4, 40.3]
	$BWF_{PD} - 2_{PD}$	0.21 [0.26, 0.16]	10.9 [8.3, 13.5]
	$BWF_{MA} - 5_{MA}$	0.32 [0.39, 0.26]	15.7 [12.5, 18.9]
	$BWF_{MA} - 2_{MA}$	0.11 [0.14, 0.08]	5.1 [3.5, 6.7]
	$BWF_{MA} - BWF_{PD}$	0.28 [0.38, 0.17]	13.2 [7.9, 18.7]

Abbreviations are: 2_{PD} , 2 s predetermined average; 5_{PD} , 5 s predetermined average; 2_{MA} , 2 s moving average; 5_{MA} , 5 s moving average; BWF_{PD} , value obtained from a predetermined time point after the data was smoothed with the Butterworth filter; BWF_{MA} , single peak/nadir value within each 40-s sprint/recovery cycle.

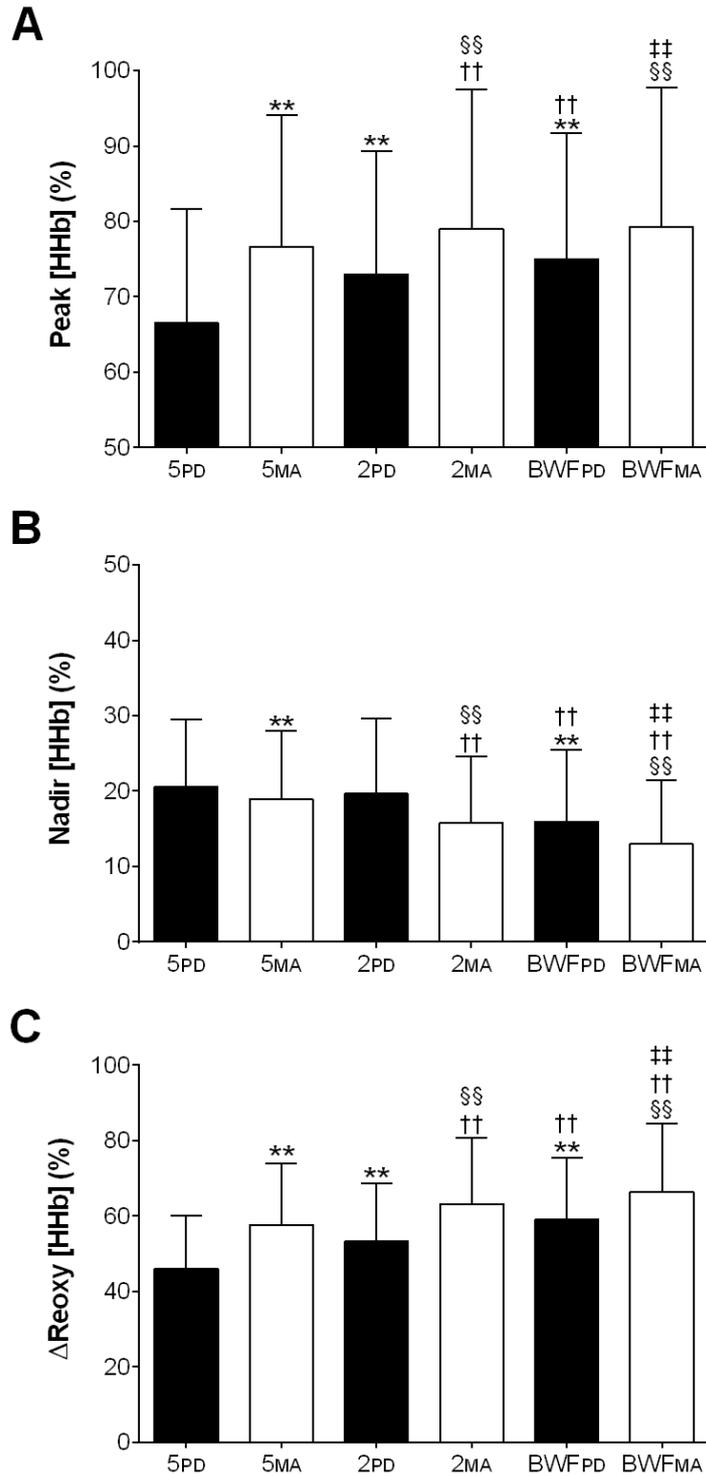


Figure 3.4: Mean and standard deviation of deoxy-haemoglobin concentration changes ([HHb]) over the entire repeated-sprint protocol determined from the different analysis methods. (A) Peak [HHb] averages of each analysis method. (B) Nadir [HHb] averages of each analysis method. (C) Δ Reoxy calculated from peak and nadir [HHb] of the different analysis methods. The number of symbols one and two represent a difference at the $P < 0.05$ and $P < 0.01$ level respectively (Holm-Šídák test). Symbols denote a difference from 5_{PD}, *; 2_{PD}, †; 5_{MA}, §; BWF_{PD}, ‡. No significant difference was found between BWF_{MA} - 2_{MA} ($P = 0.4348$), and 2_{PD} - 5_{PD} ($P = 0.0524$).

3.4 DISCUSSION

Results indicate that by using predefined averaging windows to analyse the NIRS signal within sprint and recovery periods, $\Delta\%[\text{HHb}]$ peaks and nadirs are consistently underestimated compared to a moving average, regardless of the window length. Subsequently, muscle reoxygenation between efforts is underestimated using 5_{PD} and 2_{PD} compared to 5_{MA} and 2_{MA} . However, a drawback of using the arithmetic mean is that each data point contributes equally to the average, which allows outlying data points to bias the result (Dawson & Trapp, 2004). To overcome this, we applied a 10th order zero-lag low-pass Butterworth filter to the NIRS signal, which incorporates a weighted mean from several data points across the signal. Correlation and residual analysis revealed the Butterworth filter attenuated the “noise” yet maintained the integrity of the raw data (Figure 3.3). Since NIRS responses are used as a surrogate for metabolic perturbations, detecting the magnitude of change is critical for assessing the influence of interventions and environmental factors. Thus, it appears that a digital filter combined with a rolling approach for determining peaks and nadirs of the NIRS signal, is the best method for accurate interpretation of oxygenation trends in repeated-sprint exercise.

There were clear differences in peak $\Delta\%[\text{HHb}]$ means between the predefined averaging methods. The difference between 2_{PD} and 5_{PD} can be attributed to the length of the averaging window. At sprint onset, there is a sharp rise in $\Delta\%[\text{HHb}]$ from rest that peaks at sprint cessation or shortly thereafter (Figure 2 and (Buchheit et al., 2009; Faiss et al., 2013; Ohya et al., 2013; Ohya et al., 2015; Racinais et al., 2007)). In our representative data, $\Delta\%[\text{HHb}]$ continued to increase ~40% in the final 5 s of the first sprint which led to a significant 10% reduction in peak $\Delta\%[\text{HHb}]$ for 5_{PD} compared with

2_{PD} (Table 3.1). Consequently, an averaging duration that encapsulates a sharp rise within the window length will underestimate the amplitude of $\Delta\%[\text{HHb}]$ change.

It is clear from Figure 2 that $\Delta\%[\text{HHb}]$ continued to increase after the predefined averaging window. This would tend to underestimate the peak $\Delta\%[\text{HHb}]$ response. To overcome this potential confounding factor, we applied both a 5- and 2-s moving average to the NIRS signal. The 2_{MA} and 5_{MA} yielded 15% and 8% greater peaks compared to 2_{PD} and 5_{PD}, respectively. Since maximal $\Delta\%[\text{HHb}]$ may occur either at, or immediately after sprint cessation, identification of peaks using a moving average will always capture this maximal deoxygenation. Therefore, studies that only employed predetermined averaging windows (Billaut & Buchheit, 2013; Billaut et al., 2013; Buchheit & Ufland, 2011; Sandbakk et al., 2015; Smith & Billaut, 2010, 2012) may have underestimated the true magnitude of $\Delta\%[\text{HHb}]$ change induced by repeated-sprint exercise. To more accurately represent muscle oxygenation changes in response to repeated-sprint exercise, values need to be determined from a moving average approach (Buchheit et al., 2009; Faiss et al., 2013; Jones et al., 2015; Ohya et al., 2013; Ohya et al., 2015; Willis et al., 2017). However, a moving average may not be appropriate when exercise protocols incorporate other muscular activity around repeated-sprint bouts. For example, some authors have used low-intensity exercise during recovery periods between sprints (Buchheit et al., 2009; Ohya et al., 2013; Ohya et al., 2015), and others have used running-based protocols, which impose eccentric loading during the negative acceleration phase post-sprint (Buchheit et al., 2009; Buchheit & Ufland, 2011; Galvin et al., 2013). Another limitation is that when using arithmetic means (i.e., 5_{PD} and 2_{PD}; 5_{MA} and 2_{MA}), equal weight is given to all data points, which can lead to the result being distorted by outliers (Dawson & Trapp,

2004). But in the field of repeated-sprint exercise, an arithmetic mean is commonly employed to smooth perturbations in NIRS data.

To address this methodological limitation, we used a Butterworth filter to smooth the data, and obtained $\Delta\%[\text{HHb}]$ peak from both predetermined time points within each sprint and from a rolling approach. The BWF_{PD} approach yielded 13 and 3% greater peaks than both 5_{PD} and 2_{PD} methods, respectively. Since BWF_{PD} used the last value within each sprint (in the current instance, the 100th value of a 10-s sprint sampled at 10 Hz), this was the highest $\Delta\%[\text{HHb}]$ value achieved within each 10-s sprint period. Similarly, peak $\Delta\%[\text{HHb}]$ determined by BWF_{MA} was 19% greater than 5_{MA} . However, only a trivial difference was found between BWF_{MA} filtering and 2_{MA} . Various studies have applied digital filters to smooth biomechanical and biological data (Robertson & Dowling, 2003; Schlichthärle, 2011; Winter, 2009), yet few authors have used a filter to smooth a NIRS signal during repeated-sprint exercise (Faiss et al., 2013; Sandbakk et al., 2015). When a low-pass filter is used, a f_c is chosen so that lower signal frequencies remain and higher frequencies (noise) are attenuated (Yu et al., 1999). A low-pass Butterworth filter attenuates signal power above a specified f_c , but also included a weighted average across several data points (Elmer & Martin, 2009), which leads to lag in the signal output. This temporal shift can be removed by running the filter a second time in the reverse direction (zero-lag). Repeated-sprint exercise represents a particularly salient challenge to $\Delta\%[\text{HHb}]$ signal due to the rapid changes in duty cycle. Our results suggest that an arithmetic mean underrepresents $[\text{HHb}]$ peak in most cases, and that both BWF_{PD} and BWF_{MA} better reflect peak $[\text{HHb}]$ after sprint exercise.

Differences in nadir $\Delta\%[\text{HHb}]$ were less clear between the averaging methods. Since means were calculated on the flatter portion of the NIRS signal during the late stage

of recovery, differences between averaging methods were minimal. There was an 8% and 20% lower $\Delta\%[\text{HHb}]$ nadir from both a 5_{MA} and 2_{MA} compared to 5_{PD} and 2_{PD} respectively (Table 2). While the difference between nadir 5_{MA} and 5_{PD} did not reach the typically adopted threshold for statistical significance of $P < 0.05$ (“trivial” standardised effect), we reported a small standardised effect (20% relative difference, $P < 0.0001$) between the 2_{MA} and 2_{PD} analysis methods. The 5 s averaging windows may not have the sensitivity to detect subtle differences between the nadir determined from predetermined and moving averages. It appears that a short moving average is better suited at detecting changes in nadir $\Delta\%[\text{HHb}]$. Since the restoration of NIRS variable towards baseline has become a surrogate for metabolic recovery between sprints (Billaut & Buchheit, 2013; Bowtell et al., 2014; Buchheit et al., 2009; Buchheit & Ufland, 2011; Ohya et al., 2013), an accurate depiction of this variable is necessary to assess metabolic perturbations leading to greater peripheral fatigue. Additionally, the detection of the magnitude of change has important implications for assessing the potency of training programs and environmental factors. Unless the nadir of the signal is obtained from a rolling approach, the magnitude of $[\text{HHb}]$ recovery will be underestimated.

The ΔReoxy is determined from both peak and nadir $\Delta\%[\text{HHb}]$ responses, hence, the analysis method that yields the greatest peaks and nadirs will have the greatest ΔReoxy . Consequently, we observed clear and substantial differences between all ΔReoxy analysis methods apart from BWF_{MA} vs 2_{MA} , with a range of relative differences from 5% to 31%. Studies reporting ΔReoxy from predetermined windows (Billaut & Buchheit, 2013; Buchheit & Ufland, 2011) have likely underestimated reoxygenation capacity. The most accurate representation of ΔReoxy would come from works that have chosen a

moving average approach for the determination of peaks and nadirs (Faiss et al., 2013; Ohya et al., 2015).

Irrespective of the method, a narrow window for analysis (2 s) allows reporting greater magnitudes of response than a longer window (5 s). However, care should be taken when using a narrow analysis window. It contains less data from which the average is calculated, which increases the risk that outliers bias the calculated mean (Dawson & Trapp, 2004). Such a methodological pitfall is displayed in the sprint phase of Figure 2 A, where a small number of data points are far above the characteristics of the surrounding data. A narrow analysis window would place greater weight on these individual data points in the mean compared to a larger window (Dawson & Trapp, 2004). Currently, there is no consistency on the length of the analysis window. In some cases, a window as large as 5 s (Billaut & Buchheit, 2013; Buchheit et al., 2009; Smith & Billaut, 2010, 2012) and as small as 1 s (Ohya et al., 2013; Ohya et al., 2015) has been used. When a Butterworth filter was employed, both peak and nadir values (Faiss et al., 2013) and a 2-s average (Sandbakk et al., 2015) have been used. However, by using a Butterworth filter, a single data point from the resulting output can be used with assurance that it reflects the characteristics of the surrounding data. Though our choice to use a Butterworth filter was based on previous research (Faiss et al., 2013; Sandbakk et al., 2015; Willis et al., 2017), other smoothing/filtering techniques which eliminate outliers may also yield similar results. Readers should also be aware that these data and analytical methods were collected during isokinetic sprints where cadence was constrained to 120 rpm, and, although muscle oxygenation patterns appear similar, one may exert caution when analysing NIRS signal in non-isokinetic conditions where cadence is influenced by gear ratio and neuromuscular fatigue.

3.5 CONCLUSION

NIRS-derived variables in sprint exercise are subject to rapid and large perturbations. Furthermore, during cyclic movements such as running or cycling, there are relatively large oscillations in the [HHb] signal response due to mechanical effects of muscle contraction on local blood flow. This requires appropriate smoothing of the signal to avoid either over or underestimation of peaks and nadirs. Sprint and recovery means calculated over a 1-5 s window in predetermined time frames are often reported (Billaut & Buchheit, 2013; Billaut et al., 2013; Buchheit et al., 2009; Buchheit et al., 2012; Buchheit & Ufland, 2011; Cheng et al., 2013; Dupont et al., 2004; Galvin et al., 2013; Racinais et al., 2007; Sandbakk et al., 2015; Smith & Billaut, 2010, 2012), however, there remains little consistency between studies examining muscle oxygenation during repeated-sprint exercise. The current results reveal that moving averages derive greater changes in muscle oxygenation than means calculated from predetermined time points. Hence, this method is less prone to underestimation of the maximum rate of de- and reoxygenation. However, since these calculations are susceptible to signal bias from outliers, especially when a shorter averaging window is used (Dawson & Trapp, 2004), we recommend using a digital filter (Faiss et al., 2013; Sandbakk et al., 2015; Willis et al., 2017) or other smoothing/filtering techniques prior to analysis. We also suggest that future studies should avoid predetermined analysis windows, and focus on the determination of a single value for peaks and nadirs from a rolling approach.

**CHAPTER FOUR: EFFECTS OF INSPIRATORY
LOADING ON LOCOMOTOR AND RESPIRATORY
MUSCLE OXYGENATION TRENDS**

4.1 INTRODUCTION

Repeated-sprint exercise is characterized by brief periods of “maximal” exertion, interspersed with incomplete recovery periods. Over the course of a repeated-sprint series, there is a progressive reduction in both peak and mean power output, with a plateau in the latter sprints (Gaitanos et al., 1993; Racinais et al., 2007). Phosphocreatine (PCr) hydrolysis and anaerobic glycolysis are heavily relied on as a rapid source of adenosine triphosphate (ATP) replenishment in sprint exercise (Gaitanos et al., 1993; Parolin et al., 1999). Additionally, the aerobic system plays a significant role in maintaining repeated-sprint performance. Resynthesis of PCr and removal of inorganic phosphate is exclusively through oxidative processes (Harris et al., 1976), and sensitive to oxygen (O₂) availability (Sahlin et al., 1979). Therefore, the inability to maintain power production is due to ATP/PCr depletion, accumulation of metabolic by-products such as inorganic phosphate, and a decrease in ATP turnover rate with an increase in oxidative phosphorylation (Bergström & Hultman, 1991; Gaitanos et al., 1993; Hogan et al., 1999). Maintaining O₂ delivery to the locomotor muscles during repeated-sprint exercise is therefore an important mediating factor of performance.

Near-infrared spectroscopy offers the possibility to explore O₂ balance (delivery vs. consumption) in skeletal muscle during sprint activity in real time. Deoxyhaemoglobin ([HHb]) and oxyhemoglobin ([O₂Hb]) rise and fall respectively, proportional to an increase in metabolic activity in the underlying tissue. When NIRS has been used in repeated-sprint exercise, relative changes in [HHb] are primarily examined, because it is considered to be independent of blood volume (De Blasi et al., 1993; Grassi et al., 2003); and assumed to reflect venous [HHb] to provide an estimate of muscular oxygenation (DeLorey et al., 2003; Grassi et al., 2003). At sprint onset, there is a rapid increase of

vastus lateralis [HHb] which trends back towards baseline during the between sprint rest periods (Buchheit et al., 2009; Racinais et al., 2007; Smith & Billaut, 2010). Other than muscle deoxygenation during exercise, reoxygenation between sprints is often examined as a primary measure to describe locomotor muscle O₂ availability and therefore quality of metabolic recovery (Billaut & Buchheit, 2013). Improving this capacity can have positive effects for repeated-sprint performance (Buchheit & Ufland, 2011; Galvin et al., 2013; Jones et al., 2015), whereas a decreased reoxygenation is associated with performance impairments (Billaut & Buchheit, 2013; Buchheit et al., 2009; Dupont et al., 2004). However, there has been no examination whether the O₂ cost associated with hyperpnoea influences locomotor muscle oxygenation trends in repeated-sprint exercise.

The respiratory muscles require ~10-15% of total pulmonary oxygen uptake ($\dot{V}O_2$) during high-intensity exercise, and a considerable portion of cardiac output in order to maintain adequate O₂ delivery to these muscles (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992). An elevated work of breathing during high-intensity exercise promotes competition between locomotor and respiratory muscles for available cardiac output (Dempsey et al., 2006). In fact, the addition of an inspiratory load to artificially increase the work of breathing during maximal exercise (>95% $\dot{V}O_{2peak}$) clearly limits endurance capacity via decreased limb perfusion and O₂ delivery, mediated by sympathetically-activated vasoconstriction in the locomotor muscles (Harms et al., 1997; Harms et al., 1998). However, at moderate intensities (50-75% $\dot{V}O_{2peak}$) there is no change in vascular resistance or blood flow to the locomotor muscles (Wetter et al., 1999), suggesting that exercise intensity is an important mediator of locomotor vasoconstriction when the work of breathing is high.

Exercising at $\sim 94\% \dot{V}O_{2\text{peak}}$, there is no further rise in vastus lateralis [HHb] with the addition of a moderate respiratory load (both inspiratory and expiratory) (Kowalchuk et al., 2002). It was only when partial arterial occlusion was applied that an increase in [HHb] compensated for reduced blood flow (Harms et al., 1997; Kowalchuk et al., 2002). This implies that there was no meaningful reduction of blood flow away from locomotor muscles, and that muscle O_2 extraction from arterial blood remained consistent despite an elevated work of breathing. But when the work of breathing was elevated further with heavy inspiratory load, an increase in vastus lateralis [HHb] during constant load exercise occurred (Turner et al., 2013). It appears that along with exercise intensity, the external load imposed on the respiratory muscles plays a role in blood flow redistribution (Turner et al., 2013). It is currently unclear if an elevated work of breathing has an influence on reoxygenation capacity in repeated-sprint exercise. Therefore, we aimed to determine the potentially deleterious impact of an elevated work of breathing on $\dot{V}O_2$, tissue oxygenation trends and mechanical output during repeated-sprint exercise.

4.2 METHODS

4.2.1 Subjects

Ten males from a variety of athletic backgrounds were recruited for this study (team sports, road cycling, combat sports, CrossFit). These subjects were chosen because they were accustomed to producing “all-out” bouts of exercise. Nine of the 10 subjects who participated in this research, also participated in the research of the previous chapter ([Chapter Three](#)). Subjects self-reported to be healthy and with no known neurological, cardiovascular or respiratory diseases. After being fully informed of the requirements, benefits, and risks associated with participation, each subject gave written informed

consent. Ethical approval for the study was obtained from the institutional Human Research Ethics Committee and the study conformed to the declaration of Helsinki. Subject characteristics are presented in Table 4.1.

Table 4.1: Subject Characteristics (n = 10).

Measure	Value
Age (years)	25.5 ± 3.6
Height (cm)	184.00 ± 7.69
Body mass (kg)	81.45 ± 8.29
$\dot{V}O_{2\text{peak}}$ (L·min ⁻¹)	4.40 ± 0.36
$\dot{V}O_{2\text{peak}}$ (mL·min ⁻¹ ·kg ⁻¹)	54.4 ± 5.9
$\dot{V}_{E\text{peak}}$ (L·min ⁻¹)	173.6 ± 26.9

Values are mean ± SD. Abbreviations are: $\dot{V}O_{2\text{peak}}$, peak pulmonary oxygen uptake; $\dot{V}_{E\text{peak}}$, pulmonary ventilation at $\dot{V}O_{2\text{peak}}$.

4.2.2 Experimental Design

Subjects visited the laboratory on seven occasions. During visit one, subjects completed a pulmonary function (Ultima CPX, MGC Diagnostics, Minnesota, USA) and respiratory muscle strength tests (MicroRPM, Micro Medical, Hoechberg, Germany) (see procedures below). Once completed, subjects then performed a familiarization trial of a ramp exercise test. Visit two had subjects complete a ramp exercise test to exhaustion for the determination of $\dot{V}O_{2\text{peak}}$. During visit three, subjects were familiarized with the repeated-sprint protocol. This involved completing the same exercise protocol that was used in later experimental sessions, but the inspiratory load was applied for the first two sprints only. In visits five and six, subjects completed the repeated-sprint exercise in a randomized, cross-over design with no restriction to their breathing (CTRL) and with inspiratory loading (INSP). The seventh trial (MATCH) consisted of ten work-matched intervals to that of the INSP trial. All exercise testing was performed on an electronically-

braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) and expired gases collected on a breath-by-breath basis (COSMED Quark CPET; Cosmed, Rome, Italy). Trials were conducted at the same time of day and separated by 3-7 days. Subjects were asked to refrain from exercise and strenuous activity for 48 h preceding all testing sessions.

Table 4.2: Pulmonary function and respiratory muscle strength

Measure	Raw	%Predicted
FVC (L)	6.0 ± 0.7	103 ± 8.8
FEV1 (L)	4.9 ± 0.5	99.7 ± 8.1
FVC/FEV1	79.1 ± 6.6	95.5 ± 7.5
MVV (L·min ⁻¹)	212.9 ± 24.7	110.9 ± 10.2
MIP (cmH ₂ O)	-146.6 ± 19.2	
MEP (cmH ₂ O)	151.8 ± 35.8	

Values are reported as mean ± SD. Abbreviations are: FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; MVV, maximum voluntary ventilation; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure.

4.2.3 Maximal Ramp Exercise

A maximal ramp cycling ergometer test was performed to determine $\dot{V}O_{2\text{peak}}$. The exercise test was initiated at a work rate of 0 W for 3 min. This was followed by an increase in work rate of 1 w every 2 s (30 W·min⁻¹) until volitional exhaustion or until cadence fell 10 rpm below self-selected rate (Burnley, Doust, & Vanhatalo, 2006). Peak $\dot{V}O_2$ was determined as the highest 30 s average prior to exercise termination. The corresponding \dot{V}_E at $\dot{V}O_{2\text{peak}}$ was deemed to be $\dot{V}_{E\text{peak}}$.

4.2.4 Repeated-sprint Exercise

After arriving at the laboratory, subjects were fitted with NIRS probes and a heart rate monitor. Testing was performed with the cycle ergometer set to “isokinetic” mode (120 rpm). In this mode, a variable resistance is applied to the flywheel

proportional to the torque produced by the subjects to constrain their pedalling rate. Below 120 rpm, no resistance is applied to the flywheel. This mode was chosen to avoid cadence-induced changes in mechanical power production. Cadence was fixed for every sprint so that exercise-induced changes in mechanical power and physiological responses were not influenced by cadence (Gotshall et al., 1996; Tomas, Ross, & Martin, 2010). The handlebars and seat were individually adjusted to each subjects' characteristic and feet secured using toe cages and retention straps fitted to the ergometer. Crank arm length was standardized to 175 mm. Visual feedback of power output was not available to the subjects during any sprint. The cycle ergometer software provides power and cadence at 4 Hz. After a 7-min warm-up consisting of 5 min of unloaded cycling at 60-70 rpm and two 4 s sprints (separated by 1 min), subjects rested for another 2.5 min before the repeated-sprint protocol was initiated. The repeated-sprint protocol was ten 10 s sprints separated by 30 s passive rest (5.5 min). Subjects were instructed to give an "all-out" effort for every sprint and verbally encouraged throughout to promote a maximal effort. Each sprint was performed in the seated position and initiated with the crank arm of the dominant leg at 45°. Before sprint one, subjects were instructed to accelerate the flywheel to 95 rpm over a 15-s period and assume the ready position 5 s before the commencement of the test. This ensured that each sprint was initiated with the flywheel rotating at ~90 rpm so that subjects could quickly reach 120 rpm. To minimise the chance of a protective pacing strategy, the first 10 s sprint of the repeated-sprint series was examined to ensure that peak power output exceeded that of the two preceding 4 s sprints. In every instance, peak power output was highest during sprint one of the repeated-sprint series. Data was exported into Excel for the calculation of mechanical work completed and power production for individual sprints, and over the entire protocol. After sprint one, five and ten, subjects were asked to rate on a 6-20 scale the

difficulty of exercise (RPE_{Exercise}) and difficulty of breathing (RPE_{Breath}). In every case, subjects were asked the questions “how difficult is exercise?” and “how difficult is breathing?”.

Inspiratory loading was achieved by placing a plastic disk with a 10-mm opening over the inspiratory side of a two-way non-rebreathing valve (Hans Rudolph inc., Kansas, United States of America) attached to the distal end of the breath-by-breath gas sampling line and turbine. The inspiratory load was applied after warm-up, 1 min before the commencement of the repeated-sprint protocol. Work matched exercise was conducted by performing ten 10 s bouts of exercise separated by 30 s of passive rest. This was achieved by reproducing the mean power output of the corresponding sprint repetition from the INSP trial. The cycle ergometers isokinetic function cannot be used when controlling for power output, therefore, subjects were asked to maintain cadence at 120 RPM during each interval.

4.2.5 Metabolic and Ventilatory Measurements

Subjects wore a silicone facemask to which the breath-by-breath gas sampling line and turbine were attached. The analyser was calibrated before each test against known gas concentrations and the turbine volume transducer was calibrated using a 3 L syringe (Cosmed, Rome, Italy). Data was then exported into Excel so that $\dot{V}O_2$ and \dot{V}_E could be inspected for errant data points. Editing data was performed to remove the occasional errant breaths caused by for example swallowing or coughing which were considered not be reflective the metabolic responses to exercise. These errant data points were removed by the same researcher in every case before values greater than 4 standard deviations from the local mean were removed (Lamarra, Whipp, Ward, & Wasserman, 1987; Rossiter et al., 2000). A 5-breath rolling average was then applied for the calculation of

peak and nadir for both $\dot{V}O_2$ and \dot{V}_E for every 40-s sprint/recovery period to give a single value for each sprint and recovery phase. Inspiratory volume (IV), breathing frequency (f_b), end-tidal O_2 partial pressure ($P_{ET}O_2$), end-tidal CO_2 partial pressure ($P_{ET}CO_2$) was averaged to give one value for each 40-s period. Because the facemask was removed immediately after the tenth sprint, only maximum values were calculated over the first 10 s. Mouth pressure (P_m) was recorded continuously at 50 Hz with a pressure transducer (Honeywell, New Jersey, United States of America) attached to the saliva port of the non-rebreathing valve via Tygon tubing. Representative data from one subject of the effects of inspiratory muscle loading on P_m is displayed in Figure 4.1. Mean inspiratory and expiratory P_m was then calculated as an index of respiratory muscle work as well as mean peak inspiratory P_m . For statistical analysis, inspiratory P_m was converted to positive values and presented in the results as such. Heart rate (HR) and arterial oxygen saturation (estimated by fingertip pulse oximetry; SpO_2) was sampled on a breath-by-breath basis integrated into the COSMED system.

4.2.6 Near-infrared Spectroscopy

Subjects were instrumented with two NIRS probes to assess muscle oxygenation (Oxymon MKIII, Artinis, the Netherland). The first probe was fixed over the distal part of the vastus lateralis muscle belly approximately 15 cm above the proximal border of the patella of the dominate leg. The second probe was fixed over the left 6th intercostal space at the anterior axillary line of the serratus anterior to assess changes in the accessory respiratory muscles. Probes were held in place with black plastic spacers secured to the skin using double-sided stick disks and shielded from light using black self-adhesive elastic bandage. Optode spacing was set to 4.5 cm and 3.5 cm for vastus lateralis and respiratory muscles, respectively. Skinfold thickness was measured between the emitter

and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose tissue thickness covering the muscle. The skinfold thickness for vastus lateralis (1.19 ± 0.69 cm) and respiratory muscles (1.12 ± 0.44 cm) was less than half the distance between the emitter and the detector in every case. A modified form of the Beer-Lambert law was used to calculate micromolar changes in tissue [HHb] and [O₂Hb] across time using the received optical density from continuous wavelengths of NIR light. A differential pathlength factor of 4.95 was used (Smith & Billaut, 2010; Subudhi et al., 2007). Tissue saturation index was used as an index of tissue oxygenation ($TSI = [O_2Hb] \div ([O_2Hb] + [HHb])$), expressed in %), which reflects the dynamic balance between O₂ supply and O₂ consumption in the tissue microcirculation and is independent of near-infrared photon pathlength in tissue. Tissue saturation index was determined for both the vastus lateralis (TSI_{VL}) and respiratory muscles (TSI_{RM}). We also chose to focus our analysis on $\Delta[HHb]$ to allow comparisons to previous research because $\Delta[HHb]$ is independent of changes in total haemoglobin (De Blasi et al., 1993) and taken to reflect venous [HHb] which provides an estimate of muscular oxygen extraction (DeLorey et al., 2003; Grassi et al., 2003), and because $\Delta[O_2Hb]$ is influenced by rapid blood volume and perfusion variations caused by forceful muscle contractions (De Blasi et al., 1993; Takaishi et al., 2002).

Data were acquired at 10 Hz. A 10th order zero-lag low-pass Butterworth filter was applied to the data to remove artefacts and smooth pedalling induced fluctuations; the resulting output was used for analysis (Rodriguez, Townsend, Aughey, & Billaut, 2018). The application of the filter was conducted in the *R* environment (R Core Team, 2016) using the *signal* package (Signal developers, 2013). Values were then normalized to femoral artery occlusion so that 0% represented a 5-s average immediately prior the occlusion and 100% represented the maximum 5 s average. To obtain one value per

sprint and recovery for vastus lateralis [HHb] (HHb_{VL}) and TSI_{VL}, peaks and nadirs were identified for each period using a rolling approach. Time to peak HHb_{VL} (TTP_{HHb}) was also calculated as the time from sprint onset to peak HHb. Reoxygenation capacity (Δ Reoxy, %) and reoxygenation rate (Reoxy rate, %·s⁻¹) were also calculated as the change from sprint to recovery. Baseline for the respiratory muscles was established before warm-up while seated quietly on the cycle. Exercise values are expressed as percent change from baseline. Because there were no clear peaks and nadirs in the respiratory muscle [HHb] (HHb_{RM}) and TSI_{RM} signals, an average was calculated for of each 40-s sprint/recovery period.

4.2.7 Statistical Analysis

Data in text and figures are presented as mean \pm standard deviation. A custom made spreadsheet was used to analyse the effects of INSP and MATCH on laboratory measurements (Hopkins, 2006b). All measures, other than $\dot{V}O_2$, SpO₂, RPE and NIRS responses (except for TTP_{HHb}), were log-transformed before analysis then back-transformed to express the changes in percent units and standardized effects. Relative changes (%) and standardized effects are expressed with 90% confidence limits (90% CL). Practical significance was assessed by calculating Cohen's d effect size (ES) (Cohen, 1988). Standardized effect sizes of <0.2, 0.2-0.5, 0.5-0.8, >0.8 were considered to as trivial, small, moderate and large respectively and presented with 90% CL. Probabilities were also calculated to establish if the chance the true (unknown) differences were lower, similar or higher than the smallest worthwhile change (ES = 0.2). Effects were not considered meaningful if there was <75% probability of being substantially positive/negative relative to the smallest worthwhile change. If the chance of having higher/lower values than the smallest worthwhile difference was both >5%, the true

difference was assessed as *unclear*. For clear effects, the likelihood that the true effect was substantial were assessed qualitatively as follows: *likely* (75 to <95%), *very likely* (95-99.5%), *almost certainly* (>99%) (Batterham & Hopkins, 2006; Hopkins et al., 2009).

4.3 RESULTS

4.3.1 Mouth Pressure

Mouth pressure responses to exercise and inspiratory muscle loading are presented in Table 4.3, and representative data from a single subject are presented in Figure 4.1. Mean inspiratory P_m was greater during INSP compared to CTRL with a large effect (relative difference = 629.8%, $\pm 61.8\%$; standardised effect size = 12.75, ± 0.58). Additionally, peak inspiratory P_m was greater during Insp than CTRL with a large effect (702.4%, $\pm 70.9\%$; ES = 13.00, ± 0.55). But there was a trivial difference in expiratory P_m (-0.3%, $\pm 5.6\%$; ES = -0.03, CL ± 0.43).

Mean inspiratory P_m was lower during MATCH compared to INSP with a large effect (-91.2%, $\pm 1.0\%$; ES = -10.27, ± 0.45). Similarly, peak inspiratory P_m was lower during MATCH than INSP with a large effect (-92.4%, $\pm 0.9\%$; ES = -10.75, ± 0.50). A large effect was also present for expiratory P_m with MATCH being lower than Insp (-40.0%, $\pm 5.0\%$; ES = -4.21, ± 0.69).

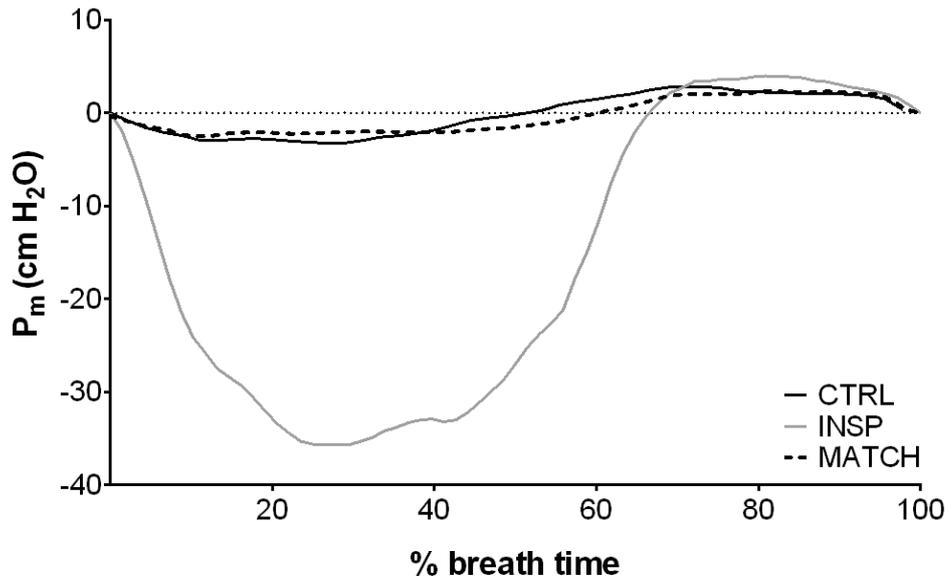


Figure 4.1: Representative data of mouth pressure during exercise. The traces represent mouth pressure (P_m) during a single breath in the Inspiratory Loading (INSP), Control (CTRL), and Work Matched (MATCH) exercise conditions.

4.3.2 Mechanical Measurements

Total work completed per sprint for each condition is presented in Figure 4.2. There was no meaningful effect of INSP on total work completed over the entire repeated-sprint protocol (56.62 ± 7.02 kJ) compared to CTRL (57.87 ± 8.02 kJ) (-2.7% , $\pm 6.4\%$; ES = -0.17 , ± 0.42). Similarly, total work developed in MATCH (55.92 ± 6.98 kJ) and INSP (-0.6% , $\pm 0.1\%$; ES = -0.04 ± 0.01) were not different. There was no meaningful effect of INSP on PPO (1097 ± 148 W) compared to CTRL (1158 ± 172 W) (-5.1% , ± 6.1 ; ES -0.30 , ± 0.35). Whereas an *almost certainly* large effect existed between MATCH (773 ± 122 W) and INSP (-29.7% , $\pm 2.3\%$; ES = -2.21 ± 0.20).

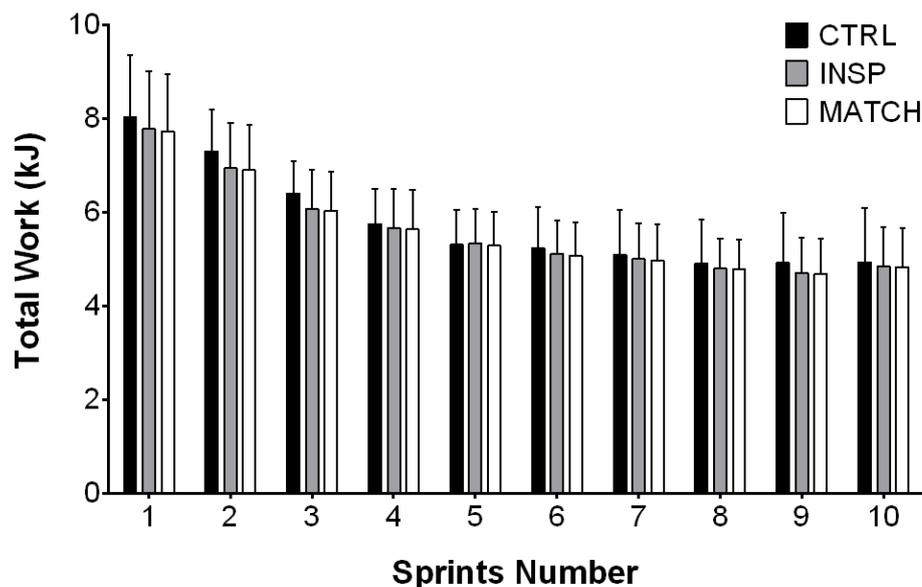


Figure 4.2: Total mechanical work per sprint performed during the sprints. The bars represent data from control (CTRL), Inspiratory Loading (INSP), and Work Match (MATCH) exercise conditions. Values are presented as mean \pm SD.

4.3.3 Physiological Responses

Responses to exercise are presented in Table 4.3 and Figure 4.3. Over the entire protocol, $\dot{V}O_2$ was greater during both sprint (4.7%, $\pm 2.7\%$; ES = 0.64, ± 0.37) and recovery (4.2%, ± 3.1 ; ES = 0.74, ± 0.55) for INSP compared to CTRL. Additionally, $\dot{V}O_2$ was lower during MATCH compared to INSP during both sprint (-21.7%, $\pm 5.0\%$; ES = -4.59, ± 1.06) and recovery (16.3%, $\pm 2.9\%$; ES = -3.30, ± 0.58). Likewise, \dot{V}_E during INSP was greater than CTRL both during sprint (-19.6%, $\pm 3.5\%$; ES = -1.13, ± 0.22) and recovery (-11.5%, ± 5.6 ; ES = -0.80, ± 0.41) compared to INSP. Throughout MATCH, \dot{V}_E was lower during both sprint (-35.8%, $\pm 9.5\%$; ES = -2.92, ± 0.98) and recovery (-33.2%, $\pm 6.2\%$; ES = -2.81, ± 0.65). There was no meaningful difference of IV between INSP and CTRL (2.8%, $\pm 4.8\%$; ES = 0.16, ± 0.27). On the other hand, IV was *almost certainly* lower during MATCH compared to INSP (-15.6%, $\pm 5.1\%$; ES = -0.91, ± 0.32). There was an *almost certainly* large effect of INSP on R_f compared to CTRL (-21.2%, $\pm 4.7\%$; ES = -1.41, ± 0.35). Additionally, f_b was *very likely* lower during MATCH compared to INSP (-20.8%, $\pm 9.6\%$; ES = -1.53, ± 0.79).

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Muscle Oxygenation Trends*

During Insp, $P_{ET}O_2$ was lower than CTRL (-3.7%, $\pm 2.3\%$; ES = -1.71, ± 0.65), and lower during MATCH compared to CTRL (-7.4%, ± 2.3 ; ES = -2.81, ± 0.91). Conversely, $P_{ET}CO_2$ was higher during INSP compared to CTRL (9.1%, $\pm 4.0\%$; ES = 1.22, ± 0.51), and higher during MATCH compared to INSP (7.7%, ± 4.2 ; ES = 1.03, ± 0.54). There were *unclear* differences for S_pO_2 between both INSP and CTRL (-0.1%, ± 0.5 ; ES = -0.10, ± 0.43), and, MATCH and INSP (0.2%, $\pm 0.4\%$; ES = 0.16, ± 0.34).

Differences for HR were *unclear* between INSP and CTRL (1.0%, $\pm 4.4\%$; ES = 0.11, ± 0.50). However, there was a clear *almost certainly* large effect between MATCH and INSP (-15.6%, ± 3.3 ; ES = -2.66, ± 0.61).

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Table 4.3: Physiological responses to the repeated-sprint exercise. The columns include data from Control (CTRL), Inspiratory Loading (INSP), and Work Match (MATCH) exercise conditions. Data was averaged over the entire 5.5 min repeated-sprint protocol.

Variable	CTRL	INSP	MATCH
Inspiratory P _m (cmH ₂ O)	2.3 ± 0.2	17.1 ± 2.8***	1.5 ± 0.3###
Peak inspiratory P _m (cmH ₂ O)	3.5 ± 0.3	28.1 ± 4.8***	2.1 ± 0.4###
Expiratory P _m (cmH ₂ O)	2.0 ± 0.2	2.0 ± 0.1	1.2 ± 0.2###
Sprint $\dot{V}O_2$ (% $\dot{V}O_{2peak}$)	90.7 ± 6.72	95.4 ± 4.32 **	73.7 ± 6.1 ###
Recovery $\dot{V}O_2$ (% $\dot{V}O_{2peak}$)	70.7 ± 5.2	74.9 ± 4.5 *	58.6 ± 4.3 ###
Sprint \dot{V}_E (L·min ⁻¹)	161.5 ± 27.5	129.1 ± 16.8 ***	83.4 ± 15.2 ###
Recovery \dot{V}_E (L·min ⁻¹)	101.4 ± 13.9	89.7 ± 11.4 **	60.2 ± 10.0 ###
IV (L)	3.0 ± 0.5	3.1 ± 0.5	2.6 ± 0.4 ###
f _b (b·min ⁻¹)	48.1 ± 7.8	37.8 ± 5.0 ***	30.2 ± 6.0 ##
P _{ET} O ₂ (mmHg)	117.7 ± 2.3	113.4 ± 2.8 ***	105 ± 5.1 ###
P _{ET} CO ₂ (mmHg)	35.5 ± 2.3	38.6 ± 2.6 ***	41.6 ± 3.2 ##
S _p O ₂ (%)	97 ± 1	97 ± 1	97 ± 1
HR (b·min ⁻¹)	153 ± 12	154 ± 9	131 ± 12 ###
RPE _{Exercise} (AU)			
Sprint 1	15 ± 3	14 ± 2	12 ± 2 ###
Sprint 5	17 ± 2	17 ± 2	13 ± 2 ###
Sprint 10	18 ± 2	18 ± 2	13 ± 2 ###
RPE _{Breath} (AU)			
Sprint 1	12 ± 2	15 ± 2 ***	11 ± 2 ###
Sprint 5	15 ± 1	18 ± 2 ***	12 ± 2 ###
Sprint 10	16 ± 2	19 ± 1 ***	12 ± 2 ###

Values are reported as mean ± SD. Abbreviations are: P_m, mouth pressure; $\dot{V}O_2$, pulmonary oxygen uptake; \dot{V}_E , pulmonary ventilation; IV, inspiratory volume; f_b, respiratory frequency; P_{ET}O₂, partial pressure of end-tidal oxygen; P_{ET}CO₂, partial pressure of end-tidal carbon dioxide; S_pO₂, arterial oxygen saturation estimated by pulse oximetry; HR, heart rate; RPE_{Exercise}, rating of perceived exertion for exercise; RPE_{Breath}, rating of perceived exertion for breathing. The symbols represent comparisons between INSP and CTRL (*), INSP and MATCH (#). The number of symbols; one, two and three denote likely, very likely and almost certainly respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size.

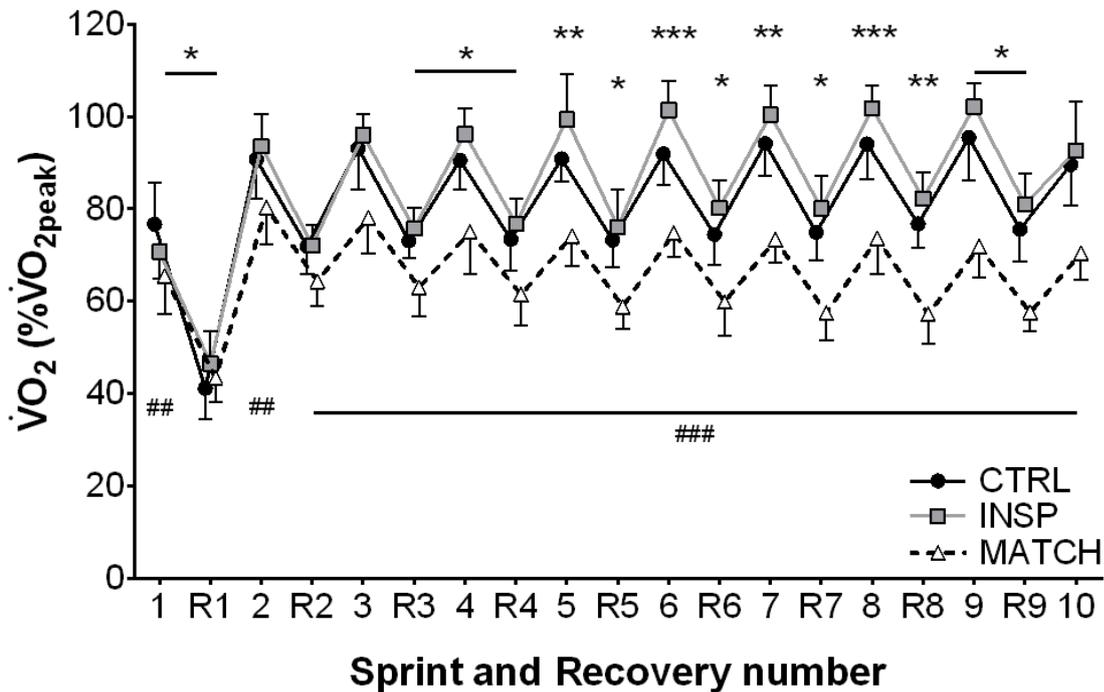


Figure 4.3: Sprint and recovery pulmonary oxygen uptake ($\dot{V}O_2$) expressed as a percentage of $\dot{V}O_{2peak}$ for Control (CTRL), Inspiratory Loading (INSP) and Worked Matched (MATCH) exercise. The symbols represent comparisons between INSP and CTRL (*), INSP and MATCH (#). The number of symbols; one, two and three denote likely, very likely and almost certainly respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size. Results are mean \pm SD.

4.3.4 Muscle Oxygenation

Responses to exercise are presented in Table 4.4 and, Figure 4.4 and Figure 4.5. Differences were *unclear* between INSP and CTRL for TSI_{RM} (1.0%, $\pm 7.5\%$), but MATCH was *very likely* lower than INSP (22.1%, $\pm 12.5\%$). Average HHb_{RM} was *likely* greater during INSP compared to CTRL (9.0%, $\pm 7.5\%$). Conversely, HHb_{RM} was lower during MATCH compared to INSP (-19.6%, $\pm 6.0\%$).

Differences of sprint TSI_{VL} (-0.3%, $\pm 9.5\%$) and recovery TSI_{VL} (-1.1%, $\pm 7.0\%$) were *unclear* between INSP and CTRL. Similarly, the differences between INSP and CTRL for both sprint HHb_{VL} (-1.1%, ± 5.1) and recovery HHb_{VL} (-2.7%, $\pm 5.4\%$) were *unclear*. There was no meaningful difference in TTP_{HHb} between INSP and CTRL (-5.8%, ± 6.0).

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There was also no meaningful difference in ΔReoxy between INSP and CTRL (4.7%, ± 8.3). Additionally, there was an *unclear* difference in Reoxy rate (0.0%, ± 0.3).

In MATCH exercise, differences in TSI_{VL} were unclear compared to INSP for both sprint (2.2%, ± 6.7), and recovery (-1.4%, ± 7.6) phases. Additionally, there was no meaningful difference in sprint HHb_{VL} (-1.1%, ± 5.1), and an *unclear* difference for recovery HHb_{VL} (3.0%, ± 5.7). The TTP_{HHb} was greater during MATCH than INSP (12.0%, ± 14.4). There were also *unclear* differences in ΔReoxy (-5.2%, ± 11.5), and Reoxy rate (0.1%, ± 0.4) between MATCH and INSP.

Table 4.4: Mean near-infrared spectroscopy responses to repeated-sprint exercise. The columns include data from Control (CTRL), Inspiratory Loading (INSP), and Work Match (MATCH) exercise conditions.

Variable	CTRL	INSP	MATCH
TSI_{RM} (%)	40.34 \pm 19.01	41.93 \pm 20.73	63.50 \pm 8.21 ##
HHb_{RM} (%)	28.94 \pm 19.85	37.96 \pm 16.41 *	18.36 \pm 13.60 ###
Sprint TSI_{VL} (%)	36.66 \pm 25.91	36.38 \pm 20.29	38.57 \pm 16.48
Recovery TSI_{VL} (%)	77.02 \pm 12.79	75.95 \pm 14.29	74.57 \pm 7.13
Sprint HHb_{VL} (%)	83.65 \pm 11.61	82.56 \pm 16.22	85.11 \pm 12.23
Recovery HHb_{V} (%)	30.63 \pm 9.82	27.90 \pm 13.43	30.90 \pm 8.67
TTP_{HHb} (s)	13.0 \pm 3.3	12.3 \pm 3.4	13.8 \pm 3.7 #
ΔReoxy (%)	49.94 \pm 20.3	54.66 \pm 19.24	49.45 \pm 21.08
Reox rate ($\% \cdot \text{s}^{-1}$)	2.16 \pm 0.78	2.20 \pm 0.75	2.27 \pm 0.79

Values are reported as mean \pm SD. Abbreviations are: TSI_{RM} , respiratory muscle tissue saturation index (n = 8); HHb_{RM} , respiratory muscle deoxyhaemoglobin; TSI_{VL} , vastus lateralis tissue saturation index; HHb_{VL} , vastus lateralis deoxyhaemoglobin; TTP_{HHb} , time to peak deoxyhaemoglobin; ΔReoxy , reoxygenation; Reoxy rate, reoxygenation rate. The symbols represent comparisons between INSP and CTRL (*), INSP and MATCH (#). The number of symbols; one, two and three denote likely, very likely and almost certainly respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size.

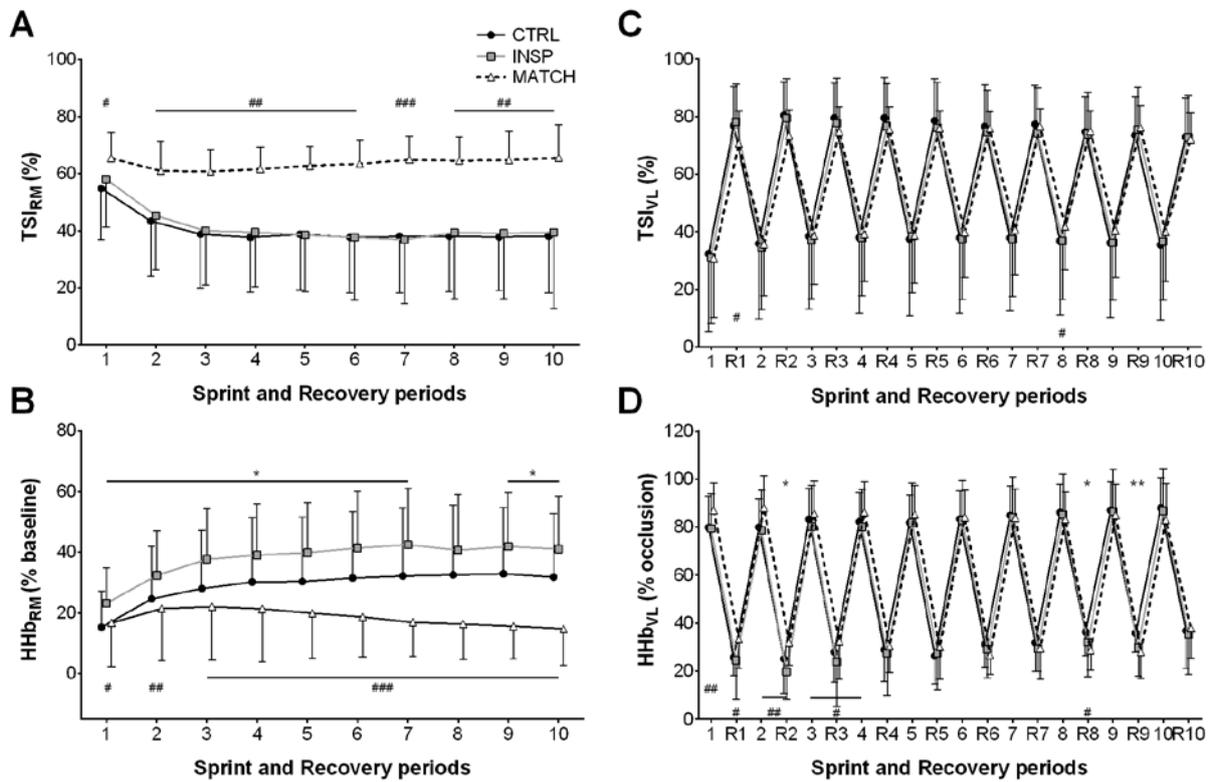


Figure 4.4: Near-infrared spectroscopy responses to repeated-sprints during the Control (CTRL) Inspiratory Loading (INSP) and Work Matched (MATCH) trials. (A) Respiratory muscle tissue saturation index (TSI_{RM}) (n = 8). (B) Respiratory muscle deoxy haemoglobin (HHb_{RM}) expressed as percent change from baseline (n = 10). (C) Vastus lateralis tissue saturation index (TSI_{VL}). (D) Vastus lateralis deoxy haemoglobin (HHb_{VL}) expressed relative to occlusion values. Values are presented as mean ± SD. The symbols represent comparisons between INSP and Control (*), INSP and MATCH (#). The number of symbols; one, two and three denote likely, very likely and almost certainly respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size.

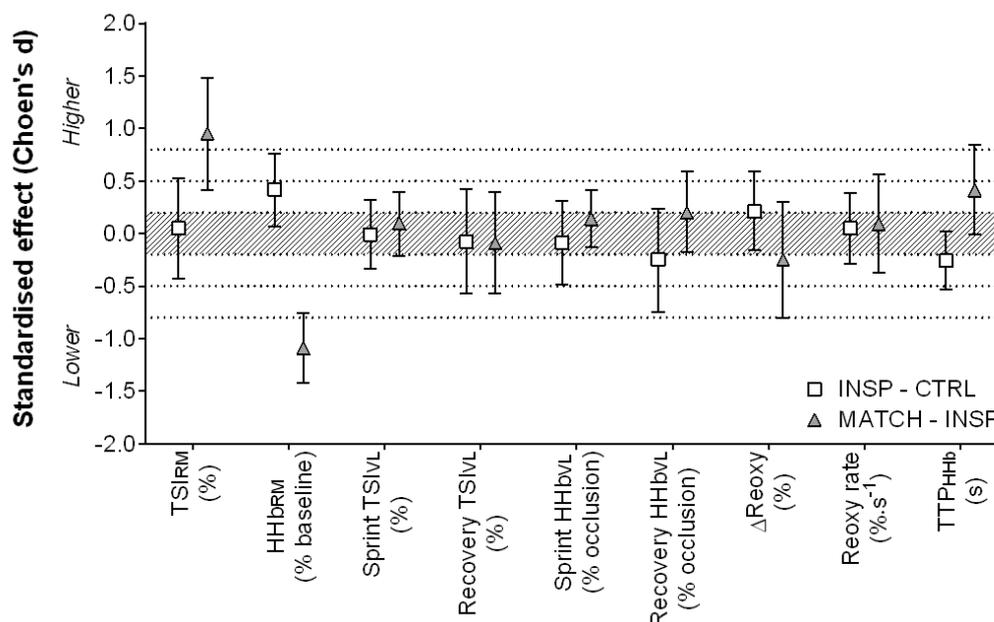


Figure 4.5: Standardised effects (Cohen's d) with 90% confidence intervals for near-infrared spectroscopy variables comparing Inspiratory Loading (INSP) to Control (CTRL), and Work Matched exercise (MATCH) to INSP. Shaded area indicates a trivial effect, and dotted lines thresholds for small, moderate and large effects. Abbreviations are: TSI_{RM}, respiratory muscle tissue saturation index; HHb_{RM}, respiratory muscle deoxyhaemoglobin; TSI_{VL}, vastus lateralis tissue saturation index; HHb_{VL}, vastus lateralis deoxyhaemoglobin; ΔReoxy, vastus laterals reoxygenation; Reoxy rate, vastus laterals reoxygenation rate; TTP_{Hb}, time to peak deoxyhaemoglobin.

4.3.5 Rating of Perceived Exertion

Perceptions of exercise and breathing difficulty are presented in Table 4.4. There was a *likely* trivial effect of INSP on RPE_{Exercise} compared to CTRL after sprint 1 (-0.2%, ±0.5%; ES = -0.08, ±0.21), *unclear* effects after sprint 5 (0.3%, ±1.1%; ES = 0.14, ±0.52), and *unclear* effects after sprint 10 (0.4%, ±1.0%; ES = 0.19, ±0.49). On the other hand, RPE_{Exercise} during MATCH was *almost certainly* lower than INSP after sprint 1 (-2.1%, ±0.8%; ES = -0.98, ±0.37), sprint 5 (-4.4%, ±1.3%; ES = -2.78, ±0.79), and 10 (-5.6%, ±1.2%; ES = -3.43, ±0.73).

During INSP, RPE_{breath} was *almost certainly* greater than CTRL after sprint 1 (2.8%, ±0.7; ES = 1.46, ±0.37), sprint 5 (2.4%, ±1.0%; ES = 1.79, ±0.77), and sprint 10 (2.7%, ±1.2; ES = 1.32, ±0.57). Similarly, MATCH was *almost certainly* lower than INSP

after sprint 1 (-4.0%, $\pm 1.1\%$; ES = -2.09, 90% CL ± 0.57), sprint 5 (-6.1%, $\pm 2.0\%$; ES = -4.54, ± 1.50), and sprint 10 (-6.8%, $\pm 1.6\%$; ES = -3.93, ± 0.95).

4.4 DISCUSSION

This study examined the physiological responses in repeated-sprint exercise to heightened respiratory muscle work, in particular, the oxygenation trends of both respiratory and vastus lateralis muscles. The addition of inspiratory loading increased mouth pressure and respiratory muscle O₂ utilization. However, this had no meaningful impact on blood arterial O₂ saturation and tissue oxygenation trends within the vastus lateralis muscle. We interpret these findings to suggest during maximal intermittent work, O₂ delivery and demands of the respiratory and locomotor muscles can be maintained.

4.4.1 Work of Breathing and Respiratory Muscle Oxygenation

Hyperpnoea during high-intensity exercise requires a considerable portion of whole-body $\dot{V}O_2$ to support the metabolic demands of the respiratory muscles (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992), and is increased when an inspiratory load is added (Harms et al., 1998). In the present study, $\dot{V}O_2$ was elevated by 4-5% during both the sprint and recovery phases of the repeated-sprint protocol when an inspiratory load was added. This occurred even with no meaningful difference in total work between INSP and CTRL. Responses in HHb_{RM} lend support to the notion of increased O₂ utilization by the respiratory muscles. The addition of an inspiratory load increased oxygen utilization of the respiratory muscles probably to accommodate an elevated work of breathing, as demonstrated during endurance exercise (Turner et al., 2013; Wetter et al., 1999). Furthermore, there were *unclear* differences in TSI_{RM} suggesting that [O₂Hb] was higher to MATCH the demands for O₂ delivery of the respiratory muscles. Previous studies have

shown similar changes in HHb_{RM} in response to inspiratory loading (Turner et al., 2013), and resistive breathing (Nielsen, Boesen, & Secher, 2001). The present data further suggest maintenance of respiratory muscle [O₂Hb] with inspiratory loading. But this can have negative consequences for exercise tolerance and the development of peripheral fatigue if blood flow is redirected away from the active limbs, and towards the respiratory muscles to meet the metabolic demands of breathing.

During continuous high-intensity exercise when the work of breathing is high, there can be an increase in vascular resistance and a reduction in limb perfusion (Harms et al., 1997; Harms et al., 1998; Sheel et al., 2001). It is believed that this is a protective mechanism aiming at maintaining adequate blood flow and O₂ supply to the respiratory muscles. An accumulation of metabolites in the respiratory muscles stimulate group IV afferent discharge in the respiratory muscles (J. M. Hill, 2000), leading to sympathetically mediated efferent discharge and vasoconstriction in the locomotor muscles (Harms et al., 1997; St Croix et al., 2000). Though there is evidence of this in sustained high-intensity exercise, such evidence does not exist in repeated-sprint exercise. The intermittent nature of repeated-sprint exercise may have sufficient recovery time to prevent the accumulation of fatigue inducing metabolites recovery O₂ debt in the respiratory muscles. Moreover, respiratory muscle fatigue does not appear to be induced by repeated-sprint exercise (Minahan et al., 2015). The deleterious effects of respiratory muscle work may be more prominent in a repeated-sprint protocol with shorter between sprint recovery periods (i.e. 10 s sprint and 20 s rest (Faiss et al., 2013; Willis et al., 2017)). But to date, no examination exists exploring the role of respiratory muscle work in repeated-sprint exercise and the effects on locomotor muscle oxygenation.

4.4.2 Locomotor Muscle Oxygenation

Inspiratory loading had no discernible effects on sprint HHb_{VL} despite a considerable increase in the work of breathing and respiratory muscle O₂ utilization. The evolution of vastus lateralis deoxygenation is a rapid increase at sprint onset, and then plateaus with sprint repetition (Buchheit et al., 2009; Racinais et al., 2007; Smith & Billaut, 2010). This suggests that a maximal level of O₂ extraction in the locomotor muscles is achieved (Esaki et al., 2005). However, a higher secondary ceiling point to vastus lateralis deoxygenation has been observed when repeated-sprint exercise has been performed in simulated altitude (normobaric hypoxia) (Billaut & Buchheit, 2013). This can be in part explained by a compensatory increase in muscle O₂ extraction to negate a reduced O₂ availability (Legrand et al., 2005). If vastus lateralis O₂ availability had been impacted in the present study by an elevated work of breathing, it would have been expected for sprint HHb_{VL} to be greater during INSP. Nevertheless, muscle deoxygenation per se may play a limited role in prolonged repeated-sprint performance. But muscle O₂ availability during recovery appears to be more influential in maintaining performance (Billaut & Buchheit, 2013). The capacity to reoxygenate between sprints is highly sensitive to O₂ availability, and underpins metabolic recovery between sprint bouts (Billaut & Buchheit, 2013; Buchheit et al., 2009; Jones et al., 2015). Even with the addition of an inspiratory load which increased respiratory muscle O₂ utilization vastus lateralis O₂ delivery was maintained. It appears that the cardiovascular system can adjust to support the metabolic O₂ demands of both the respiratory and locomotor muscles during repeated-sprint exercise.

Exercise intensity is an important mediator of blood flow redirection. Increasing the work of breathing artificially at submaximal exercise intensities (up to 75% $\dot{V}O_{2peak}$)

has been shown to increase whole-body $\dot{V}O_2$ with no change in limb blood flow (Wetter et al., 1999). On the other hand, when an inspiratory load is added during maximal exercise, blood flow to the locomotor muscles is compromised (Harms et al., 1997; Harms et al., 1998). During maximal exercise, cardiac output is limited and its ability to supply adequate blood flow to both locomotor and respiratory muscles is challenged. Consequently, part of the available blood is directed away from the limbs and towards the respiratory muscles to support the elevated work of breathing.

Though repeated-sprint exercise is known to elicit $\dot{V}O_2$ of >90% of peak, it is not sustained throughout the entire protocol (Buchheit et al., 2009; Dupont, Blondel, & Berthoin, 2003), and skeletal muscle O_2 extraction fluctuates between efforts and recovery (Billaut & Buchheit, 2013). In the current study, sprint pulmonary $\dot{V}O_2$ fluctuated between 90% and 70% of $\dot{V}O_{2peak}$ during sprint and recovery phases, respectively, but increased by ~4.5% when the inspiratory load was added (Figure 4.3). Even though participants in the present study were asked to produce maximal “all-out” efforts, the load on the cardiovascular system remained submaximal (Buchheit et al., 2009; Dupont et al., 2003). Subsequently, competition for available cardiac output between locomotor and respiratory muscles was minimized. When limb blood flow has been attenuated with inspiratory loading, there is no accommodating increase in $\dot{V}O_2$ (Harms et al., 1997; Harms et al., 1998). This presumably occurs when the prescribed exercise intensity is sufficient to elicit sustained $\dot{V}O_{2peak}$, and therefore, no further increase can occur. In the present study, maintenance of TSI_{RM} suggested that there was a rise in O_2 delivery proportional to the additional metabolic work of the respiratory muscles. Because there were no differences in HR, it is improbable that cardiac output increased to accommodate the additional O_2 demand. Demands may have been met by a

redirection of blood flow from less active regions towards the respiratory muscles (Ogata et al., 2007; Peltonen et al., 2013).

Hyperventilation was present in both CTRL, and to a lesser degree, INSP trials, which may have had a protective effect on limb O₂ delivery. Hyperventilation is associated with an increase in alveolar ventilation disproportionate to $\dot{V}O_2$ (pressure of alveolar O₂ increases), and $\dot{V}CO_2$ (pressure of alveolar CO₂ decreases) (Forster et al., 2012; Sheel & Romer, 2012). This is a potential mechanism associated with high-intensity exercise which can constrain a fall in arterial O₂ and pH (Forster et al., 2012; Whipp & Ward, 1998). Despite a reduction of \dot{V}_E in a state of heightened O₂ demand during the INSP trial, S_pO₂ was maintained with no signs of arterial hypoxia. In studies where vastus lateralis tissue oxygenation was impaired during exercise with resistive breathing and inspiratory loading, there was a small degree of arterial hypoxemia (Nielsen et al., 2001; Turner et al., 2013). Exercise-induced arterial hypoxemia is a known limiting factor of exercise (Dempsey & Wagner, 1999), and preventing it with supplemental O₂ can attenuate peripheral muscle fatigue (Romer, Haverkamp, Lovering, Pegelow, & Dempsey, 2006). The respiratory muscle work may only be influential to exercise if a significant degree of arterial O₂ desaturation has also occurred. Though exercise-induced arterial hypoxemia has been demonstrated to be incurred with repeated-sprint exercise (Smith & Billaut, 2010), there was no evidence of its occurrence in the present study. Meaningful changes in repeated-sprint oxygenation trends from an elevated work of breathing may only occur if exercise-induced arterial hypoxemia is also present.

The level of inspiratory loading may have also had an influence on the outcomes in this study. In previous work, inspiratory loading was achieved by reducing the inspiratory aperture to 10 mm and 8 mm (Turner et al., 2013). Only with the smaller

opening that changes in [HHb] of the exercise limb were detected. Similarly, when resistive breathing has been used, the most noticeable changes in tissue oxygenation trends occurred when the aperture was reduced to 4.5 mm (Nielsen et al., 2001). The inspiratory work in the present study may have been too low to induce a respiratory muscle metaboreflex. However we do not believe this to be the case since peak P_m in our experiment was similar to the previous work when an 8-mm aperture was used (30.7 ± 6.6 cmH₂O vs. 28.1 ± 4.8 cmH₂O) (Turner et al., 2013). Additionally \dot{V}_E was considerably higher than previous work (Nielsen et al., 2001; Turner et al., 2013) which would have contributed to a heightened work of breathing.

4.4.3 Worked Matched Exercise

To our knowledge, this is the first time repeated work matched bouts of exercise have been used to examine the demands of repeated-sprint exercise under altered metabolic conditions. Regardless of a similar degree of vastus lateralis tissue deoxygenation incurred during the work matched sprints, the physiological load placed on the cardiovascular system was considerably lower. This is evidenced by the consistently lower $\dot{V}O_2$, in part, due to markedly lower respiratory muscle O₂ utilization. But more importantly, $\dot{V}O_2$ was heavily influenced by how exercise was prescribed. Matching total work was achieved by replicating mean power output for each sprint, and therefore was lacking maximal acceleration and power production associated with sprint exercise. Reliance on intramuscular ATP and PCr hydrolysis would have been reduced (Gaitanos et al., 1993; Glaister, 2005), and metabolic perturbations associated with maximal exercise minimized (Gaitanos et al., 1993; Glaister, 2005; Hogan et al., 1999; Parolin et al., 1999). Despite a substantial decrease in the O₂ cost associated with the work of breathing and sprint exercise, there was no substantial difference in $\Delta Reoxy$ nor

Reoxy rate. This implies that tissue reoxygenation was maximal in all exercise conditions whatever the respiratory challenge. It appears that there exists some degree of reserve in the cardiovascular system that is called upon to maintain O₂ delivery to both the respiratory and locomotor muscle. Therefore, the O₂ cost of breathing in repeated-sprint cycling is unlikely to have a meaningful negative impact on locomotor O₂ transport.

4.5 CONCLUSION

An important factor of repeated-sprint performance is the reoxygenation capacity between sprint bouts (Billaut & Buchheit, 2013; Buchheit et al., 2009; Buchheit & Ufland, 2011). We further tested this mechanism by increasing the work of breathing, which is known to negatively influence limb blood flow and O₂ delivery at least in endurance exercise (Aaron, Seow, et al., 1992; Dempsey et al., 2006; Harms et al., 1997; Sheel et al., 2001). The present data demonstrate that the addition of inspiratory loading did no impair O₂ delivery to the vastus lateralis. When maximal exercise is interspersed with short rest periods, the cardiovascular system appears to maintain O₂ delivery to both the locomotor and respiratory muscle in a state of heightened metabolic demands.

**CHAPTER FIVE: INFLUENCE OF ACUTE
ARTERIAL HYPOXEMIA ON RESPIRATORY
MUSCLE OXYGENATION**

5.1 INTRODUCTION

Repeated-sprint ability describes the capacity to recover from and maintain sprint (≤ 10 s) performance during subsequent “all-out” efforts (Girard, Bishop, & Racinais, 2013). Phosphocreatine hydrolysis and anaerobic glycolysis are primary sources of rapid ATP replenishment in repeated exercise (Gaitanos et al., 1993; Parolin et al., 1999). However, the time course of metabolic recovery exceeds the rest period characterised by repeated-sprint exercise (Gaitanos et al., 1993; Harris et al., 1976). Resulting in a progressive reduction in both peak and mean power output, with a plateau in the latter sprints (Gaitanos et al., 1993; Racinais et al., 2007). Resynthesis of phosphocreatine and removal of inorganic phosphate is derived exclusively through oxidative processes and is sensitive to muscle oxygen (O_2) availability (Harris et al., 1976; Hogan et al., 1999; Sahlin et al., 1979). Therefore, underpinning the ability to maintain performance over multiple sprints is the capacity to deliver oxygen to the locomotor muscles between efforts (Billaut & Buchheit, 2013; Buchheit & Ufland, 2011).

The balance of muscle O_2 delivery vs. extraction can be elucidated with the use of near-infrared spectroscopy (NIRS). Concentrations deoxyhaemoglobin ([HHb]) and oxyhaemoglobin ([O_2 Hb]) rise and fall respectively, proportional to an increase in metabolic activity in the underlying tissue. However, analysis is often restricted to [HHb] as it is less sensitive to blood volume changes and provides an estimate of muscular oxygenation (De Blasi et al., 1993; DeLorey et al., 2003; Grassi et al., 2003). At sprint onset, there is a rapid increase of vastus lateralis [HHb] (deoxygenation) which trends back towards baseline during intersprint rest periods (reoxygenation) (Buchheit et al., 2009; Racinais et al., 2007; Smith & Billaut, 2010). But there is limited work examining if the O_2 cost of hyperpnoea influences repeated-sprint oxygenation trends.

During high-intensity exercise, the O₂ cost of hyperpnoea and cardiac output distribution devoted to the respiratory muscle accounts for 10-15% of total pulmonary O₂ uptake ($\dot{V}O_2$) (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992; Harms et al., 1998). To ensure the required O₂ demands are met, sympathetically mediated vasoconstriction of the locomotor muscles promote blood flow redistribution towards respiratory musculature (respiratory muscle metaboreflex) (Dempsey et al., 2006; Harms et al., 1997; Sheel et al., 2001; St Croix et al., 2000). This mechanism in part contributes to the peripheral muscle fatigue that is incurred during high-intensity exercise, and is exaggerated by fatiguing contractions of the respiratory muscles (Romer, Lovering, et al., 2006). But the data presented in [Chapter 4](#) do not support this phenomenon occurring in repeated-sprint exercise, where despite an elevated work of breathing and respiratory muscle O₂ utilisation, vastus lateralis oxygenation was maintained. The deleterious effects of respiratory muscle work may be more apparent in acute hypoxia. It has been demonstrated that hypoxia leads to elevated pulmonary ventilation and work of breathing compared to normoxia (Cibella et al., 1996; Reeves, Welsh, & Wagner, 1994). By alleviating the hypoxia-induced rise in work of breathing, the rate development of peripheral fatigue can be attenuated (Amann, Pegelow, et al., 2007). The associated negative consequence of respiratory muscle work are seemingly amplified in acute hypoxia, likely mediated by a hastening activation of the respiratory muscle metaboreflex (Amann, Pegelow, et al., 2007; Dempsey et al., 2006).

Negative effects of hypoxia on exercise performance and the development of peripheral fatigue during repeated sprints is fairly well established (Billaut & Buchheit, 2013; Billaut et al., 2013; Smith & Billaut, 2010, 2012). Arterial hypoxemia specifically limits reoxygenation capacity between sprint efforts to constrain metabolic recovery

(Billaut & Buchheit, 2013). However, it is currently unclear what influence acute hypoxia has on respiratory muscle oxygenation, and balance between locomotor and respiratory muscle oxygenation. Therefore, the purpose of the present study was to examine the effect of severe acute arterial hypoxemia on respiratory muscle oxygenation during repeated-sprint exercise. Hypoxemia was induced via a reduction in the fraction of inspired O₂ (F_IO₂), and oxygenation of the vastus lateralis and intercostal muscle were simultaneously measured with NIRS. It was reasoned that both vastus lateralis and intercostal muscle oxygenation would be impaired during exercise, mediated by arterial oxygenation and the work of breathing.

5.2 METHODS

5.2.1 Subjects

Ten males from a variety of athletic backgrounds were recruited to participate in this study (team sports, road cycling, heavy resistance training). These subjects were chosen because they were accustomed to producing “all-out” bouts of exercise. Subjects self-reported to be healthy and with no known neurological, cardiovascular or respiratory diseases. After being fully informed of the requirements, benefits, and risks associated with participation, each subject gave written informed consent. Ethical approval for the study was obtained from the institutional Human Research Ethics Committee and the study conformed to the declaration of Helsinki.

Table 5.1: Subject characteristics.

Measure	Value
Age (year)	26.0 ± 3.6
Body mass (kg)	78.6 ± 9.4
Height (m)	178.3 ± 7.5
$\dot{V}O_{2\text{peak}}$ (mL·min ⁻¹ ·kg ⁻¹)	48.42 ± 6.92

Values are mean ± SD. Abbreviations are: $\dot{V}O_{2\text{peak}}$, peak pulmonary oxygen uptake.

5.2.2 Experiment Design

On a preliminary visit, participants were familiarised with an incremental exercise test used in the following session. In the next session, the incremental exercise test was performed to exercise tolerance. On the following two sessions, subjects completed the same repeated-sprint protocol used in the main testing sessions for familiarisation. The main testing sessions were performed in a randomised order in normoxia and hypoxia. Trials were conducted at the same time of day and separated by 3-7 days. Subjects were asked to refrain from exercise and strenuous activity for 48 h preceding all testing sessions. All exercise testing was performed on an electronically-braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) and expired gases collected on a breath-by-breath basis (COSMED Quark CPET; Cosmed, Rome, Italy).

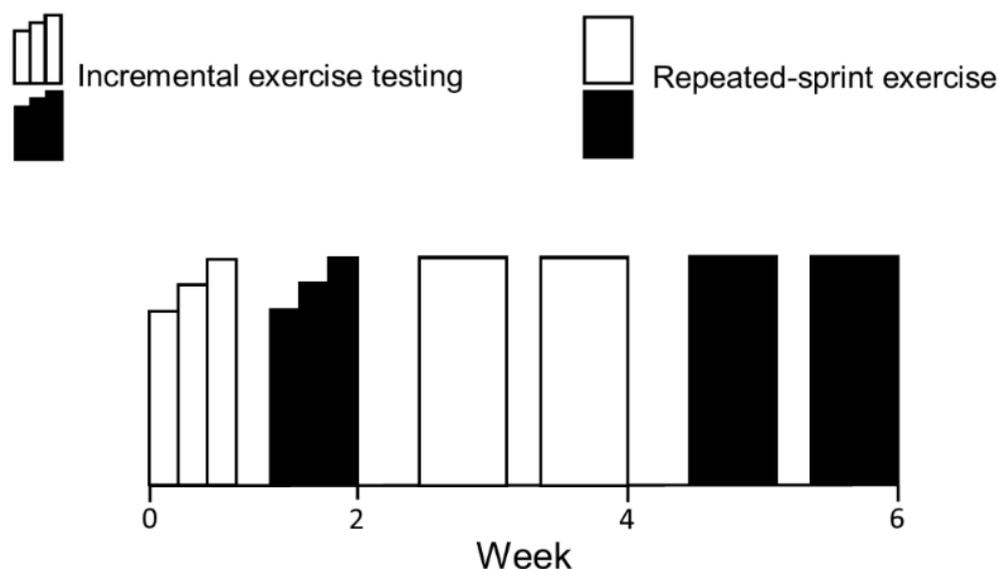


Figure 5.1: Study 3 design. Familiarisation trials are represented by the open squares, whereas experimental trials are represented by the filled squares.

5.2.3 Incremental Exercise Testing

An incremental exercise test was performed for the determination of peak pulmonary oxygen uptake ($\dot{V}O_{2\text{peak}}$). The test was initiated at a work rate of 0 W for 3 min, followed by an increase in work rate 1 W every 2 s ($30 \text{ W}\cdot\text{min}^{-1}$) until volitional exhaustion or until the cadence fell below 10 RPM self-selected pedalling rate (Burnley et al., 2006). Peak 30 s average was calculated and used to represent $\dot{V}O_{2\text{peak}}$.

5.2.4 Repeated-sprint Exercise

Trials were performed in a semi-random single-blind order, ensuring a balance of normoxic and hypoxic trials. All testing was conducted within a 23.92 m² environmental exercise laboratory. The F_iO_2 was 0.2084 ± 0.005 and 0.1455 ± 0.0031 for normoxia and hypoxia testing session respectively. After arriving at the laboratory, subjects were fitted with NIRS probes and a heart rate monitor. Testing was performed with the cycle ergometer set to isokinetic mode (120 rpm). Cadence was fixed for every sprint so that exercise-induced changes in mechanical power and physiological responses were not influenced by cadence (Gotshall et al., 1996; Tomas et al., 2010). After a 7-min

warm-up consisting of 5 min of unloaded cycling at 60-70 rpm and two 4 s sprints (separated by 1 min), subjects rested for another 2.5 min before the repeat-sprint protocol was initiated. The repeat-sprint protocol was ten 10 s sprints separated by 30 passive rest (5.5 min). Subjects were instructed to give an “all-out” effort for every sprint and verbally encouraged throughout to promote a maximal effort. Each sprint was performed in the seated position and initiated with the crank arm of the dominant leg at 45°. Before sprint one, subjects were instructed to accelerate the flywheel to 95 rpm over a 15-s period and assumed the ready position 5 s before the commencement of the test. This ensured that each sprint was initiated with the flywheel rotating at ~90 rpm so that subjects could quickly reach 120 rpm. To minimise the chance of a protective pacing strategy, the first 10 s sprint of the repeated-sprint series was examined to ensure that peak power output exceeded that of the two preceding 4 s sprints. In only one instance was the peak power produced by a subject below that of the preceding sprints. Consequently the subject was asked to immediately terminate the sprint activity and passively rest for 5 min before the repeated-sprint protocol was restarted. The handlebars and seat were individually adjusted to each subjects’ characteristic. Four subjects used their own clipless pedals and cycling shoes, the remaining six had their feet secured using toe cages and retention straps fitted to the ergometer. Crank arm length was standardized to 175 mm. Visual feedback of power output was not available to the subjects during any sprint. The cycle ergometer software provides power and cadence at 4 Hz. Data was exported in to excel for the calculation of mechanical work completed and power production for individual sprints, and over the entire protocol.

5.2.5 Metabolic and Ventilatory Measurements

Subjects wore a silicone facemask which the breath-by-breath gas sampling line and turbine were attached. The analyser was calibrated before each test against known gas concentrations (normoxia: 16% O₂ and 5% CO₂; hypoxia: 7% O₂ and 5% CO₂) and the turbine volume transducer was calibrated using a 3 L syringe (Cosmed, Rome, Italy). Data was then exported into Excel so that $\dot{V}O_2$ could be inspected for errant data points. Editing data was performed to remove the occasional errant breaths caused by for example swallowing or coughing which were considered not be reflective the metabolic responses to exercise. These errant data points were removed by the same researcher in every case before values greater than 4 standard deviations from the local mean were removed (Lamarra et al., 1987; Rossiter et al., 2000). A 5-breath rolling average was then applied for the calculation of peak and nadir for every 40-s sprint/recovery period to give a single value for each sprint and recovery phase. Respiratory frequency (f_b) was averaged over the entire sprint protocol to give one value for each subject per trial. Because the facemask was removed immediately after the tenth sprint, only maximum values were calculated over the first 10 s. Mouth pressure (P_m) was recorded continuously at 50 Hz with a pressure transducer (Honeywell, New Jersey, United States of America) attached to the saliva port of the non-rebreathing valve via Tygon tubing (Hans Rudolph inc., Kansas, United States of America). Mean inspiratory P_m was then calculated as an index of respiratory muscle work. An index of inspiratory muscle force development was also calculated for each exercise trial ($\int P_m \times f_R$) (Witt et al., 2007). For statistical analysis, inspiratory P_m was converted to positive values and presented in the results as such. Arterial oxygen saturation (estimated by fingertip pulse oximetry; S_pO_2) and heart rate (HR) was sampled on a breath-by-breath basis integrated into the COSMED system.

5.2.6 Near-infrared Spectroscopy

Subjects were instrumented with two NIRS probes to assess muscle O₂ status (Oxymon MKIII, Artinis, The Netherlands). The first probe was fixed over the distal part of the vastus lateralis muscle belly approximately 15 cm above the proximal border of the patella. The second was fixed over the sixth intercostal space at the anterior axillary line to assess changes in the accessory respiratory muscles. Probes were held in place with black plastic spacers secured to the skin using double sided stick disks and shielded from light using a black self-adhesive elastic bandage. Optode spacing was set to 4.5 cm and 3.5 cm for vastus lateralis and respiratory muscles respectively. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose tissue thickness covering the muscle. The skinfold thickness for vastus lateralis (1.19 ± 0.69 cm) and respiratory muscles (1.12 ± 0.44 cm) was less than half the distance between the emitter and the detector in every case. A differential pathlength factor of 4.95 was used (Smith & Billaut, 2010; Subudhi et al., 2007). Data was acquired at 10 Hz. A 10th order zero-lag low-pass Butterworth filter was applied to the data to remove artefacts and smooth pedalling induced fluctuations; the resulting output was used for analysis (Rodriguez et al., 2018). The application of the filter was conducted in the *R* environment (R Core Team, 2016) using the *signal* package (Signal developers, 2013). Vastus lateralis deoxyhaemoglobin was normalised to femoral artery occlusion so that 0% represented a 5-s average immediately prior the occlusion and 100% represented the maximum 5 s average. Peaks and nadirs were then identified within every 40-s sprint/recovery period to represent each sprint and recovery phase respectively (HHb_{VL}). Reoxygenation capacity (Δ Reoxy, %) was also calculated as the change from peak to nadir. Respiratory muscle oxyhaemoglobin (O₂Hb_{RM}) and deoxyhaemoglobin (HHb_{RM}) were expressed as an absolute change from baseline. A 2 min

resting baseline was established before warm-up while seated quietly on the cycle. Because there were no clear peaks and nadirs in the HHb_{RM} signal, an average was calculated for of each 40-s sprint/recovery period.

5.2.7 Statistical Analysis

Data in text and figures are presented as mean \pm standard deviation. Custom made spreadsheets were used to analyse the effects of hypoxia on laboratory measurements (Hopkins, 2006b). To assess the difference between trials, analysis was performed using the post-only crossover spreadsheet. All measures, other than SpO₂, and NIRS variables were log-transformed before analysis then back-transformed to express the changes in percent units and standardized effects. Relative changes (%) and effect size statistics are expressed with 90% confidence limits (90% CL). Practical significance was assessed by calculating Cohen's d effect size (ES) (Cohen, 1988). Standardized effect sizes of <0.2, >0.2 – 0.5, >0.5 – 0.8, <0.8 were considered to as trivial, small, moderate and large respectively and presented with 90% CL. Probabilities were also calculated to establish if the chance the true (unknown) differences were lower, similar or higher than the smallest worthwhile change (ES = 0.2). Effects were not considered meaningful if there was <75% probability of being substantially positive/negative relative to the smallest worthwhile change. If the chance of having higher/lower values then the smallest worthwhile difference was both >5%, the true difference was assessed as *unclear*. For clear effects, the likelihood that the true effect was substantial were assessed qualitatively as follows: *likely* (75 to <95%), *very likely* (95 – 99.5%), *most likely* (>99%) (Batterham & Hopkins, 2006; Hopkins et al., 2009).

5.3 RESULTS

Mechanical work recorded during the repeated-sprint tests are displayed in Figure 5.2. There was no meaningful difference in total work between hypoxia and normoxia (relative difference = -2.9%, 90% CL \pm 5%; standardised effect size = -0.21, 90% CL \pm 0.37). There was also a *trivial* difference in peak power during sprint one output between the conditions (-2.1%, \pm 5.7; ES = -0.11, \pm 0.32).

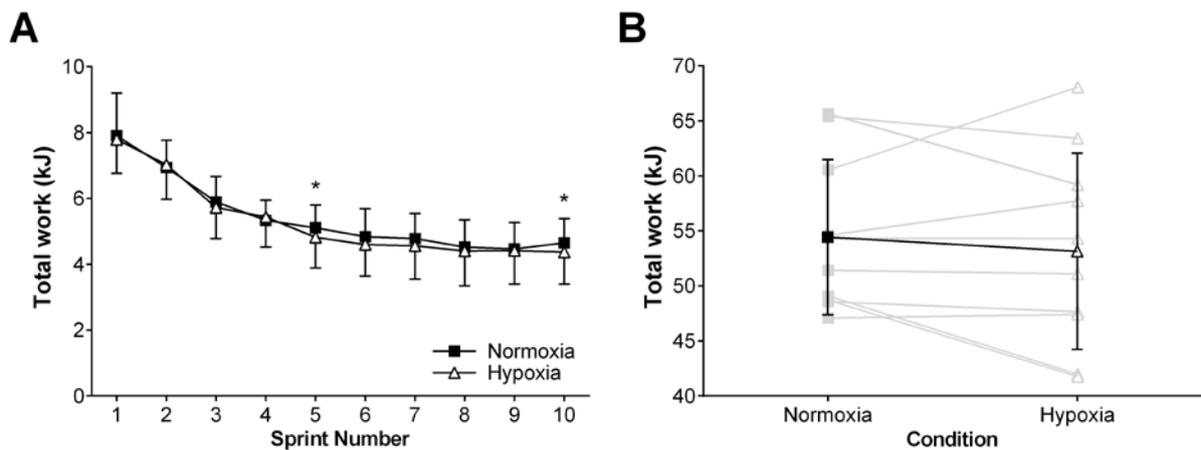


Figure 5.2: Total mechanical work completed during repeated-sprint exercise in Normoxia and Hypoxia. Mean total work per sprint (A) and, individual and mean total work completed over the entire protocol (B). The number of symbols (*); one, two and three denote likely, very likely and *most likely* respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size. Results are mean \pm SD.

Physiological responses to exercise are presented in Table 5.2. Each sprint and recovery $\dot{V}O_2$ is displayed in Figure 5.3. Overall, sprint $\dot{V}O_2$ in hypoxia was *likely* lower than normoxia. But no clear difference was observed during recovery (Table 5.2).

*Respiratory Muscle Work and Tissue Oxygenation
Trends During Repeated-sprint Exercise*

Table 5.2: Physiological responses to repeated-sprint exercise in Normoxia and Hypoxia.

	Normoxia (mean ± SD)	Hypoxia (mean ± SD)	Relative difference (% [90% CI])	Effect size (Cohen's d [90% CI])
SpO ₂ (%)	96 ± 2	87 ± 3	-9.7 [11.4, -8.1]	-5.70 [-6.67, 4.73]***
HR (bpm) n = 9	151 ± 12	156 ± 11	2.0 [-0.1, 4.2]	0.22 [-0.01, 0.46]
Sprint $\dot{V}O_2$ (mL·min ⁻¹ ·kg ⁻¹)	44.99 ± 5.49	42.65 ± 5.61	-5.3 [-8.4, -2.2]	-0.40 [-0.64, -0.16]*
Recovery $\dot{V}O_2$ (mL·min ⁻¹ ·kg ⁻¹)	37.02 ± 6.19	35.28 ± 6.10	-4.7 [-8.7, -0.6]	-0.27 [-0.50, -0.03]
P _{ET} O ₂ (mmHg)	118 ± 3	75 ± 3	-36.5 [-37.6, -35.5]	-17.59 [-18.21, -16.96] ***
P _{ET} CO ₂ (mmHg)	34 ± 3	31 ± 3	-7.4 [-9.9, -4.8]	-0.89 [-1.21, -0.57] ***
IV (L)	2.72 ± 0.50	2.70 ± 0.51	-0.6 [-4.0, 2.9]	-0.03 [-0.20, 0.14]
f _b (b·min ⁻¹)	52.01 ± 15.32	51.40 ± 10.91	0.1 [-5.8, 6.4]	0.00 [-0.21, 0.22]
Inspiratory P _m (cmH ₂ O)	2.10 ± 0.33	2.20 ± 0.45	3.9 [-3.6, 12.0]	0.22 [-0.21, 0.66]
∫P _m × f _b	68.17 ± 13.85	70.48 ± 12.76	3.7 [-4.2, 12.3]	0.17 [-0.20, 0.53]

Abbreviations are: P_m, mouth pressure; ∫P_m × f_b, inspiratory muscle force development; $\dot{V}O_2$, pulmonary oxygen uptake; IV, inspiratory volume; f_b, respiratory frequency; P_{ET}O₂, end-tidal oxygen; P_{ET}CO₂, end-tidal carbon dioxide; SpO₂, arterial oxygen saturation; HR, heart rate. The number of symbols (*); one, two and three denote *likely*, *very likely*, and *most likely* respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size.

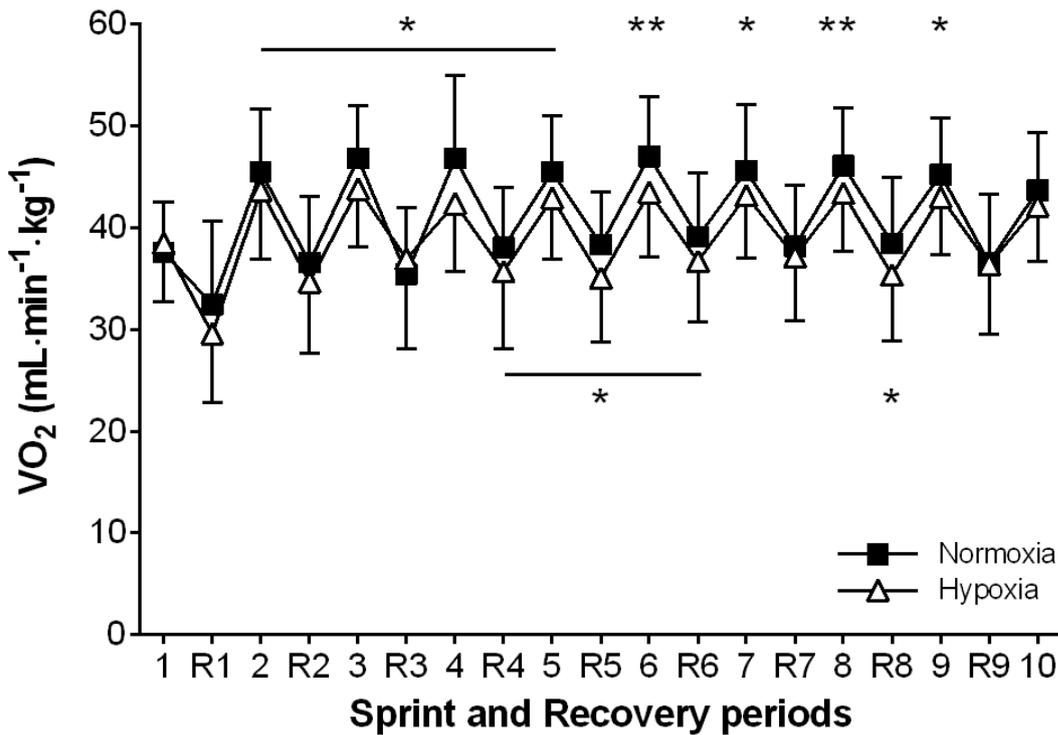


Figure 5.3: Sprint and recovery pulmonary oxygen uptake ($\dot{V}O_2$) during Normoxia and Hypoxia repeated-sprint exercise trials. Abbreviations are: O_2Hb_{RM} , respiratory muscle oxyhaemoglobin; HHb_{RM} , respiratory muscle deoxyhaemoglobin; tHb , respiratory muscle total haemoglobin; HHb_{VL} , vastus lateralis deoxyhaemoglobin; $\Delta Reoxy$, reoxygenation. The number of symbols (*); one, two and three denote likely, very likely and most likely respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size. Results are mean \pm SD.

Respiratory muscle and vastus lateralis NIRS responses are presented in Table 5.3, Figure 5.4 and Figure 5.5. Sprint and recovery HHb_{VL} were *likely* and *very likely* greater in the sprint and recovery periods respectively during hypoxic exercise compared to normoxia. On the other hand, there were no clear differences between the conditions for any of the respiratory muscle derived variables.

Respiratory Muscle Work and Tissue Oxygenation Trends During Repeated-sprint Exercise

Table 5.3: Near-infrared spectroscopy responses to repeated-sprint exercise in Normoxia and Hypoxia.

	Normoxia (mean \pm SD)	Hypoxia (mean \pm SD)	Relative difference (% [90% CI])	Effect size (Cohen's d [90% CI])
Respiratory muscles				
O ₂ Hb _{RM} (μ m)	-11.07 \pm 4.01	-12.07 \pm 5.09	-0.1 [-2.9, 0.9]	-0.23 [-0.67, 0.21]
HHb _{RM} (μ m)	9.48 \pm 6.81	10.38 \pm 8.04	0.9 [-0.8, 2.6]	0.12 [-0.11, 0.35]
tHb _{RM} (μ m)	-1.59 \pm 4.75	-2.18 \pm 7.12	-0.6 [-3.6, 2.4]	-0.11 [-0.69, 0.46]
Vastus lateralis				
Sprint HHb _{VL} (%)	74.95 \pm 16.85	85.08 \pm 9.56	9.2 [0.2, 18.0]	0.50 [0.01, 0.98] *
Recovery HHb _{VL} (%)	25.81 \pm 8.28	39.89 \pm 14.81	14.1 [4.9, 23.3]	1.55 [0.54, 2.57] **
Δ Reoxy (%)	50.14 \pm 13.82	45.19 \pm 13.05	-5.0 [-10.7, 0.8]	-0.33 [-0.71, 0.05]

Abbreviation are: O₂Hb_{RM}, respiratory muscle oxyhaemoglobin; HHb_{RM}, respiratory muscle deoxyhaemoglobin; tHb, respiratory muscle total haemoglobin; HHb_{VL}, vastus lateralis deoxyhaemoglobin; Δ Reoxy, reoxygenation. The number of symbols (*); one, two and three denote *likely*, *very likely* and *most likely* respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size.

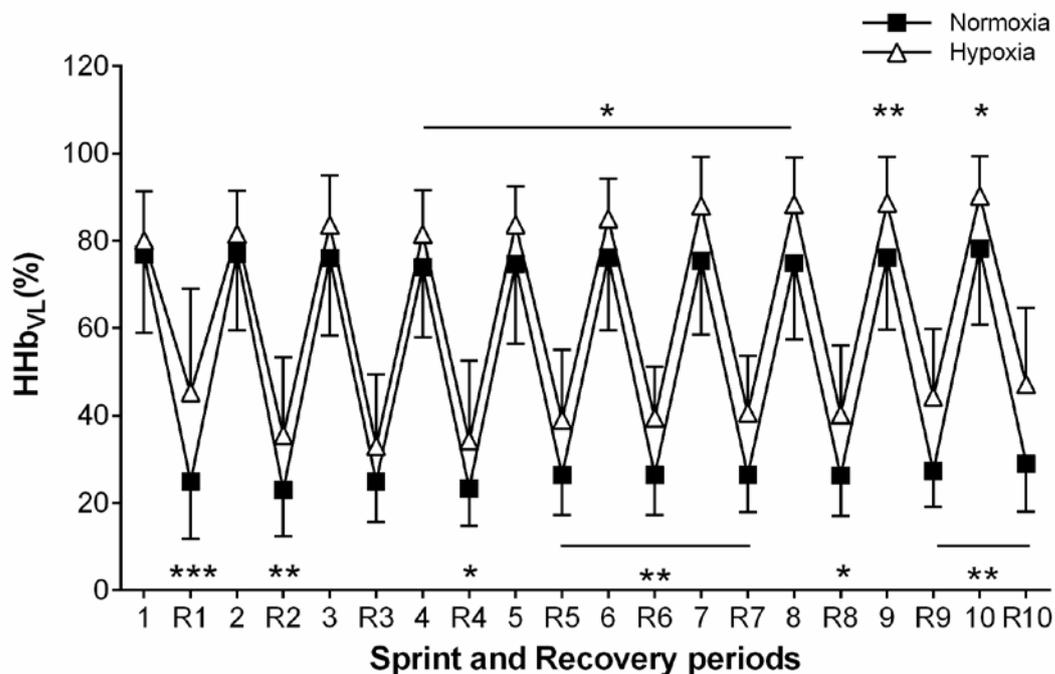


Figure 5.4: Vastus lateralis deoxyhaemoglobin (HHb_{VL}) during repeated-sprint exercise in normoxia and hypoxia. The number of symbols (*); one, two and three denote likely, very likely and most likely respectively, that the chance of the true effect exceeds a small (-0.2-0.2) effect size. Results are mean \pm SD.

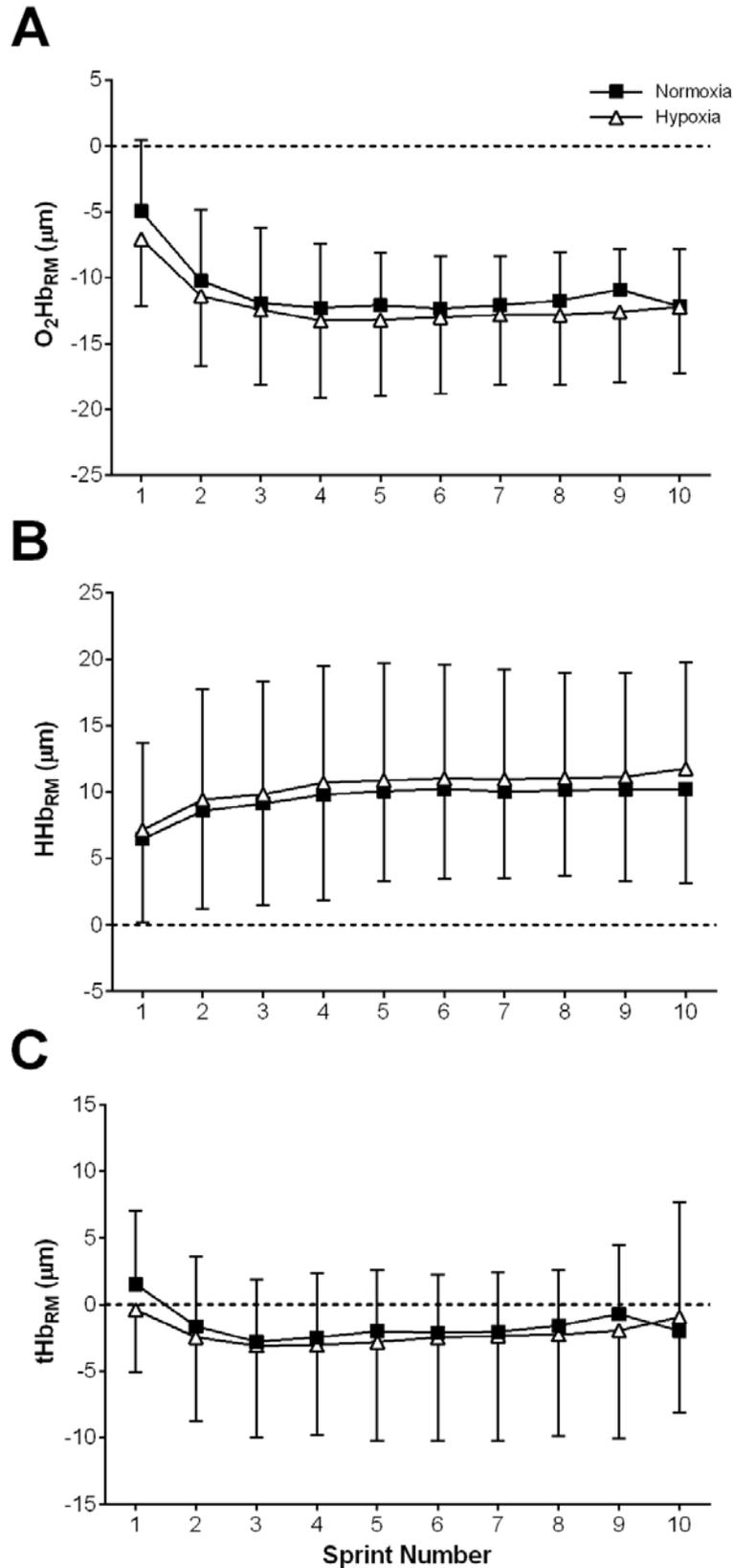


Figure 5.5: Respiratory muscle oxygenation trends during repeated-sprint exercise in Normoxia and Hypoxia expressed as an absolute change from baseline (dotted horizontal line). (A) Respiratory muscle oxyhaemoglobin ($\text{O}_2\text{Hb}_{\text{RM}}$); (B) respiratory muscle deoxyhaemoglobin (HHb_{RM}); (C) respiratory muscle total haemoglobin (tHb_{RM}). There was no clear effect of Hypoxia on respiratory muscle oxygenation. Results are mean \pm SD.

5.4 DISCUSSION

The present study is the first to report on the influence of a reduction in F_{iO_2} on respiratory muscle oxygenation trends during repeated sprint exercise. Despite substantial arterial hypoxemia, respiratory muscle oxygenation was maintained to a similar level to that of normoxic exercise. This was in contrast to the impairment of vastus lateralis oxygenation that was demonstrated here, and in previous research (Billaut & Buchheit, 2013).

Acute hypoxia typically has no discernible effects on isolated sprint performance (Girard, Brocherie, & Millet, 2017). However, as sprints are repeated, the effects of reduced O_2 availability typically become more apparent (Billaut & Buchheit, 2013; Billaut et al., 2013; Smith & Billaut, 2012). Surprisingly, arterial hypoxemia did not negatively affect total mechanical work to the same extent as previous research. A non-meaningful -2.9% reduction in total work performed was observed, which was considerably less than previous research using a similar protocol (Billaut & Buchheit, 2013; Smith & Billaut, 2010, 2012). It is probable that there was an element of pacing from two subjects, despite receiving strong verbal encouragement throughout the sprint exercise. More typical responses were demonstrated in the other eight subjects. There is the chance that the pacing strategy adopted may have impacted the results. However, this is unlikely because of the more typical responses demonstrated in the other measures.

Consistent with others, sprint HHb_{VL} was higher during the hypoxic trials compared to normoxia (Billaut & Buchheit, 2013). Similarly, recovery HHb_{VL} was negatively affected by hypoxia. It is plausible that the metabolic demands of exercise were similar between conditions. The additive effect of arterial hypoxemia to skeletal muscle O_2 extraction, is the likely source of the elevated HHb_{VL} during exercise (Costes et al.,

1996). Whereas recovery HHb_{VL} (and to a lesser extent ΔReoxy) is solely linked to limited muscle O_2 delivery between sprint efforts (Billaut & Buchheit, 2013). Changes in muscle oxygenation trends in these ways is representative of a mismatch between O_2 supply and extraction. Importantly, vastus lateralis O_2 availability during recovery is a strong determining factor of metabolic recovery, and therefore performance decline in repeated-sprint exercise (Gaitanos et al., 1993; Harris et al., 1976; Parolin et al., 1999). Impairment of O_2 transport was also represented by the $\sim 5\%$ reduction in $\dot{V}\text{O}_2$ during hypoxic exercise that was shown here (Figure 5.3), and similarly by others (Bowtell et al., 2014). It is fairly well established that hypoxia results in a linear decrease in $\dot{V}\text{O}_{2\text{peak}}$ (Martin & O'Kroy, 1993; Wehrlin & Hallén, 2006). Therefore, the lower $\dot{V}\text{O}_2$ represented a similar, or even greater fraction of the maximal O_2 utilisation relative to blunted $\dot{V}\text{O}_{2\text{peak}}$ (Mazzeo, 2008). Therefore, in order to meet the metabolic demands of the sprint intervals, non-oxidative ATP resynthesis, specifically PCr hydrolysis, must increase to compensate (Hogan et al., 1999). Contrary to the clear changes in vastus lateralis oxygenation, respiratory muscle oxygenation appears to be unaffected by hypoxia.

Despite clear differences in arterial O_2 saturation (87% vs. 96%), respiratory muscle oxygenation responses were similar between the hypoxic and normoxic exercise trials. This distinct lack of difference suggests that hypoxia no further compromises O_2 delivery to the respiratory muscles. However, this contrasts with others who have shown progressive deoxygenation of the respiratory muscles in response to hypoxia. During voluntary isocapnic hyperpnoea and inspiring a hypoxic gas mixture ($\text{F}_i\text{O}_2 = 0.10\text{-}0.11$), deoxygenation of the sternocleidomastoid and intercostal muscles is exaggerated compared to normoxia (Katayama et al., 2015). Our contradictory results could be explained by the stark difference in hypoxic gas mixtures used ($\text{F}_i\text{O}_2 = 0.10$ vs. 0.15), and

resulting S_{pO_2} . In the present study, S_{pO_2} averaged 87%, compared to the 82% exhibited in the previous research (Katayama et al., 2015). There is some evidence in resting rats that respiratory muscle blood flow increases to compensate for arterial hypoxemia, serving to protect O_2 supply (Kuwahira, Gonzalez, Heisler, & Piiper, 1993). If arterial hypoxemia was greater, there is the possibility for exaggerated respiratory muscle deoxygenation. However, the degree of arterial hypoxemia induced in the present study does not appear to exaggerate respiratory muscle respiratory deoxygenation compared to similar exercise in normoxia.

Hypoxia is a known potent stimulant of hyperpnoea, which consequently elevates the work of breathing (Cibella et al., 1996; Reeves et al., 1994). As shown in the previous chapter ([Chapter 4](#)) and by others (Turner et al., 2013), an elevated work of breathing amplifies respiratory muscle deoxygenation. Since there were no meaningful differences in either ventilation patterns (f_b and IV), or inspiratory pressure generation (P_m and $\int P_m \times f_b$) in the present study, O_2 cost of hyperpnoea was likely similar between conditions (Aaron, Johnson, et al., 1992; Dominelli, Render, Molgat-Seon, Foster, & Sheel, 2014). The results provide evidence that neither the work of breathing, nor the O_2 cost of exercise hyperpnoea were significantly influenced by the F_{iO_2} used in this study. Intercostal NIRS responses look to only respond proportionally to the metabolic activity of hyperpnoea, and free from influence of hypoxemia (Costes et al., 1996; Ferrari et al., 2004; Katayama et al., 2015).

The evidence that the intercostal muscle oxygenation was not influenced by hypoxia, but vastus lateralis oxygenation trends were, has important implications for the ability to perform repeated-sprints. It is unlikely that cardiac output increased to meet the demands of the additional muscle work because 1) HR was similar between the

conditions. 2) stroke volume does not increase with work rates above 40-60% of $\dot{V}O_{2\text{peak}}$ (Higginbotham et al., 1986), and subjects in present research were exercising at 70-90% of $\dot{V}O_{2\text{peak}}$. It appears that O_2 delivery was preferentially distributed to the intercostal muscles to constrain an excessive decrease in respiratory muscle oxygenation. The work of breathing is estimated to account for 10-15% of total O_2 uptake during high-intensity exercise (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992; Harms et al., 1998). In order to maintain O_2 supply to these essential muscles, blood flow is directed away from the locomotor muscles by the sympathetic nervous system (Dempsey et al., 2006; Harms et al., 1997; Sheel et al., 2001; St Croix et al., 2000). For these reasons, the O_2 cost of exercise hyperpnoea during repeated-sprint exercise could be a contributor of impaired vastus lateralis oxygenation in hypoxia. Vastus lateralis oxygenation is implicated as an important mediating factor for the metabolic recovery between sprint efforts (Harris et al., 1976; Hogan et al., 1999; Sahlin et al., 1979), and therefore performance (Billaut & Buchheit, 2013; Buchheit et al., 2009). Reducing the O_2 cost of hyperpnoea is a potential pathway of enhancing blood flow and O_2 availability for the locomotor muscle in hypoxic environments. There is evidence that inspiratory muscle training attenuates the O_2 cost of voluntary hyperpnoea (Turner et al., 2012), and improves the self-selected recovery time between repeated-sprints (Romer et al., 2002b). However, in [Chapter 4](#) it was demonstrated that increasing respiratory muscle O_2 utilisation with inspiratory loading has no discernible effects of vastus lateralis oxygenation. Competition for available O_2 is potentially minimised by the brief periods of passive rest between sprints. As theorised by Dempsey et al. (2006), locomotor muscle fatigue is exacerbated when a high work of breathing is accompanied by sustained high-intensity exercise. But more work is still needed exploring the role of hypoxia in locomotor and respiratory muscle oxygenation trends.

5.5 CONCLUSION

As demonstrated in the past, vastus lateralis deoxygenation during repeated-sprint exercise is exaggerated by hypoxia. On the other hand, with a similar level of inspiratory pressure development, hypoxia did not affect intercostal muscle oxygenation. Blood flow appears to be preferentially distributed to the respiratory muscles in order to maintain O₂ delivery proportional to metabolic activity.

**CHAPTER SIX: EFFECTS OF INSPIRATORY
MUSCLE TRAINING ON LOCOMOTOR AND
RESPIRATORY MUSCLE OXYGENATION
TRENDS**

6.1 INTRODUCTION

The energy requirement and oxygen (O_2) cost of breathing increase relative to pulmonary ventilation (\dot{V}_E) (Aaron, Johnson, et al., 1992). During moderate intensity-exercise, the oxygen cost of breathing is 3-6%, and increases to 10-15% of pulmonary oxygen uptake ($\dot{V}O_2$) during maximal exercise (Aaron, Seow, et al., 1992; Harms et al., 1998). However, competition between locomotor and respiratory musculature for available blood and O_2 delivery flow can develop (Dempsey et al., 2006; Harms et al., 1997; Vogiatzis et al., 2009). The work of breathing incurred during high-intensity exercise causes locomotor vasoconstriction and a reduction in O_2 perfusion. (Harms et al., 1997; Mortensen, Damsgaard, Dawson, Secher, & Gonzalez-Alonso, 2008; Vogiatzis et al., 2009). This effect is mediated by an accumulation of metabolites in the respiratory muscles stimulating group IV afferent discharge (J. M. Hill, 2000), leading to sympathetically mediated efferent discharge in the locomotor muscles (Harms et al., 1997; St Croix et al., 2000). When both exercise hyperpnoea and locomotor work are high, the respiratory and locomotor muscle can compete for a limited cardiac output to maintain adequate O_2 supply for metabolic activity.

Whole body exercise in acute hypoxia hastens the development of peripheral fatigue compared to exercise in normoxia (Amann, Romer, et al., 2006; Billaut et al., 2013). Hypoxemia also stimulates \dot{V}_E , increasing the respiratory muscle load and accelerates the development of locomotor and respiratory muscle fatigue (Amann, Pegelow, et al., 2007; Babcock, Johnson, et al., 1995; Verges et al., 2010). Even when the ventilatory demands are matched between normoxia and hypoxia by exercising at a lower work rate, diaphragm fatigue is greater in hypoxia (Gudjonsdottir et al., 2001; Vogiatzis et al., 2007b). The accelerated rate of fatigue development is brought on by a

persistent respiratory muscle deoxygenation beyond that experienced in normoxia (Katayama et al., 2015). Moreover, the work of breathing incurred in hypoxia also has an influence on the development of peripheral fatigue. When the respiratory muscles are unloaded in hypoxia by ~70% with proportional assist ventilation, locomotor muscle fatigue can be attenuated by up to 40% compared to normoxia (Amann, Pegelow, et al., 2007). Therefore, specific training targeting the respiratory muscle is a potential pathway to overcome detrimental effects of hypoxemia.

Inspiratory muscle training (IMT) is associated with enhanced exercise performance during the Yo-Yo intermittent recovery test (Lomax, Grant, & Corbett, 2011; Nicks, Morgan, Fuller, & Caputo, 2009), time trials (Romer et al., 2002c; Salazar-Martínez et al., 2017; Volianitis et al., 2001), constant load cycling (Bailey et al., 2010; Mickleborough et al., 2010), and repeated-sprint exercise (Romer et al., 2002b). By improving the strength of the respiratory muscle, the relative intensity of breathing at any given \dot{V}_E decreases. Consequently, reducing the intensity of hyperpnoea would attenuate the accumulation of metabolites to blunt the respiratory muscle metaboreflex (McConnell & Lomax, 2006; Witt et al., 2007), reduce the O₂ cost of hyperpnoea (Turner et al., 2012), and lessen respiratory muscle fatigue in both normoxia and hypoxia (Downey et al., 2007). For these reasons, IMT is a possible method of alleviating the detrimental of an elevated work of breathing. But the application of IMT in repeated-sprint exercise is limited (Romer et al., 2002b).

Underpinning the ability to maintain performance during repeated-sprint exercise, is the capacity to deliver O₂ to the locomotor muscles in the short rest periods between sprints (Billaut & Buchheit, 2013; Buchheit & Ufland, 2011). When repeated sprints are performed in hypoxia, this capacity is severely impacted (Billaut & Buchheit,

2013). Surprisingly, respiratory muscle oxygenation can be maintained, and potentially mediated by preferential blood flow distribution to the respiratory muscles ([Chapter 4](#)). Reducing the O₂ cost of exercise hyperopia with IMT may increase the available O₂ to be utilised by the respiratory muscles. After a 6-week intervention, self-selected recovery time between sprints is reduced (Romer et al., 2002b), however data regarding locomotor O₂ delivery was not reported. Therefore, the underlying mechanisms for the enhanced quality of recovery between bouts after IMT remain unclear. Moreover, the application of IMT to repeated-sprint exercise performed in hypoxia has yet to be examined. Therefore, the aim of this study is to examine the effects of IMT on skeletal muscle tissue oxygenation during repeated-sprint exercise. Additionally, IMT will be examined for its influence in minimising the deleterious of hypoxia.

6.2 METHODS

6.2.1 Subjects

Ten males from a variety of athletic backgrounds were recruited to participate in this study. These subjects were the same group that chose to participate in the research presented in the previous chapter ([Chapter Five](#)), and chosen because they were accustomed to producing “all-out” bouts of exercise. Subjects self-reported to be healthy and with no known neurological, cardiovascular or respiratory diseases. After being fully informed of the requirements, benefits, and risks associated with participation, each subject gave written informed consent. Ethical approval for the study was obtained from the institutional Human Research Ethics Committee and the study conformed to the declaration of Helsinki.

Table 6.1: Subject characteristics. Inspiratory Muscle Training (IMT) and Control groups.

	Control	IMT
Age (year)	24.8 ± 2.4	27.2 ± 2.2
Body mass (kg)	77.0 ± 10.3	80.2 ± 9.3
Height (m)	177.6 ± 6.8	179.0 ± 9.0
$\dot{V}O_{2\text{peak}}$ (mL·min ⁻¹ ·kg ⁻¹)	58.91 ± 8.04	55.94 ± 5.97
$\dot{V}O_{2\text{peak}}$ (L·min ⁻¹)	3.84 ± 0.49	3.77 ± 0.52
FVC (L)	5.11 ± 0.99	5.92 ± 1.00
FVC (%predicted)	103.6 ± 9.6	105.0 ± 10.3
FEV ₁ (L)	4.50 ± 0.61	4.57 ± 0.70
FEV ₁ (%predicted)	98.0 ± 8.0	98.8 ± 10.5
MIP (cmH ₂ O)	133 ± 25	116 ± 41

Values are mean ± SD. Abbreviations are: $\dot{V}O_{2\text{peak}}$, peak pulmonary oxygen uptake; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; MIP, maximal inspiratory pressure.

6.2.2 Experimental Design

During the first visit, participants completed respiratory muscle and pulmonary function testing; and were familiarised with an incremental exercise test used in the following session. In the next session, the incremental exercise test was performed in normoxia to exercise tolerance. On the following two sessions, subjects completed familiarisations of the repeated-sprint protocol used in the main testing sessions. The main testing sessions were performed in a randomised order in normoxia and hypoxia. All exercise testing was performed on an electronically-braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) and expired gases collected on a breath-by-breath basis (COSMED Quark CPET; Cosmed, Rome, Italy).

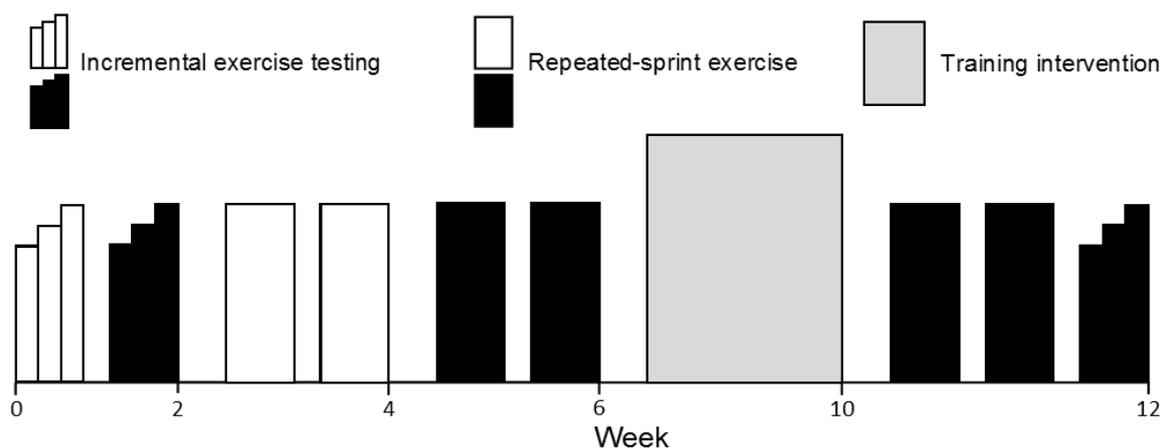


Figure 6.1: Study 4 design. Familiarisation trials are represented by the open squares, whereas experimental trials are represented by the filled squares.

6.2.3 Incremental Exercise Testing

An incremental exercise test was performed in normoxia for the determination of peak pulmonary oxygen uptake ($\dot{V}O_{2\text{peak}}$). The test was initiated at a work rate of 0 W for 3 min, followed by an increase in work rate 1 W every 2 s ($30 \text{ W}\cdot\text{min}^{-1}$) until volitional exhaustion or until the cadence fell below 10 RPM self-selected pedalling rate (Burnley et al., 2006). Peak 30 s average was calculated and used to represent $\dot{V}O_{2\text{peak}}$.

6.2.4 Repeated-sprint Exercise

Trials were performed in a single-blind semi-random order, ensuring a balance of normoxic and hypoxic trials pre- and post-testing. All testing was conducted within a 23.92 m^2 environmental exercise laboratory, and environmental hypoxia was achieved via nitrogen dilution. Fraction of inspired O_2 for pre-testing was 0.2084 ± 0.005 and 0.1455 ± 0.0031 for normoxia and hypoxia respectively. Post-testing, the fraction of inspired O_2 was 0.2071 ± 0.0022 and 0.1443 ± 0.0036 , for normoxia and hypoxia respectively.

After arriving at the laboratory, subjects were fitted with NIRS probes and a heart rate monitor. Testing was performed with the cycle ergometer set to isokinetic

mode (120 RPM). Cadence was fixed for every sprint so that exercise-induced changes in mechanical power and physiological responses were not influenced by cadence (Gotshall et al., 1996; Tomas et al., 2010). After a 7-min warm-up consisting of 5 min of unloaded cycling at 60-70 RPM and two 4 s sprints (separated by 1 min), subjects rested for another 2.5 min before the RS protocol was initiated. The RS protocol was ten 10 s sprints separated by 30 passive rest (5.5 min). Subjects were instructed to give an “all-out” effort for every sprint and verbally encouraged throughout to promote a maximal effort. Each sprint was performed in the seated position and initiated with the crank arm of the dominant leg at 45°. Before sprint one, subjects were instructed to accelerate the flywheel to 95 rpm over a 15-s period and assume the ready position 5 s before the commencement of the test. This ensured that each sprint was initiated with the flywheel rotating at ~90 rpm so that subjects could quickly reach 120 rpm. To minimise the chance of a protective pacing strategy, the first 10 s sprint of the repeated-sprint series was examined to ensure that peak power output exceeded that of the two preceding 4 s sprints. In only one instance during pre-testing was the peak power produced by a subject below that of the preceding sprints. Consequently the subject was asked to immediately terminate the sprint activity and passively rest for 5 min before the repeated-sprint protocol was restarted. The handlebars and seat were individually adjusted to each subjects’ characteristic and feet secured using toe cages and retention straps fitted to the ergometer. Crank arm length was standardized to 175 mm. Visual feedback of power output was not available to the subjects during any sprint. The cycle ergometer software provides power and cadence data at 4 Hz. Data was exported into excel for the calculation of mechanical work completed and power production for individual sprints, and over the entire protocol. Inspiratory loading was achieved by placing a plastic disk with a 10-mm opening over the inspiratory side of a two-way non-rebreathing valve (Hans Rudolph inc.,

Kansas, United States of America) attached to the distal end of the breath-by-breath gas sampling line and turbine. The inspiratory load was applied after warmup, 1 min before the commencement of the RS protocol.

6.2.5 Metabolic and Ventilatory Measurements

Subjects wore a silicone facemask which the breath-by-breath gas sampling line and turbine were attached. The analyser was calibrated before each test against known gas concentrations (normoxia: 16% O₂ and 5% CO₂; hypoxia: 7% O₂ and 5% CO₂) and the turbine volume transducer was calibrated using a 3 L syringe (Cosmed, Rome, Italy). Data was then exported into Excel so that $\dot{V}O_2$ could be inspected for errant data points. Editing data was performed to remove the occasional errant breaths caused by for example swallowing or coughing which were considered not be reflective the metabolic responses to exercise. These errant data points were removed by the same researcher in every case before values greater than 4 standard deviations from the local mean were removed (Lamarra et al., 1987; Rossiter et al., 2000). A 5-breath rolling average was then applied for the calculation of peak and nadir for every 40-s sprint/recovery period to give a single value for each sprint and recovery phase. Breathing frequency (f_b) was averaged over the entire sprint protocol to give one value for each subject per trial. Because the facemask was removed immediately after the tenth sprint, only maximum values were calculated over the first 10 s. Mouth pressure (P_m) was recorded continuously at 50 Hz with a pressure transducer (Honeywell, New Jersey, United States of America) attached to the saliva port of the non-rebreathing valve via Tygon tubing. Representative data from one subject of the effects of inspiratory muscle loading on P_m is displayed in Figure 1. Mean inspiratory and expiratory P_m were then calculated as an index of respiratory muscle work as well as mean peak inspiratory P_m . An index of inspiratory muscle force

development was also calculated for each exercise trial ($\dot{V}P_m \times f_b$) (Witt et al., 2007). For statistical analysis, inspiratory P_m was converted to positive values and presented in the results as such. Arterial oxygen saturation (estimated by fingertip pulse oximetry; S_{pO_2}) and heart rate (HR) was sampled on a breath-by-breath basis integrated into the COSMED system.

6.2.6 Near-infrared Spectroscopy

Subjects were instrumented with two NIRS probes to assess muscle O_2 status (Oxymon MKIII, Artinis, The Netherlands). The first probe was fixed over the distal part of the vastus lateralis muscle belly approximately 15 cm above the proximal border of the patella. The second was fixed over the sixth intercostal space at the anterior axillary line to assess changes in the accessory respiratory muscles. Probes were held in place with black plastic spacers secured to the skin using double sided stick disks and shielded from light using a black self-adhesive elastic bandage. Optode spacing was set to 4.5 cm and 3.5 cm for vastus lateralis and respiratory muscles respectively. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose tissue thickness covering the muscle. The skinfold thickness for vastus lateralis (1.19 ± 0.69 cm) and respiratory muscles (1.12 ± 0.44 cm) was less than half the distance between the emitter and the detector in every case. A differential pathlength factor of 4.95 was used (Smith & Billaut, 2010; Subudhi et al., 2007). Data was acquired at 10 Hz. A 10th order zero-lag low-pass Butterworth filter was applied to the data to remove artefacts and smooth pedalling induced fluctuations; the resulting output was used for analysis (Rodriguez et al., 2018). The application of the filter was conducted in the *R* environment (R Core Team, 2016) using the *signal* package (Signal developers, 2013). Vastus lateralis deoxyhaemoglobin was normalised to femoral artery occlusion so

that 0% represented a 5-s average immediately prior the occlusion and 100% represented the maximum 5 s average. Peaks and nadirs were then identified within every 40-s sprint/recovery period to represent each sprint and recovery phase respectively (HHb_{VL}). Reoxygenation capacity (Δ Reoxy, %) was also calculated as the change from peak to nadir. Respiratory muscle oxyhaemoglobin (O₂Hb_{RM}) and deoxyhaemoglobin (HHb_{RM}) were expressed as an absolute change from baseline. Baseline was established before warm-up while seated quietly on the cycle. Because there were no clear peaks and nadirs in the HHb_{RM} signal, an average was calculated for of each 40-s sprint/recovery period.

6.2.7 Inspiratory Muscle Training

Following pre-intervention testing, subjects were randomly assigned to either Inspiratory Muscle Training (IMT) or Control to complete 4 weeks of training using POWERbreathe® (light resistance) pressure threshold device (POWERbreathe®, HaB International Ltd, UK). Subjects were naïve that a Control group existed, but were informed that the study was investigating the effects of strength (IMT) vs. endurance (Control) respiratory muscle training. The IMT group completed 30 inspiratory efforts twice per day (AM and PM) 7 days per week at a pressure threshold corresponding with 50% MIP (Romer et al., 2002b; Volianitis et al., 2001). Once participants could complete 30 breaths comfortably, they were instructed to increase the pressure threshold. The Control group completed 60 breaths once per day (AM or PM) 7 days per week at a pressure threshold corresponding with 15% MIP and remained at this level for the entirety of the intervention period (Romer et al., 2002b; Volianitis et al., 2001). All subjects were instructed to initiate each breath from residual volume until reaching their total lung capacity, and to perform the manoeuvre in a slow controlled manner to prevent

hypocapnia. On a weekly basis, subjects visited the laboratory for respiratory muscle strength testing. Subjects also completed a diary to monitor compliance to training and daily exercise. Exercise load was determined by the product of session RPE (11-point scale) and session duration, and expressed as arbitrary units (AU) (Foster, 1998). The exercise load was summed to create a weekly exercise lode.

6.2.8 Statistical Analysis

Data in text and figures are presented as mean \pm standard deviation. Custom spreadsheets were used to analyse the effects of training on laboratory measurements (Hopkins, 2006b). To assess the difference between groups at baseline, and the effects of training within groups, analysis was performed using the post-only crossover spreadsheet. If the within group training effects were deemed to be meaningful, analysis of the between group training effects were assessed using the pre-post parallel group spreadsheet. All measures, other than S_{pO_2} , RPE, and HHb_{VL} were log-transformed before analysis then back-transformed to express the changes in percent units and standardized effects. Because O_2Hb_{RM} values were negative (relative to baseline), they were inversed before log-transformation. Results were reversed to preserve the direction of O_2Hb_{RM} . Relative changes (%) and effect size statistics are expressed with 90% confidence limits (90% CL). Practical significance was assessed by calculating Cohen's d effect size (ES) (Cohen, 1988). Standardized effect sizes of <0.2 , $>0.2 - 0.5$, $>0.5 - 0.8$, >0.8 were considered to as trivial, small, moderate and large respectively and presented with 90% CL. Probabilities were also calculated to establish if the chance the true (unknown) differences were lower, similar or higher than the smallest worthwhile change (ES = 0.2). Effects were not considered meaningful if there was $<75\%$ probability of being substantially positive/negative relative to the smallest worthwhile change. If the chance

of having higher/lower values than the smallest worthwhile difference was both >5%, the true difference was assessed as *unclear*. For clear effects, the likelihood that the true effect was substantial were assessed qualitatively as follows: *likely* (75 to <95%), *very likely* (95 – 99.5%), *almost certainly* (>99%) (Batterham & Hopkins, 2006; Hopkins et al., 2009). Pearson's product-moment correlation (r) was computed in the R environment (R Core Team, 2016) to assess the degree of the relationship between MIP and total work completed. The following criteria were adopted to interpret the magnitude of the correlation between variables: ≤ 0.1 , trivial; $>0.1 - 0.3$, small; $>0.3 - 0.5$, moderate; $>0.5 - 0.7$, large; $>0.7 - 0.9$, very large; and $>0.9 - 1.0$, almost perfect (Hopkins et al., 2009).

6.3 RESULTS

6.3.1 Adherence to Training and Exercise Load

The mean prescribed pressure threshold level of the two groups is presented in Figure 6.2 A. The Control group was expected to complete 28 sessions over 4 weeks, and the IMT group 56 sessions. There was a 99% and 94% adherence in the Control and IMT groups respectively. Weekly and total exercise load over the 4-week intervention period is presented in Figure 6.2 B. There was no meaningful difference in total exercise load between the groups (relative difference =13.7%, 90% CL $\pm 108.7\%$; standardised effect size = 0.19, 90% CL ± 1.23).

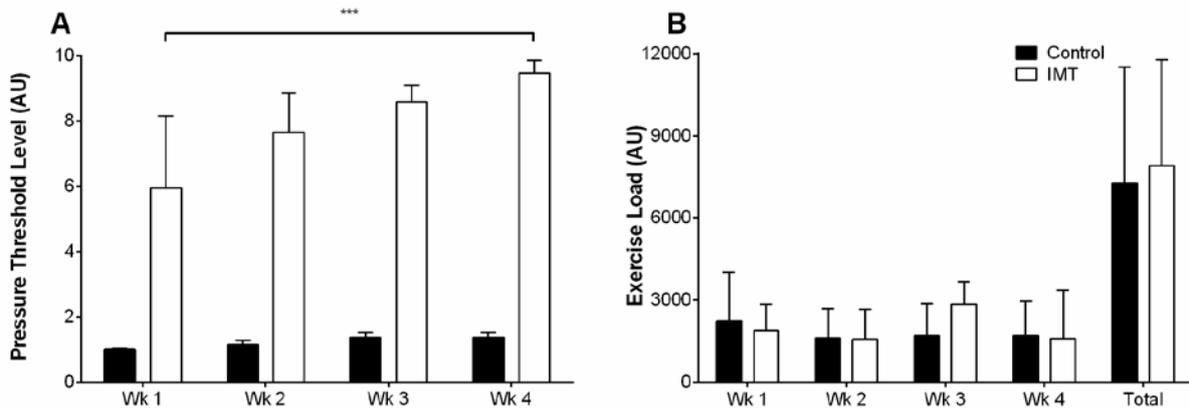


Figure 6.2: Pressure threshold level (A) and exercise load (B) presented as mean \pm SD for the Inspiratory Muscle Training (IMT) and Control groups. The symbols (*) represent a between group comparison that the chance of the true effect exceeds a small (-0.2 - 0.2) effect size. The number of symbols one, two and three denote likely, very likely, and almost certainly respectively, of the likelihood of the effect being substantial.

6.3.2 Respiratory Muscle and Pulmonary Function

Weekly relative change in maximal inspiratory mouth pressure (MIP) from baseline is presented in Figure 6.3. Differences between groups were *unclear* at baseline (-16.4%, \pm 31.9%; ES = -0.53, \pm 1.11). After the intervention period, MIP increased 34.7%, \pm 25.6% in the IMT group above the Control group (ES = 0.93, \pm 0.59; *very likely*). At baseline, differences in FVC between IMT and Control were *unclear* (2.2%, \pm 24.8; ES = 0.11, \pm 1.19). After the intervention changes were *unclear* in both the IMT (-0.8%, \pm 7.9%; ES = -0.04, \pm 0.37) and Control groups (-9.6, \pm 12.5; ES = -0.50, \pm 0.68). Differences in FEV₁ at baseline between IMT and Control were *unclear* (1.4%, \pm 12.6%; ES = 0.08, \pm 0.74). Changes after the intervention period were *unclear* in both the IMT (0.5%, \pm 10.0%; ES = 0.02, \pm 0.50) and Control groups (-11.4%, \pm 12.6%; ES = -0.72, \pm 0.97).

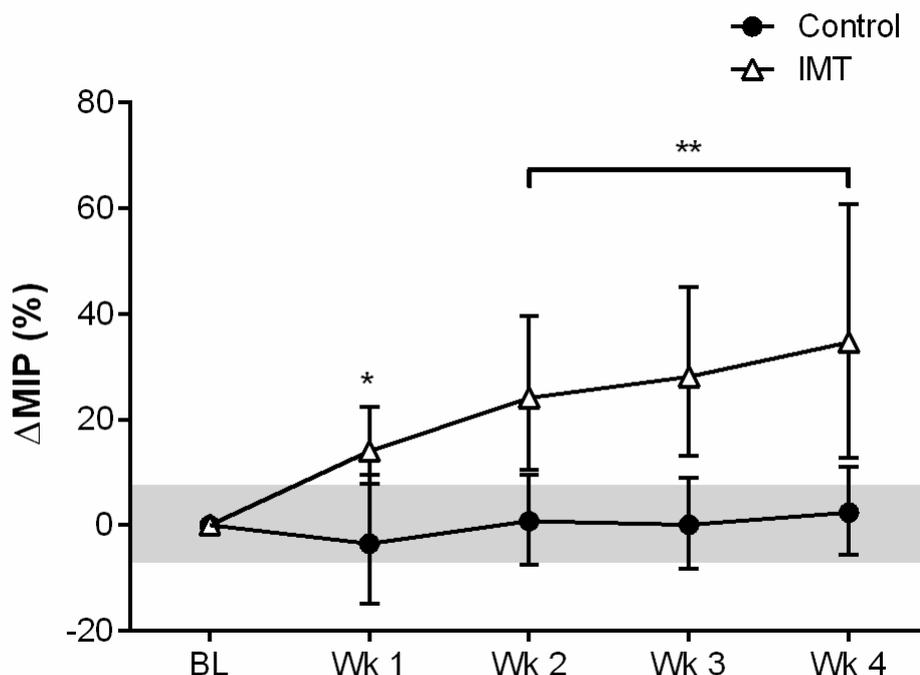


Figure 6.3: Relative change of maximal inspiratory mouth pressure (MIP) from baseline (BL) after each week of the intervention period for the Inspiratory Muscle Training (IMT) and Control groups with 90% CL. The grey shaded area represents the smallest worthwhile change as a percent. The symbols (*) represent a within group comparison relative to BL that the chance of the true effect exceeds a small (-0.2-0.2) effect size. The number of symbols one and two denote likely and very likely respectively, of the likelihood of the effect being substantial.

6.3.3 Incremental Exercise

After the intervention period, there was no meaningful change in $\dot{V}O_{2peak}$ in the IMT group (-3.4%, $\pm 6.0\%$; ES = -0.21, ± 0.39). Additionally, the change was *unclear* in the Control group (-10.8%, $\pm 16.8\%$; ES = -0.73, ± 1.20).

6.3.4 Repeated-sprint Exercise

Total mechanical work completed for both training groups for Normoxia and Hypoxia is presented in Figure 6.4, and the physiological responses to repeated-sprint exercise are presented in Table 6.2. There were *unclear* effects of IMT on total work completed during both the NM (-5.9%, $\pm 20.6\%$; ES = -0.40, ± 1.42), and HY trials (0.9%, $\pm 4.1\%$; ES = 0.03, ± 0.11). Similarly, changes in the CON group after the intervention period were *unclear* in NM (-1.9%, $\pm 3.2\%$; ES = -0.11, ± 0.30); and not meaningful in HY (-

6.5%, $\pm 9.4\%$; ES = $-0.32, \pm 0.48$). Muscle oxygenation effects to inspiratory muscle training are presented in Figure 6.5 and Figure 6.6. In Hypoxia, vastus lateralis ΔReoxy was 9.3%, $\pm 5.4\%$ greater post intervention in the Control group. This translated to a *likely* moderate between group effect ($-11.2\%, 12.0\%$; ES = $-0.78, 0.84$). The relationship between MIP and total work is presented in Figure 6.6. There was a small correlation between MIP and total work in normoxia, and a moderate correlation in hypoxia.

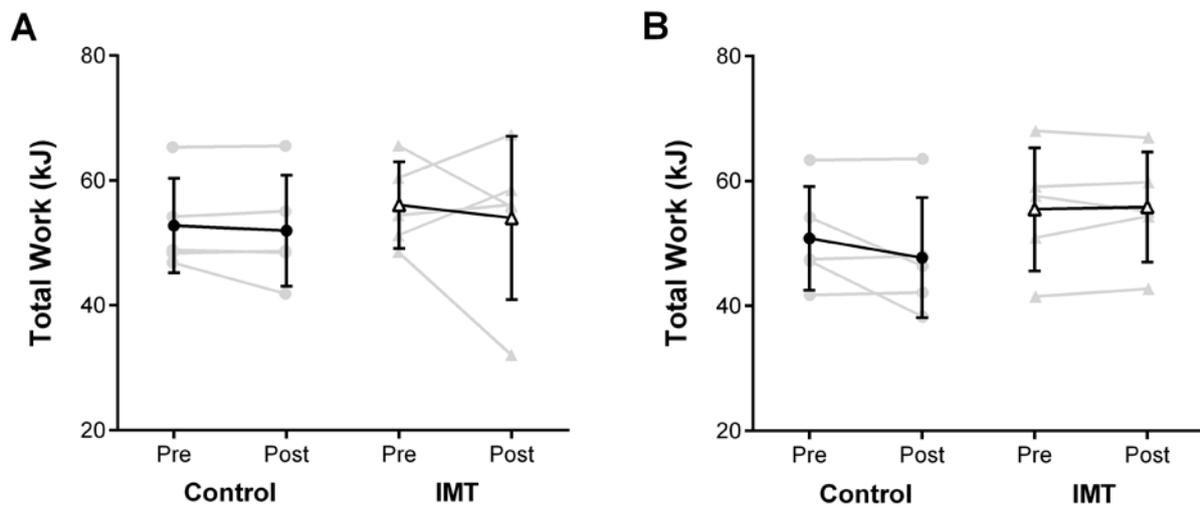


Figure 6.4: Total mechanical work completed during repeated-sprint exercise pre- and post-intervention in Normoxia (A) and Hypoxia (B) for both the Control and Inspiratory Muscle Training (IMT) groups. Individual total mechanical work for each subject is represented by the grey lines, and mean \pm SD is represented by the black lines.

*Respiratory Muscle Work and Tissue Oxygenation
Trends During Repeated-sprint Exercise*

Table 6.2: Physiological responses to repeated-sprint exercise pre-and post-Inspiratory muscle training.

	Control		IMT	
	Pre	Post	Pre	Post
Normoxia				
Peak $\dot{V}O_2$ (L·min ⁻¹)	3.62 ± 0.39	3.57 ± 0.40	3.45 ± 0.53	3.52 ± 0.52
Nadir $\dot{V}O_2$ (L·min ⁻¹)	2.96 ± 0.44	2.91 ± 0.26	2.83 ± 0.49	2.76 ± 0.43
S _P O ₂ (%)	96 ± 1	97 ± 2 *	96 ± 2	95 ± 3 †
Peak P _m (cmH ₂ O)	2.77 ± 0.61	2.83 ± 0.87	3.06 ± 0.43	3.06 ± 0.54
∫P _m × f _b	65.67 ± 12.74	63.30 ± 14.36	70.67 ± 15.93	67.81 ± 12.69
Hypoxia				
Peak $\dot{V}O_2$ (L·min ⁻¹)	3.31 ± 0.28	3.29 ± 0.26	3.44 ± 0.39	3.35 ± 0.30
Nadir $\dot{V}O_2$ (L·min ⁻¹)	2.83 ± 0.31	2.71 ± 0.20	2.74 ± 0.36	2.58 ± 0.31
S _P O ₂ (%)	87 ± 3	87 ± 4	86 ± 2	88 ± 4
Peak P _m (cmH ₂ O)	2.84 ± 0.76	2.85 ± 0.62	3.24 ± 0.67	3.06 ± 0.41
∫P _m × f _b	68.92 ± 12.99	66.90 ± 8.59	72.04 ± 13.84	67.73 ± 8.35

Abbreviations are: $\dot{V}O_2$, pulmonary oxygen uptake; S_PO₂, arterial oxygen saturation; P_m, inspiratory mouth pressure; ∫P_m × f_b, inspiratory muscle force development. The * symbol indicates substantial within group effects. Whereas the † symbol indicated a substantial between group effect. The symbols denote a likely chance of the true effect exceeds a small (-0.2 – 0.2) effect size.

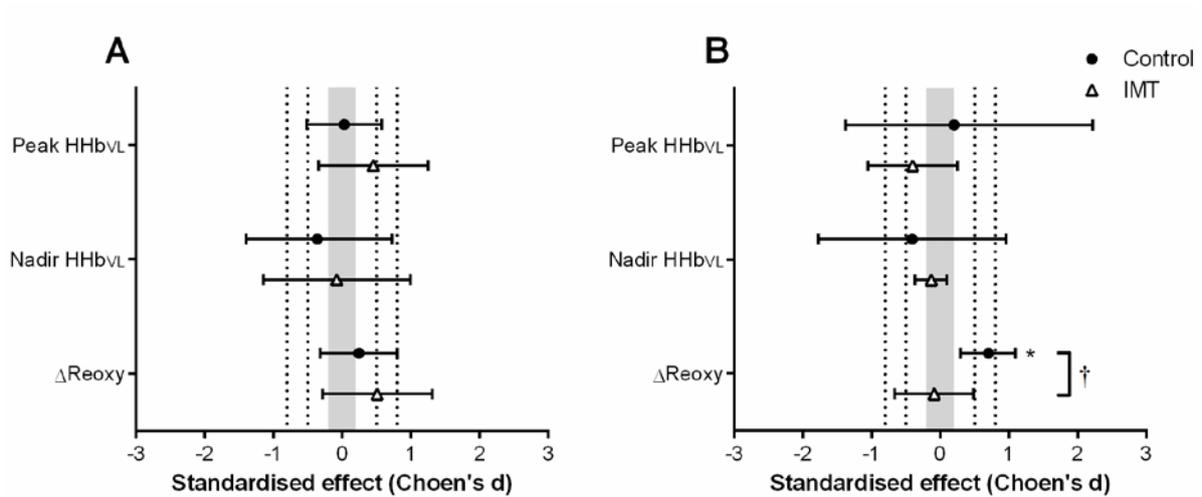


Figure 6.5: Standardised effects for the change in locomotor muscle oxygenation responses to repeated-sprint exercise in normoxia (A) and hypoxia (B) for both the Control and Inspiratory Muscle Training (IMT) groups. The grey shaded area represents the smallest worthwhile change in standardized units. The dotted lines denote the thresholds for small, moderate and large effects. The * symbol indicates substantial within group effects. Whereas the † symbol indicated a substantial between group effect. The number of symbols one and two denote likely and very likely respectively, of the likelihood of the effect being substantial.

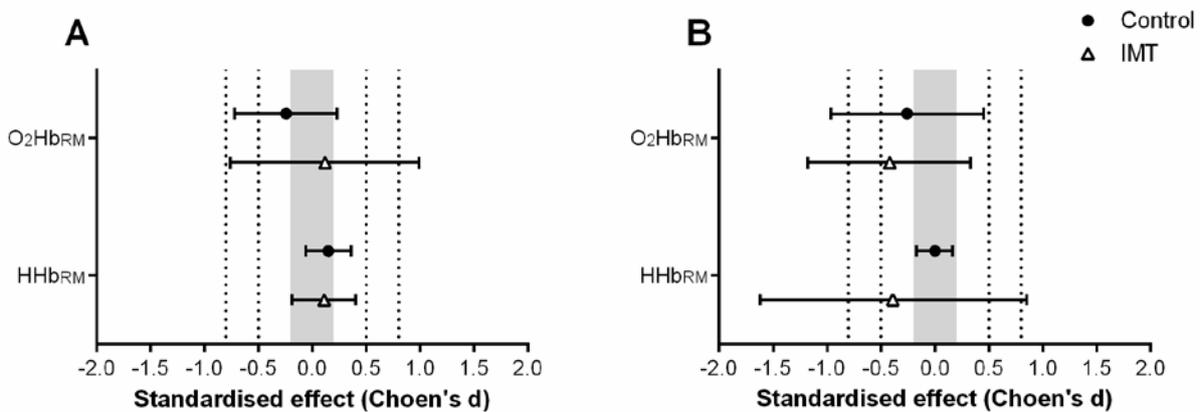


Figure 6.6: Standardised effects for the change in respiratory muscle oxygenation responses to repeated-sprint exercise in normoxia (A) and hypoxia (B) for both the Control and Inspiratory Muscle Training (IMT) groups. The grey shaded area represents the smallest worthwhile change in standardized units. The dotted lines denote the thresholds for small, moderate, and large effects.

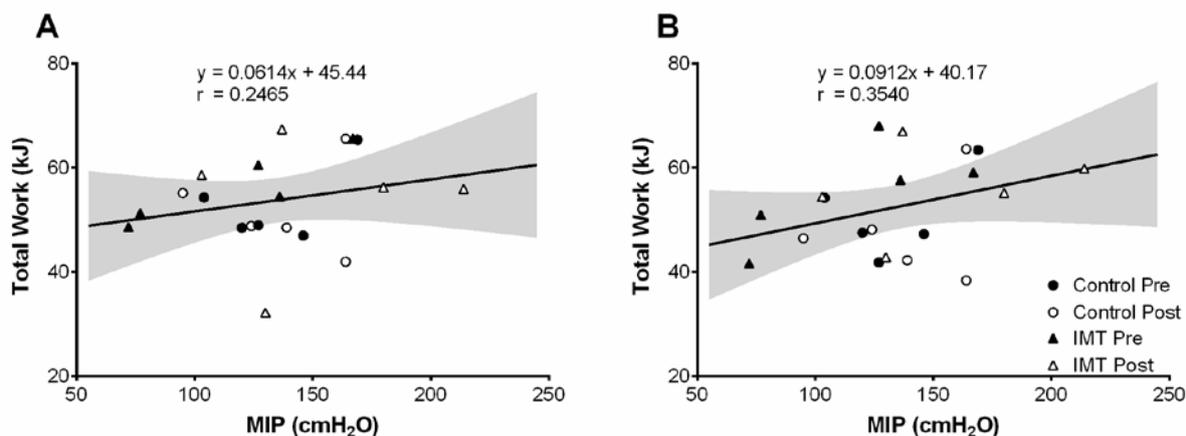


Figure 6.7: Relationship between maximal inspiratory mouth pressure (MIP) and total work completed in normoxia (A) and hypoxia (B) for the Inspiratory Muscle Training (IMT) and Control groups. Linear regression models represented by the solid line with 95% confidence intervals shown by the grey shaded area.

6.4 DISCUSSION

We investigated the effects of IMT on locomotor and respiratory muscle oxygenation trends, and performance in repeated-sprint exercise. The training intervention successfully increased MIP, but there was no substantial change in muscle oxygenation or repeated sprint performance. Though there was a small and moderate correlation of MIP and total work completed in normoxia and hypoxia, the increase in MIP from baseline was not large enough to translate to any meaningful change in repeated-sprint performance. Based on the regression equation, MIP would need to increase 52% for the change in total work to exceed the smallest worthwhile change in normoxia for this population. Whereas a smaller change of 38% in MIP is needed for a meaningful difference in hypoxia

6.4.1 Respiratory muscle and pulmonary function adaptation

After the end of the intervention period, the IMT group increased their MIP by 34.6%. While the control group has a non-meaningful change of 2.4%. The training program we used is a common form of training that has produced similar changes in

pressure-generating capacity of the respiratory muscles (Downey et al., 2007; Lomax et al., 2011; Romer et al., 2002b; Salazar-Martínez et al., 2017; Turner et al., 2012). This change in respiratory muscle strength is likely the result of diaphragm hypertrophy induced by the IMT. Diaphragm thickness has been found to have a moderate correlation with mouth pressure generation capacity (Orrey, Unger, & Hanekom, 2014). After a similar training protocol of IMT, diaphragm thickness can increase up to ~10%, and MIP by 24% after 4 weeks of training (Downey et al., 2007). A longer training intervention could produce greater results. However, the effectiveness of IMT appears to diminish over time. Over an 11-week training period, MIP has been demonstrated to increase by 45% (Volianitis et al., 2001). But by week 4, MIP had already increased by 41%. Therefore, extending the training in the present study is unlikely to have provided and added benefits that could be translated to enhanced repeated-sprint performance.

No meaningful changes in pulmonary function (FVE and FEV₁) were induced by the training intervention in the present study. This is consistent with others who have shown no change in pulmonary function following IMT in healthy subjects (Romer et al., 2002b; Salazar-Martínez et al., 2017; Turner et al., 2012), and those with chronic pulmonary conditions (Shahin, Germain, Kazem, & Annat, 2008; Turner et al., 2011). These data support the specificity of IMT, that adaptations are localised to the pressure generating capacity of the respiratory muscles. The effectiveness of IMT in clinical populations is not a change in pulmonary function, but an unloading of the respiratory muscle to alleviate sensations of dyspnoea (el-Manshawi, Killian, Summers, & Jones, 1986; Turner et al., 2011).

6.4.2 Repeated-sprint performance and Tissue Oxygenation

Contrary to others (Romer et al., 2002b), IMT did not alter repeated-sprint ability. Increasing the strength of the inspiratory muscles can act as a form of respiratory muscle unloading, lowering the O₂ cost of voluntary hyperpnoea (Turner et al., 2012), and delay the activation of respiratory muscle metaboreflex (McConnell & Lomax, 2006; Witt et al., 2007). Because of the strong link between locomotor muscle O₂ delivery and recovery from multiple sprint activity (Billaut & Buchheit, 2013), IMT is an appealing training regime to enhance performance. As such, IMT has attenuated self-selected recovery time, with no concurrent change in 20 m sprint times (Romer et al., 2002b). This implies that recovery was accelerated, potentially linked to the perceptual and metabolic changes that were observed during submaximal exercise. The difference in sprint exercise protocols may have played a role in the discrepancies of performance outcomes following IMT. Romer et al (2002b) used sprints over a fixed distance of 20 m, which in average was covered in ~3.18 s. This represents approximately one third the duration of the sprints in the present study. The effectiveness of IMT may be limited to repeated-sprint exercise that consists of sprints <5 s. Sprints of a longer duration may cause physiological disturbances that surpass the effectiveness of IMT. That unloading the respiratory muscle by increasing their relative strength, and the associated decrease in O₂ cost of hyperpnoea, appears to be insignificant in repeated-sprints lasting 10 s.

Considering inspiratory muscle force development was similar to pre-post intervention, a smaller fraction of the maximal pumping capacity would have been utilized in the present study. Despite the presumed reduction in O₂ cost, and relative intensity of breathing, there was no change in oxygenation trends. Arterial hypoxemia is a strong limiting factor in locomotor tissue reoxygenation (Billaut & Buchheit, 2013).

Hypoxemia also stimulates \dot{V}_E , and hastens the development of respiratory muscle fatigue via deoxygenation of these muscles (Babcock, Johnson, et al., 1995; Katayama et al., 2015; Verges et al., 2010). But with IMT the relative intensity of hyperpnoea can be lessened, blunting the development of fatigue (Downey et al., 2007), and reducing the O₂ cost of voluntary hyperpnoea (Turner et al., 2012). Regardless of evidence supporting IMT to alleviate O₂ competition, it did not translate to any change in muscle oxygenation, or performance.

Attenuating the relative intensity of hyperpnoea through IMT decrease the O₂ cost of ventilation, especially at a higher \dot{V}_E (Turner et al., 2012). Despite this evidence, there was no clear change in oxygenation trends after the intervention period in either locomotor or respiratory muscles in the present study. The lack of change in muscle oxygenation observed after a period of IMT is consistent with previous research also using maximal exercise (Turner et al., 2016). This may be in part due to the cardiovascular strain placed on the system at high workloads. At work rates above 80% $\dot{V}O_{2max}$, cardiac output and mean arterial blood pressure begin to plateau (Vogiatzis et al., 2009). As a result, limb and respiratory muscle blood flow is constrained, or even slightly reduced (Mortensen et al., 2008; Vogiatzis et al., 2009). An overriding vasoconstriction plays a functional role in maintaining blood pressure by constraining blood flow to the active muscles (Saltin, Radegran, Koskolou, & Roach, 1998). Combined, these data demonstrate that a ~30% increase in MIP does not alter the relative intensity of hyperpnoea in a meaningful way as to reduce the O₂ cost of breathing in maximal exercise.

The intermittent nature of repeated-sprint exercise may also explain the ineffectiveness of IMT. During the sprint and recovery phases, $\dot{V}O_2$ and locomotor muscle

O₂ extraction fluctuates between sprint efforts (Billaut & Buchheit, 2013; Buchheit et al., 2009; La Monica et al., 2016). Therefore, there is limited competition for available cardiac output to meet the demands of both locomotor and respiratory muscles. In the previous chapter ([Chapter 5](#)), I demonstrated that inspiratory loading does not compromise locomotor muscle oxygenation, achieved by a ~4% increase in $\dot{V}O_2$ to accommodate the elevated work of breathing. It appears that there is enough reserved in the cardiovascular system to meet the demands of both locomotor and respiratory muscles, and the respiratory muscles are not sufficiently challenged to warrant IMT as a method of improving repeated-sprint exercise.

6.4.3 Limitations

Though the diaphragm muscle is the primary respiratory flow generator, a direct measurement of oxygenation is difficult due to the complex arrangement of the respiratory muscles. Therefore, accessory intercostal muscles are relied on for an index of global respiratory muscle oxygenation (Katayama et al., 2015; Turner et al., 2016). Additionally, the complicated arrangement of the respiratory muscles it is not possible to apply arterial occlusion to these muscles. Instead [O₂Hb] and [HHb] were measured as an absolute change from baseline. While NIRS yields information on muscle O₂ perfusion and extraction in the underlying tissue, the technique employed does not provide a measurement of blood volume. Using the light absorbing tracer indocyanine green dye, muscle blood flow can be determined (Vogiatzis et al., 2009). Limits in the current study restrict the ability to draw conclusions on the distribution of cardiac output after IMT.

Lastly, respiratory muscle pressure generation and work completed can be directly calculated with the use of oesophageal balloons (Verges et al., 2010; Vogiatzis et al., 2009). In the present study, a non-invasive measurement of respiratory muscle force

development ($\dot{V}P_m \times f_b$) was used (Witt et al., 2007). Therefore, clear conclusions on the effects of IMT on the work of breathing after the intervention period are difficult.

6.5 CONCLUSION

This is the first study to investigate the effects of pressure threshold IMT on skeletal muscle tissue oxygenation and repeated-sprint cycling exercise. Following 4 weeks of IMT, a 34.7% increase in MIP did not alleviate O₂ competition between locomotor and respiratory muscles. Additionally, there was no ergogenic benefits of the training on repeated-sprint performance in either normoxia or hypoxia. This study does not support the use of IMT to reduce respiratory muscle O₂ utilisation, or locomotor muscle oxygenation during repeated-sprint exercise.

CHAPTER SEVEN: SUMMARY AND CONCLUSIONS

7.1 SUMMARY OF MAIN FINDINGS

The theory of competition between locomotor and respiratory muscles for available cardiac output, and therefore O₂, proposed by Dempsey and colleagues (Aaron, Seow, et al., 1992; Amann, Pegelow, et al., 2007; Dempsey et al., 2006; Harms et al., 1997) was examined in this thesis in a new model of exercise. Specifically, this thesis investigated the role respiratory muscle work plays in the balance of vastus lateralis O₂ delivery and uptake in a repeat-sprint exercise model.

Since NIRS responses are used as a surrogate for metabolic perturbations, detecting the magnitude of change in oxygenation status is critical for assessing the influence of interventions and environmental factors. Therefore, in study 1 ([Chapter Three](#)), common methodologies used to smooth and identify peaks and nadirs in vastus lateralis [HHb] were evaluated. Additionally, to overcome the limitation of an arithmetic mean, a Butterworth filter was used to smooth the raw NIRS signal. Analysis revealed that 1) the size of an averaging window (2 vs. 5 s) influences the outcomes of analysis, 2) a larger analysis window underestimates the vastus lateralis [HHb] response compared to a smaller window, and 3) values obtained from predetermined time-points underestimate the magnitude of change relative to values obtained from a rolling approach. For the most accurate representation of NIRS responses to repeated-sprint exercise, it was suggested that 1) a digital filter be used to smooth NIRS data, rather than an arithmetic mean, and 2) a rolling approach be used to determine peaks and nadirs rather than obtaining values from predetermined time-points. These recommendations were then applied to the analysis conducted in the forthcoming research chapters.

7.1.1 Work of breathing and respiratory muscle oxygenation

For the first time, respiratory muscle oxygenation has been investigated in repeated-sprint exercise. There was a progressive deoxygenation from baseline that began to plateau by sprints 3-4, which is directly related to the metabolic activity of respiratory muscles (Nielsen et al., 2001; Turner et al., 2013). It is estimated that the O_2 cost of hyperpnoea accounts for 10-15% of whole body $\dot{V}O_2$ during high-intensity exercise (Aaron, Johnson, et al., 1992; Aaron, Seow, et al., 1992; Harms et al., 1998). By elevating the work of breathing with inspiratory loading, this effect was amplified in study 2 ([Chapter Four](#)). To accommodate the heightened inspiratory work, $\dot{V}O_2$ was elevated by 4-5%. Perceptually, subjects reported a substantial increase in their difficulty of breathing, while there was no clear difference in their perception of exercise difficulty. The sensations of respiratory effort are likely derived from feedback originating at the respiratory muscles regarding tension and displacement (el-Manshawi et al., 1986). No measures of respiratory muscle fatigue were taken in this study. Therefore, it is unclear if a loss in pressure generating capacity contributed to the respiratory sensations during exercise. At a minimum, this was unlikely to occur during the control exercise condition since respiratory muscle fatigue is not induced by repeated-sprint exercise (Minahan et al., 2015). But, it is unclear if fatigue developed in the respiratory muscles during the inspiratory loading trial.

When respiratory muscle oxygenation patterns were evaluated in response to acute arterial hypoxemia, there were no clear differences when compared to normoxia (study 3, [Chapter Five](#)). There was also no meaningful differences in either ventilation patterns (f_b and IV), or inspiratory pressure generation (P_m and $\int P_m \times f_R$). Therefore, the O_2 cost of exercise hyperpnoea was likely similar between conditions (Dominelli et al.,

2014). This is supported by the evidence of a similar concurrent rise and fall of [HHb] and [O₂Hb] relative to baseline respectively. This suggests that O₂ delivery to the respiratory muscles is maintained close to the levels of normoxia. Therefore, there is minimal effect of hypoxemia on the O₂ status of the respiratory muscles during repeated-sprint exercise. Others have reported an exaggerated deoxygenation of the respiratory muscles in hypoxia during voluntary isocapnic hyperpnoea (Katayama et al., 2015). However, their hypoxia gas mixture resulted in a lower S_PO₂ of 82% compared to the average 87% in subjects in study 3. A hypoxic threshold may exist where respiratory muscle O₂ delivery can be maintained close to the rate of that during exercise in normoxia. If arterial hypoxemia was greater in study 3, further desaturation of the respiratory muscles may have been detected. The evidence in this chapter suggests that during repeated-sprint exercise, respiratory muscle O₂ status is no further compromised compared to normoxia. Perhaps there is preferential blood flow distribution which maintains constant O₂ supply to the respiratory muscles proportional to metabolic activity (Costes et al., 1996; Ferrari et al., 2004).

In study 4 ([Chapter Six](#)), the inspiratory muscles were strengthened using a pressure threshold device (POWERbreathe®, HaB International Ltd, UK), and mimicking a training protocol of previous work in the area (Downey et al., 2007; Romer et al., 2002b; Turner et al., 2012; Volianitis et al., 2001). Strengthening the respiratory muscles acts as form of respiratory muscle unloading, decreasing the O₂ cost, and relative intensity of exercise hyperopia (Downey et al., 2007; Turner et al., 2012; Witt et al., 2007). After the 4-week intervention period, subjects increased the pressure generating capacity of their inspiratory muscles by 34%, which is consistent with previous research (Downey et al., 2007; Lomax et al., 2011; Romer et al., 2002b; Salazar-Martínez et al., 2017; Turner et al.,

2012). Considering inspiratory pressure during exercise was similar pre/post intervention, a smaller fraction of the maximal pumping capacity would have been utilised. However, the change was too small to have any clear effects on respiratory muscle oxygenation in normoxia, or hypoxia. It is possible that the respiratory workload was experienced during repeated-sprint exercise is too low for inspiratory muscle training to be effective. A decrease in respiratory muscle [HHb] during “heavy” inspiratory loading has been demonstrated while cycling at 80% $\dot{V}O_{2peak}$, reflecting a decrease in O_2 extraction by the active musculature (Turner et al., 2016). However, no change in muscle oxygenation post intervention was observed during moderate inspiratory loading, or during maximal exercise (cycling at 100% $\dot{V}O_{2peak}$). This evidence, combined with the evidence of study 4, suggest that inspiratory muscle training has no effect on respiratory muscle oxygenation with the normally occurring work of breathing.

7.1.2 Influence of respiratory muscle work on vastus lateralis oxygenation trends

Respiratory muscle work has been implicated as a limiting factor of limb O_2 perfusion (Dempsey et al., 2006). However, competition between locomotor and respiratory muscle for available cardiac output does not appear to be a significant characteristic of repeated-sprint exercise. In study 2 when the work of breathing and O_2 utilisation of the respiratory muscles was elevated. But there was no clear impairment of O_2 to the locomotor muscles. The intermittent nature of repeated-sprints is likely a key mediating factor for which O_2 delivery can be maintained to both locomotor and respiratory muscles. The addition of an inspiratory loading while exercising >95% $\dot{V}O_{2peak}$, results in a decrease in limb perfusion and O_2 delivery, mediated by sympathetically-activated vasoconstriction in the locomotor muscles (Harms et al., 1997; Harms et al., 1998). Whereas at moderate intensities (50-75% $\dot{V}O_{2peak}$), there is no change

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in vascular resistance or blood flow (Wetter et al., 1999). Even though repeated-sprint exercise can elicit >90% of $\dot{V}O_2$ peak, it is not sustained throughout the entire protocol (Buchheit et al., 2009; Dupont et al., 2003). Moreover, skeletal muscle O_2 extraction oscillates between sprint and recovery phases (Billaut & Buchheit, 2013). In study 2, $\dot{V}O_2$ fluctuated between 90% and 70% of $\dot{V}O_{2peak}$ during sprint and recovery phases respectively, and increased ~4.5% when the inspiratory load was added. This fluctuation in O_2 demands may aid in minimising the competition for available cardiac output. Moreover, the fact the $\dot{V}O_2$ increased proportionally to the elevated work of breathing, represents a functional capacity of the cardiovascular system to adjust to meet the demands of additional muscular activity (Gleser, Horstman, & Mello, 1974). When limb blood flow has been demonstrated to be restricted when exercising with inspiratory loading, there is no increase in $\dot{V}O_2$ to compensate for the additional muscle work (Harms et al., 1997; Harms et al., 1998). Available room for $\dot{V}O_2$ to increase may also be a crucial factor in maintaining O_2 supply to all active muscles.

To further explore the role of O_2 availability, in study 3, the fraction of inspired O_2 was decreased to 0.1455 using an environmental exercise laboratory. Because O_2 availability during between sprint rest phases is a strong determining factor of metabolic recovery, O_2 delivery underpins the capacity to maintain sprint performance over multiple efforts (Gaitanos et al., 1993; Harris et al., 1976; Parolin et al., 1999). As had been demonstrated by others, hypoxia impaired vastus lateralis O_2 delivery compared to normoxia (Billaut & Buchheit, 2013; Smith & Billaut, 2010). On the other hand, oxygenation of the respiratory muscles was similar to normoxic exercise. Based on this evidence, it appears that O_2 delivery is preferentially distributed to the respiratory muscles to maintain pulmonary ventilation. Amann et al. (2007) has demonstrated the

link between inspiratory muscle work and the development of quadriceps fatigue during exercise in acute hypoxia. By reducing the work of breathing with proportional assist ventilation during high-intensity exercise, the amount of quadriceps fatigue is attenuated (Amann, Pegelow, et al., 2007). Because hypoxia can accelerate the development of exercise-induced diaphragm fatigue and accumulation of metabolites (Babcock, Johnson, et al., 1995; Vogiatzis et al., 2006; Vogiatzis et al., 2007b), a heightened metaboreflex may compromise limb blood flow to a larger degree than normoxia. Therefore, the respiratory muscle work incurred during repeated-sprint exercise may play a role in limiting locomotor muscle oxygenation. Alleviating the O₂ cost of hyperpnoea appears to be a pathway for enhancing limb O₂ delivery during exercise.

In study 4, inspiratory muscle training was used as a method of attenuating the relative intensity of exercise hyperpnoea, and reducing the O₂ cost of exercise hyperpnoea (Turner et al., 2016; Turner et al., 2012). This form of training has also been demonstrated to attenuate sympathetic activity linked to the respiratory muscle metaboreflex (Witt et al., 2007). Despite this evidence, there were no clear changes in vastus lateralis oxygenation trends in either normoxia, or hypoxia after the intervention period. Unloading the respiratory muscles with proportional assist ventilation can attenuate leg vascular resistance and promote leg blood flow (Harms et al., 1997; Harms et al., 1998). Considering this, it is likely that a ~30% increase in maximal inspiratory mouth pressure has a minimal effect on reducing the relative intensity of exercise hyperpnoea. Other have also shown no clear difference in locomotor muscle oxygenation trends during maximal exercise following a 6-week intervention period (Turner et al., 2016).

7.1.3 The role of respiratory muscle work on exercise performance

Respiratory muscle work appears to have trivial effects on repeated-sprint performance in normoxia. When the work of breathing was elevated in study 2 with inspiratory loading, there was a negatable difference in total work completed. In this instance, $\dot{V}O_2$ increase $\sim 4.5\%$ to accommodate the increased metabolic activity of the respiratory muscles. In instances where $\dot{V}O_2$ is unable to increase to meet the heightened O_2 demands, metabolic recovery between sprints may be compromised, and therefore subsequent sprint performance. Therefore, if O_2 demands of exercise hyperpnoea can be met without compromising limb blood flow, repeated-sprint performance should be unaffected. Even the substantial change in perception of breathing difficulty had a trivial effect of performance. The summation of sensory signals (afferent feedback and central motor drive) within the central nervous system regulate exercise intensity within tolerable limits (Hureau, Romer, & Amann, 2016). It appears that subjects can tolerate the heightened respiratory effort in an intermittent exercise model without effecting locomotor mechanical work.

The greatest potential for respiratory muscle work to influence performance, is when exercise is performed in hypoxia. Though there was no clear difference in exercise performance in this thesis, arterial hypoxemia has previously been demonstrated to have substantial effects on performance (Balsom, Gaitanos, Ekblom, & Sjodin, 1994; Billaut et al., 2013; Girard et al., 2017; Goods et al., 2014; Smith & Billaut, 2010). Performance outcomes in study 3 were likely influenced by an element of pacing in two subjects (Figure 5.2). The evidence that respiratory muscle oxygenation was maintained, while locomotor oxygenation was compromised, is suggestive of preferential blood flow distribution. Therefore, the quality of recovery between sprints may be compromised by

the favourable blood flow allocated to the respiratory muscles in hypoxia. Under these conditions, repeated-sprint performance may be impacted by respiratory muscle work.

Alleviating the relative intensity of exercise hyperpnoea with inspiratory muscle training has enhanced exercise performance during the Yo-Yo intermittent recovery test (Lomax et al., 2011; Nicks et al., 2009), time trials (Romer et al., 2002c; Salazar-Martínez et al., 2017; Volianitis et al., 2001), and constant load cycling (Bailey et al., 2010; Mickleborough et al., 2010). However, the effective of inspiratory muscle training in repeated-sprint exercise is mixed. Self-selected recovery time between sprint efforts has been demonstrated to be reduced after inspiratory muscle training (Romer et al., 2002b). On the other hand, an improvement in repeated-sprint performance has been shown to be no greater than the control group (Archiza et al., 2017). The latter finding supports the performance outcomes in study 4. Based on regression models, for the training intervention to have had meaningful effects on performance in normoxia and hypoxia, pressure generating capacity needed to increase by 52% and 38% respectively in these subjects. Since the required adaptation to enhance performance appears to be beyond the typical change (Archiza et al., 2017; Downey et al., 2007; Lomax et al., 2011; Romer et al., 2002b; Salazar-Martínez et al., 2017; Turner et al., 2012), this thesis does not support the use of inspiratory muscle training to improve repeated sprint performance.

7.2 LIMITATION OF THIS RESEARCH

- A fundamental limitation of the research presented in this thesis is the absence of direct measurements of either leg blood flow or O₂ uptake. While the NIRS techniques employed in this thesis provided information on the relationship between O₂ delivery vs. extraction, the influence of the work of breathing on O₂ transport per se remains speculative. Moreover, there are a number of limitation of NIRS measurements which

could affect the interoperation of data. 1) There is no consistency within the literature on an appropriate differential pathlength factor (DPF) to be used. However the variation in near-infrared light scattering properties in human tissue is like far more influential than the DPF if selected within an appropriate range. 2) Blood volume changed (due to muscle contraction) can influence the tissue path length. 3) Light propagation can be affected by adipose tissue (Matsushita, Homma, & Okada, 1998). 4) It is difficult to separate haemoglobin and myoglobin in the NIRS signal because the chromophores overlap in the near-infrared light spectrum (Ferrari et al., 2004).

- The level of arterial oxygenation can either be measured directly or indirectly. A direct measurement involves blood sampling via an arterial puncture. Along with arterial O₂, arterial CO₂ and pH can also be assessed (Harms et al., 1997; Romer, Haverkamp, et al., 2006). Also, by having a direct sample of arterial blood, factors that affect the oxygen haemoglobin dissociation curve (Figure 2.4) can be taken into consideration when interoperating the data. To overcome the invasive nature of arterial blood sampling, an indirect measurement of arterial oxygenation can be assessed, as was in this thesis, by pulse oximetry (SpO₂) and relies on the same technology as NIRS. The same limitation of NIRS mentioned above will also apply to pulse oximetry. Moreover, shifts in the oxygen haemoglobin dissociation curve during exercise can influence the data. Even though SpO₂ is less accurate, there is typically a <2% difference in arterial oxygenation difference compared to an arterial blood sample (Collins et al., 2015). An advantage of the indirect method is that SpO₂ can be monitored continuously throughout exercise which cannot be done with blood sampling.

- While mouth pressure was used to infer the inspiratory muscle work, it is not a direct measurement of the work of breathing (Benditt, 2005; Otis, 1954). Although mouth pressure can give an indication of inspiratory muscle force development (Witt et al., 2007), it does not account for the increased inspiratory work necessary to overcome the elastic recoil of the chest cavity at high lung volumes (B. D. Johnson, Saupe, & Dempsey, 1992). To obtain a more accurate representation of inspiratory muscle work, a balloon catheter is inserted through the nose and into the oesophagus to record transdiaphragmatic pressure (Benditt, 2005), however such technique is invasive and difficult to use during all-out sprint efforts.
- This thesis adds to the body of research on the effectiveness of inspiratory muscle training on performance and physiological responses to exercise. The training design adopted in this thesis was similar to previous work in the area (Griffiths & McConnell, 2007; Romer et al., 2002b; Salazar-Martínez et al., 2017; Turner et al., 2013). A typical training design consists of pre-testing, separated by an intervention period, and concluded by post-testing. However, this is not the strongest design to determine the magnitude of change of an intervention in a sample population. A stronger design would incorporate a double baseline separated by 4-weeks (same duration as the intervention). Including these components will help establish “normal” activity profiles of the subjects before commencing training. If participants modify their activity during the intervention, it makes it difficult to separate the effects of training from changes in activity on post-intervention outcomes. A double baseline period would also help establish thresholds for the smallest worthwhile change. Repeated respiratory muscle strength assessments prior to training would have helped establish a baseline to set training intensity and interpret training responses, and separate training effects from normal physiological variance.

- A single familiarisation prior to the experimental trials is widespread practice in exercise science research. However, there is increasing evidence that multiple familiarisation trials are required to reduce the learning effect (Higgins, James, & Price, 2014; McGawley & Bishop, 2006). In studies 1 and 2, only one single familiarisation session was performed prior to the experimental sessions. Considering the nature of study 1, an additional familiarisation would be unlikely to have influenced the outcomes. Completing a second familiarisation in study 2, however, may have reduced the potential learning effect prior to the repeated experimental trials. To overcome this limitation, repeated familiarisation trials were performed in studies 3 and 4.
- Considering the small sample size, it is likely that study 4 was underpowered. A suboptimal sample size would widen the confidence intervals and make it difficult to obtain clear outcomes (Hopkins, 2006a).

7.3 SUGGESTED FUTURE RESEARCH

- Further explore the role respiratory muscle work plays in repeated-sprint exercise by unloading the respiratory muscles with either proportional assist ventilation or a helium-O₂ inspiratory gas mixture. Using of proportional assist ventilation can reduce the work of breathing by 35-55% (Harms, 2000; Harms et al., 1997). Though the inspiratory muscle training implemented in study 4 can act as a form of respiratory muscle unloading (Turner et al., 2016; Turner et al., 2012), it is unlikely to have the same magnitude of effect as proportional assist ventilation.
- In a team a sport setting, maximal repeated accelerations/sprints do not occur in isolation. Often, the brief period of maximal exertion is interspersed with longer periods of low to moderate activity (Spencer, Bishop, Dawson, & Goodman, 2005).

- Future investigation should explore the role inspiratory muscle work plays in an intermittent activity protocol that aims to simulate team-sport running (Sweeting et al., 2017; Zois, Bishop, Fairweather, Ball, & Aughey, 2013). Repeated-sprint running may pose more of a challenge for the cardiorespiratory system to adequately oxygenate both the locomotor and respiratory muscles. Running typically elicits a greater $\dot{V}O_{2\max}$ since it utilises a larger muscle mass (upper body and postural muscles) compared to cycling (Millet, Vleck, & Bentley, 2009). Therefore, an elevated work of breathing may be a limiting factor during running exercise, whereas inspiratory muscle work appears to have negligible effects on repeated-sprint cycling
- Hyperventilation occurs when alveolar ventilation disproportionately rises relative to CO_2 production causing a decrease in the pressure of alveolar CO_2 , and increase in pressure of alveolar O_2 (Forster et al., 2012; Sheel & Romer, 2012). This is a potential mechanism associated with high-intensity exercise which can constrain a fall in arterial O_2 and pH (Forster et al., 2012; Whipp & Ward, 1998). Though this was not directly examined in this thesis, some evidence of hyperventilation occurring during repeated-sprint exercise was present. As depicted by the data of a representative subject (Figure 7.1), $P_{ET}O_2$ and $P_{ET}CO_2$ rose and fell respectively from baseline over the course of the repeated-sprint protocol. Further evidence of hyperventilation comes in study 3. Arterial hypoxemia is a potent stimulant of ventilation. However, there was no clear difference in IV , f_R , or inspiratory pressure. The wave like pattern in $P_{ET}O_2$ and $P_{ET}CO_2$ (Figure 7.1) appears to be linked to the sprinting phases of the protocol, and occurs at exercise onset. This pattern is suggestive of a locomotor respiratory coupling. Meaning that breathing frequency is entrained to the cadence of locomotor exercise (Bernasconi & Kohl, 1993; Siegmund et al., 1999). This link

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should be further investigated to determine the influencing factors exercise hyperpnoea over a variety sprint durations.

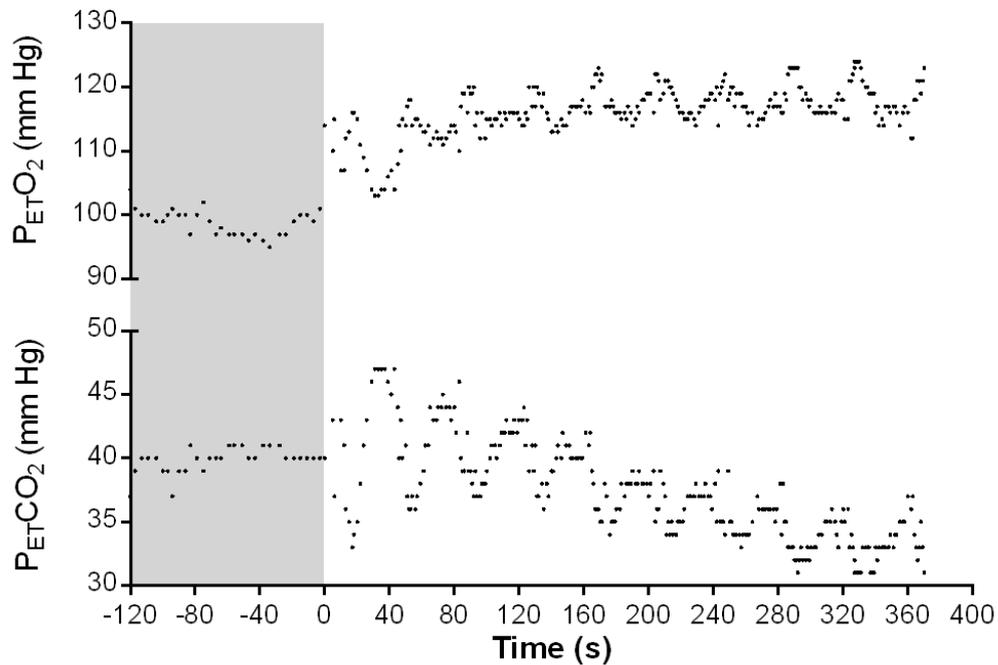


Figure 7.1: Partial pressure of end-tidal gasses oxygen (P_{ET}O₂) and carbon dioxide (P_{ET}CO₂) recorded on a breath-by-breath basis during repeated-sprint exercise. Data is from a single subject collected in study 2 during the Control exercise condition. The grey shaded area represents the 2-min baseline period observed prior to the commencement of warm-up.

- Future research should investigate the concurrent use of inspiratory loading during high-intensity interval training. The evidence presented in study 2 suggests that inspiratory loading heightens the metabolic demands of repeated-sprint exercise, but performance can be maintained. Though this thesis does not support the direct use of inspiratory muscle training as an ergogenic aid (study 4), using it in conjunction with repeated-sprint training may provide fruitful results. There is some evidence that supports the use of airflow-restriction masks during high-intensity interval training (Porcari et al., 2016). However, evidence to the contrary also exists (Sellers, Monaghan, Schnaiter, Jacobson, & Pope, 2016). More research is needed to fully explore these training methods.

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