The effects of Deep Breathing on exercise performance in humans

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Declaration

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For you Dad, showing me how to work hard and never give up, and always give that extra little bit, you've taught me more than I'll ever know!

"Don't spoil the ship for a ha'p'orth of tar"

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Abstract

'The effects of Deep Breathing on exercise performance in humans'

INTRODUCTION: Exercise is pursued by healthy, clinical and athletic populations and unquestionably, plays a pivotal role in health and wellbeing and the advancement of athletic performance. The ability to exercise at higher intensities provides a greater stimulus for cardio-pulmonary, metabolic, neuromuscular and musculoskeletal adaptations but is compromised by fatigue-limiting symptoms, both physiological and psychological, the mechanisms underpinning which are not completely understood. Recent advances in our understanding of fatigue mechanisms have identified respiratory system limitations and the potential for respiratory adaptation. Individual breathing patterns exist, functional and dysfunctional, influenced genetically, developmentally and by multiple psychophysiological mechanisms but importantly it exhibits considerable plasticity. Advances in neuroscience, have identified the potential for respiratory neuroplasticity and its' bi-directionality, with meditation style activities which incorporate deep breathing showing both functional and structural changes in neural structures and circuitry. Differences between athletes and non-athletes and males and females in both pattern and response to exercise exist. Little research has focused on the manipulation of breathing patterns which can potentially influence physiological and psychological factors in fatigue mechanisms. **AIM:** To investigate if adopting a deep breathing pattern during exercise can improve exercise performance via effects on gas exchange parameters and/or perceived exertion. **MEHODS:** Three studies examined the effect of a self-regulated, deep breathing pattern on exercise performance. Study 1 examined constant work rate (CWR) heavy intensity exercise in a heterogenous group of healthy males and females, untrained and trained runners. Study 2 assessed endurance running performance, via a lab-based maximal, incremental vVO_2peak treadmill running test. Study 3 examined the effect on high intensity interval exercise (HIIE) performance via a lab-based, treadmill interval running test to exhaustion. **RESULTS:** Study 1 showed a significant improvement in locomotor efficiency as observed by reductions in both oxygen cost and energy cost. Study 2 and Study 3 showed no significant difference in performance measures. **CONCLUSION:** Deep breathing improves locomotor efficiency in CWR, heavy intensity locomotion. No significant benefit was observed on running performance or HIIE performance in healthy male endurance athletes.

Abbreviations

ANS	Autonomic nervous system
BDNF	brain derived neurotrophic factor
BLa^-	Blood lactate concentration
BPD	Breathing pattern disorder
CI	Confidence interval
CMD	Central motor drive
CNS	Central nervous system
CO_2	Carbon dioxide
CWR	Constant work rate
DB	Deep breathing
DTE	Dual task effects
E_C	Energy cost of locomotion
EELV	End expiratory lung volume
EFL	Expiratory flow limitation
EIAH	Exercise induced arterial hypoxemia
EILV	End inspiratory lung volume
EMT	Expiratory muscle training
FEV_1	Forced expired volume in one second
FRC	Functional residual capacity
FVC	Forced vital capacity
HIIE	High intensity interval exercise
HR	Heart rate
HRV	Heart rate variability
IMT	Inspiratory muscle training
LRC	Locomotor respiratory coupling
LT	Lactate threshold
MEFR	Maximum expiratory flow rate
MEFV	Maximum expiratory flow volume
MIP	Maximum inspiratory pressure
MLSS	Maximum lactate steady state
NE	Norephinephrine

O_2	Oxygen
O_C	Oxygen cost of locomotion
P_aCO_2	Arterial partial pressure of carbon dioxide
P_ACO_2	Alveolar partial pressure of carbon dioxide
PCO_2	Partial pressure of carbon dioxide
$P_{ET}CO_2$	End tidal partial pressure of CO_2
pFRG	parafacial respiratory group
pH	Hydrogen ion concentration
PNS	Peripheral nervous system
PO_2,	Partial pressure of oxygen
preBotC	pre-Botzinger complex
RE	Running economy
RER	Respiratory exchange ratio
RM	Respiratory muscle
RMT	Respiratory muscle training
RPE-O	Overall rating of perceived exertion
RPE-R	Respiratory
RQ	Respiratory quotient
RR	Respiratory rate
RSA	Respiratory sinus arrhythmia
RSA	Respiratory sinus arrhythmia
SaO2	Arterial oxygen saturation
SB	Spontaneous breathing
SF	Stride frequency
SR	Stride rate
T_e	Expiratory time
T_i	Inspiratory time
T_i/T_{tot}	Duty cycle
TLC	Total lung capacity
T_{tot}	Total breath time
V_A	Alveolar ventilation
V_A/Q	Ventilation perfusion matching
VC	vital capacity
VCO_2	Volume of carbon dioxide expired

$\mathbf{V_D}$	Anatomical deadspace volume
$\mathbf{V_E}$	Minute ventilation
$\mathbf{V_E/VCO_2}$	Ventilatory equivalent for CO_2
$\mathbf{VO_2}$	Volume of oxygen uptake
$\mathbf{VO_2max}$	Maximum aerobic capacity
$\mathbf{VO_2peak}$	Peak volume of oxygen uptake
$\mathbf{V_{peak}}$	Peak running speed
$\mathbf{V_T}$	Tidal volume
$\mathbf{vVO_2peak}$	Velocity at VO_2peak

Glossary of Terms

Chemoreflex	the autonomic response to chemical changes
Chemosensitivity	how sensitive an organism is to chemical changes in the internal environment
Deep Breathing	a volitional breathing pattern in which depth of breath (tidal volume, V_T) is consciously increased
Hyperpnea	increased ventilation achieved by increase in tidal volume and or respiratory
Interoception	ability to sense the internal state of the body
Locomotor respiratory coupling	the coupling between the two cyclical phases of locomotion and respiration
Locomotor efficiency	the unit cost, either oxygen or energy (kcal) of movement
Metaboreflex	autonomic responses to changes in metabolite concentration
Metabosensitivity	sensitivity to changes in metabolite concentration
Neuromodulatory	the short term changes in the nervous system
Neuroplasticity	the ability of the nervous system to change with long term adaptations
Repiratory limitation	limitation of exercise capacity due to the respiratory system
Spontaneous breathing	the automatic, autonomically controlled breathing pattern
Stride	one complete locomotion cycle, from toe-off of one foot to the next toe-off of the same foot, consists of two steps
Stride Frequency	the number of strides per minute
Ventilatory efficiency	how efficient minute ventilation (V_E) is at expelling carbon dioxide (CO_2)
Ventilatory pattern	the kinetics and kinematics of breathing
Volitional breathing	the conscious overriding of autonomic control breathing to consciously impose a breathing pattern

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1. Introduction

1.1 Introduction

Exercise is pursued by healthy, clinical and athletic populations and unquestionably it plays a pivotal role in health and wellbeing, the treatment and prevention of numerous pathophysiological conditions and the advancement of athletic performance. Exercise has been extensively researched showing many physical and psychological benefits with physically active lifestyles improving cardiopulmonary and metabolic health and reducing the risk of chronic disease (Penedo and Dahn, 2005, Kilpatrick et al., 2015). While physical activity recommendations have traditionally focused on moderate intensity exercise, increased benefits are possible with exercise in the heavy and severe intensity domains (Kilpatrick et al., 2015).

The ability to exercise, especially at higher intensities is compromised by fatigue-limiting symptoms, both physiological and psychological, the mechanisms underpinning which are not completely understood. It is therefore important to understand what factors may prevent engagement in heavy and severe intensity exercise and if any strategies can ameliorate these barriers to participation. While athletes regularly engage in heavy and severe intensity exercise, it is less common in the non-athletic population. High intensity interval exercise (HIIE) has moved from the almost exclusive realm of the trained athlete to the domain of the recreational athlete, physically active adolescents and adults, and clinical populations, although with some safety concerns (Costigan et al., 2015, Gosselin et al., 2012, De Nardi et al., 2018). Recent advances have been made in our understanding of fatigue mechanisms including the identification of respiratory system limitations and the potential for respiratory system adaptations (Dempsey et al., 2008b, Dempsey et al., 2008a, Amann, 2011b). Another possible barrier to exercise adherence, and in particularly HIIE, is perceived exertion and the resultant affective feelings of motivation, mood state, arousal and exercise enjoyment (Kilpatrick et al., 2015, Seiler and Sjursen, 2004). The severe intensity domain in which HIIE takes place puts near maximal pressure on the respiratory system, the extremely high ventilation rates increasing subjective sensations of breathlessness with negative performance outcomes (Faull et al., 2018).

At the other end of the exercise spectrum is the domain of elite athletic performance where minor improvements, less than 1% in Olympic competition, can determine a gold medallist from a mere finalist (Davison et al., 2009, Davison and Williams, 2009). At the Olympic level, differences in performance are typically less than 0.5% (Wilber, 2007). For example, in the 2012 Olympic finals of the 5,000m and 10,000m, 1% of the winning times were 8.22 and 16.5 seconds respectively, separating 1^{st} from 13^{th} in the 5,000m and 1^{st} from 15^{th} in the 10,000m.

In the constant search for performance enhancements in prolonged endurance events, research has sought to train and optimise various physiological systems to improve cardiovascular, metabolic and neuromuscular function to elicit improvements in performance. Due to their influence on aerobic metabolism and energy provision during prolonged exercise, maximum aerobic capacity (VO_2max), lactate threshold (LT) and running economy (RE) have been identified as the critical determinants of endurance performance and the target of training prescription (Joyner and Coyle, 2007, Midgley et al., 2007a). Of primary focus has been the role the cardiovascular system plays in determining the aerobic capacity of the body to perform sustained high intensity exercise and how it may be trained to increase cardiac output and improve transport, extraction and utilisation of oxygen (O_2) in the active musculature. Research has also examined methods to improve its fractional utilisation ($\%VO_2max$) during competition by training neuromuscular and mechanical factors which determine economy and efficiency of movement (Joyner and Coyle, 2007). While research into these physiological systems and the different methods to train and enhance them has spanned decades, the traditional consensus has been that the respiratory system does not limit performance and as a consequence has only recently been identified as a possible performance determinant area worthy of further investigation. A possible explanation for this omission is that unlike the cardiovascular and neuromuscular system, there is a failure of the lung tissue and airways, both structurally and functionally, to adapt in response to exercise (Oyanagi et al., 2016, McKenzie, 2012) and the belief that respiratory reserves are not fully utilised (Kift and Williams, 2008, Amann, 2011b).

However, recent evidence suggests that the role of the respiratory system is far more complex and pervasive than previously thought (McKenzie, 2012). This changing view has emerged amidst growing evidence that respiratory limitation due to respiratory muscle fatigue and ventilation patterns may be detrimental to exercise performance (Dempsey et

al., 2008b, Dempsey et al., 2008a, Amann, 2011b). This is especially evident in elite athletes where very significant adaptations in other physiological systems have occurred in contrast to the static nature of the respiratory system, which is therefore placed under great stress and may no longer be adequate (Amann, 2011b, McKenzie, 2012). The respiratory system manifests in individual breathing patterns, functional and dysfunctional, influenced genetically, developmentally and by multiple psychophysiological mechanisms, but importantly it exhibits considerable plasticity (Faull et al., 2016). Advances in neuroscience, specifically neuroplasticity, has identified the potential for respiratory control neuroplasticity, with both modulatory and plastic responses. These responses shown bi-directionality, with meditation style activities which incorporate deep breathing showing both functional and structural changes in neural structures and circuitry (Holzel et al., 2011). Differences between athletes and non-athletes and between males and females, all contribute to a highly individual breathing pattern and response to exercise. There is a paucity of research into manipulation of respiratory pattern which can potentially influence both physiological and psychological factors in fatigue mechanisms.

The role of the respiratory system in many of the physiological and psychological factors contributing to the development of fatigue and ultimately to the limitation of exercise performance has been largely ignored. The consensus has been that the respiratory system does not contribute to fatigue nor does it pose a limiting factor to exercise performance in healthy populations (McKenzie, 2012). Underpinning this view was the belief that the respiratory system held considerable reserve and that elite athletes may not maximally stress it, even during maximal exercise. However, more recently the validity of this contention has been called into question (Kift and Williams, 2008) and indeed, it is now recognised that the respiratory system plays a significant role in limiting exercise performance (McKenzie, 2012, Dempsey et al., 2008b, Dempsey et al., 2008a, di Paco et al., 2017). Furthermore, the autonomic nature of respiratory control may have led to the traditional belief that it could not, should not or need not be altered. In light of a growing body of research challenging this long held dogma, emerging evidence suggests that the respiratory system may fail to meet the demands imposed during exercise and may therefore play a role in the development of fatigue, both locally and systemically, limiting exercise performance (Dempsey et al., 2008a, Dempsey et al., 2008b, McKenzie, 2012, Romer and Polkey, 2008, Amann, 2011b, Harms et al., 1997). The elimination or reduction of these negative effects on performance may potentially provide a way to further improve endurance performance.

Respiratory muscle fatigue and the metabolic cost of breathing have been identified as limiting factors to endurance exercise performance and it has been suggested that improvements in ventilatory efficiency may improve endurance performance (Guenette and Sheel, 2007b). Indeed, one of the possible mechanisms suggested for performance improvements from respiratory muscle training (RMT) has been improved mechanical efficiency of the respiratory musculature (Romer and Polkey, 2008). The ventilatory pattern adopted is a consequence of the combined and proportional influences of afferent inputs on autonomic control centres. There is considerable heterogeneity in respiratory patterns both at rest and during exercise, demonstrating that ventilatory requirements may be satisfied in varying ways and indeed some elite athletes exhibit unique ventilatory patterns during exercise (Benchetrit, 2000, Lucia et al., 2001). It is important to remember that while respiration is under autonomic control it can be consciously overridden, allowing ventilatory patterns to be altered.

Of particular importance is the evidence that the respiratory system may be trained to improve performance (Dempsey et al., 2008a, Dempsey et al., 2008b, Dempsey et al., 2006, Romer and Dempsey, 2006, Tong et al., 2008, Tong et al., 2004, Guenette and Sheel, 2007b, Amann, 2011b, Gigliotti et al., 2006, McKenzie, 2012). Advances in our understanding of respiratory control networks and respiratory neuroplasticity have also opened the door to questions about respiratory limitations and possible ways to ameliorate them (Ikeda et al., 2017, Faull et al., 2016, Faull et al., 2018, Mitchell and Johnson, 2003, Mitchell and Babb, 2006). Furthermore, new developments in our understanding of fatigue mechanisms and the role of peripheral metabolite accumulation, which the respiratory system may influence, also highlight the need to investigate this often overlooked physiological system (Amann, 2011a). Research has shown that performance can be improved by targeting the respiratory system using different strategies including the breathing of low-density gas mixtures (Tong et al., 2004), using supplemental O_2 (Amann, 2006), mechanical ventilation (Romer and Polkey, 2008) and respiratory muscle training (RMT) (Bailey et al., 2010, Illi et al., 2012) to reduce metabolic cost, decrease fatigue and prevent respiratory limitation. However, the need to find alternative ways to overcome respiratory limitations has also been identified (Romer and Polkey, 2008). With the exception of RMT which has shown improvements in respiratory muscle function, the aforementioned invasive methods are neither practical nor possible for an athletic population. An alternative exists in the natural and non-invasive method to manipulate the respiratory system via voluntary changes to the ventilatory pattern.

The autonomic ventilatory pattern adopted during exercise may fail to meet the imposed functional demands placed upon the respiratory system leading to respiratory limitation of exercise. This may be due to respiratory muscle fatigue, impaired ventilation perfusion matching (V_A/Q), impaired gas exchange, expiratory flow limitation (EFL) and/or exercise induced arterial hypoxemia (EIAH) (Wagner, 1992, McClaran et al., 1999, Dempsey et al., 2008b, Dempsey et al., 2008a). During exercise, elite athletes may maximally stress the respiratory system leading to respiratory limitation (Guenette and Sheel, 2007a, Romer and Polkey, 2008). Elite female athletes may be especially susceptible due to gender-specific anatomical variations (Dominelli et al., 2011, Guenette et al., 2009, Guenette et al., 2007, Hopkins et al., 1998, Harms and Rosenkranz, 2008b). In recognition of the negative consequences these limiting factors may have on exercise performance, research has sought to find methods to overcome them.

Pulmonary ventilation is achieved by way of inhalation and exhalation forming the ventilatory pattern which is under autonomic control. It presents the only step in the respiratory process that one may consciously manipulate to improve respiratory function. Research investigating ventilatory pattern manipulation is limited and possibly stems from the belief that autonomic control is optimal. It has focused primarily on how breathing pattern effects other exercise rhythms, namely the locomotor respiratory coupling (LRC), but alteration to breathing pattern has been tightly regulated using paced breathing strategies (Baskurt, 2012, Bernasconi and Kohl, 1993, Rabler and Kohl, 2000). This approach may be limited due to the increasing influence of chemical stimuli at increased exercise intensities (Fabre et al., 2007) and the dyspnoea and increased discomfort this may cause. This strict pacing approach may run counter to the flexibility needed to moderate the increasing drive of internal stimuli which a self-selected approach may tackle. Interestingly, it has revealed that there is a bi-directional link between exercise and locomotion (Rabler and Kohl, 2000) and a breathing pattern may not only impose inefficiencies in respiratory function but also in locomotor efficiency. This is important because if respiratory pattern is altered and it changes the locomotor pattern, it may therefore affect locomotor efficiency and running economy may be effected.

Ventilation pattern determines the mechanics and therefore influences the metabolic cost of breathing influences ventilatory efficiency. Its effects have implications both on the effectiveness in maintaining O_2, CO_2, and pH homeostasis and also the incurred cost in attempting to achieve this. An inefficient, sub-optimal ventilatory pattern may result in an

increased cost of breathing and the development of respiratory muscle fatigue which has been shown to result in competition for O_2 with locomotor muscles, negatively affecting exercise performance (Dempsey et al., 2006, Romer and Dempsey, 2006). The resultant alveolar ventilation (V_A) and flow rates are key factors in the development of respiratory limitation.

Ventilatory pattern may be altered by changing either respiratory respiratory rate (RR) or tidal volume (V_T). As outlined above, the metabolic cost for increasing V_E through RR may be inefficient and increases by means of an elevated V_T may prove more cost-effective and provide for more effective gas exchange. A direct method of altering ventilatory pattern is to change the V_T by consciously adopting a deep breathing pattern which consequently leads to a reduced RR for a fixed V_E. This alteration to operational lung volumes will alter the mechanics and cost of ventilation, have implications for gas exchange efficiency, respiratory limitation and LRC. Deep breathing has also been shown to affect the autonomic nervous system (ANS) causing sympathovagal modulation, affecting heart rate (HR) via heart rate variability (HRV), a phenomenon known as respiratory sinus arrhythmia (RSA). It also affects blood pressure, arterial oxygen saturation (S_aO_2), muscle sympathetic nerve activity (MSNA) in skeletal muscle and the peripheral microcirculation (Krasnikov et al., 2013, Yasuma and Hayano, 2004, Seals et al., 1990). The depth of breathing greatly influences this effect and maximal influence may occur at moderate lung volumes, suggesting an optimal V_T for this modulatory effect which may contribute to improvements in efficiency.

1.2 Proposed research justification

Performance improvement is a central aim in sport and exercise science, whether improving functional capacity in clinical populations, improving physical activity and cardiopulmonary fitness in healthy individuals or whether applied to the elite sports professionals. Traditional areas of research have been exhaustively investigated and the opportunities for new discoveries are ever decreasing. The promising identification of the respiratory system, a major physiological system, and developments in our understanding of its complex role and interaction with other physiological systems in exercise performance has been largely overlooked as a method of improving exercise performance. In this context, scientific study of the functions of the respiratory system and its interaction and impact on other physiological systems has the potential to provide an exciting and as of yet untapped resource that may yield performance improvements and novel training

techniques while expanding our knowledge of respiratory control and the influence respiration may play on performance.

This changing research landscape recognises the respiratory system as a contributing factor to fatigue, posing a limiting factor to endurance exercise performance. Currently there is a lack of research in the area of ventilatory pattern manipulation and how this may affect respiratory limitation, respiratory efficiency, acid-base balance and how these may influence fatigue and/or exercise performance. The investigation into ventilatory pattern efficiency and how it may be manipulated may provide a new means to enhance exercise performance especially in the athlete where the respiratory system is severely stressed, respiratory limitation is more evident and minor performance improvements can determine the difference between success and failure.

Any alternative ventilatory pattern adopted must still effectively meet the physiological demands of the body for gas exchange. It is proposed that deep breathing may achieve this via improved gas exchange and that improved ventilatory efficiency and the reduced metabolic cost of breathing and/or improving mechanical efficiency has the potential to contribute to improved running economy and/or reduce fatigue and ultimately improve exercise performance.

A larger V_T may improve gas exchange by increasing V_A/V_T, allowing more air to reach the respiratory zone and for a longer duration due to reduced RR, potentially improving V_A/Q matching and reducing any associated EIAH. Improved gas exchange may then reduce V_E and V_E associated cost, or for a given V_E, improve CO_2 removal and have a positive influence on acid-base disturbances. The reduced RR for any given V_E has the potential to avoid/moderate EFL if expiratory flow rates are decreased via greater increases in expiratory time compared to the increased expiratory volume consequent to increased V_T.

The physiological basis underlying the reduction in the cost of breathing is complex. Mechanically, the decreased muscle work may be due to a reduction in respiratory muscle work through decreased respiratory rate, possible increased elastic recoil and a more optimal and efficient respiratory muscle length (Fabre et al., 2007). The improvements in mechanical efficiency may be due to changes in locomotion (stride rate and stride length) effected by changes in respiration via the locomotor-respiratory coupling (LRC) (Rabler and Kohl, 2000).

This thesis sets out to explore the role ventilatory pattern may play in enhancing exercise performance through its influence on metabolic cost and its role in the development of respiratory limitation and fatigue and how manipulation of breathing pattern may decrease the deleterious effects of these performance limiting factors. The proposed adoption of a deep breathing pattern which utilises a greater V_T may help optimise the performance of the respiratory system, however, caution must be expressed as the ability to increase volumes are also constrained by physical properties. The area approaching the upper limits of these physical constraints may prove to be less efficient and incur an additional cost to respiration. Ventilation is highly individual and therefore the potential for changes may also be. It is possible that while some may have the capacity to alter lung volumes and flow rates while maintaining adequate V_E during exercise, others may lack the ability to increase efficiency and alterations to respiratory patterns may prove less efficient as they move into a zone of decreased inefficiency governed by lung volume, elastic properties of respiratory musculature and flow rates.

1.3 Thesis overview: aims, objectives and hypothesis

1.3.1 Thesis overview

It is proposed to study the effects if any, of a deep breathing pattern on endurance exercise performance with a series of acute interventions. The acute nature of these studies has both merits and limitations in that they allow for direct comparison of deep breathing (DB) with spontaneous breathing (SB), limiting the influence of confounding factors that other training improvements might have on observed performance improvements. The main limitation to this is the possibility that a training phase is necessary to allow the subject to adopt the altered DB pattern successfully. Three separate but inter-related studies were conducted to examine the effects of DB on performance across different intensity domains. Study 1 used an acute model to show whether direct manipulation of breathing pattern can improve performance during constant work rate (CWR) heavy intensity treadmill running in a heterogeneous group of male and female, trained and untrained subjects. This initial broad net was used to see if specific populations would respond differently to DB. The source of our funding then dictated that subsequent studies focused exclusively on endurance athletes and application to endurance running performance. Study 2 examines if endurance running performance can be improved with DB in well-trained male endurance athletes by assessing running performance indirectly with a lab-based, treadmill test. In the

final study, Study 3, a high intensity interval training model was used to assess the suitability of DB in a high intensity intermittent training setting.

Based on the literature it is necessary to measure the effects of the proposed deep breathing pattern during steady state conditions to assess running economy and also during higher intensity conditions that replicate training and competition conditions. The literature also suggests that trained endurance athletes may be at greater risk of respiratory limitation so both training status and gender need to be contrasted.

1.3.2 **Research question**

'*Can adoption of a deep breathing pattern during exercise improve exercise performance in humans?*'

1.3.3 **Aims**

- ***Evaluate the effect of deep breathing on submaximal exercise performance***
- ***Evaluate the effect of deep breathing on maximal exercise performance***
- ***Evaluate the effect of deep breathing on high intensity interval exercise (HIIE) performance***

1.3.4 **Objectives**

- ***Measure and compare the cost of locomotion under deep and spontaneous breathing during constant work rate (CWR) locomotion***
- ***Measure and compare vVO_2peak under deep and spontaneous breathing via maximal treadmill test***
- ***Measure and compare the number of HIIE repetitions completed under deep and spontaneous breathing via maximal treadmill test***

1.3.5 **Hypothesis**

'***Deep breathing can improve exercise performance by improving locomotor economy and delaying the onset of fatigue thereby improving exercise performance***'

1.4 Study 1: 'To measure the effect of deep breathing on economy of locomotion during heavy intensity exercise in trained and untrained male and female subjects'

This study measured the affect that a deep breathing pattern had on locomotor performance by assessing walking and running economy during steady state, moderate intensity exercise.

It examined a heterogeneous group of untrained and endurance trained males and females aged between 18 and 50. Following pulmonary function testing, a combined lactate threshold and VO_2peak test was used to establish exercise intensity, and subjects performed two identical 20-minute steady state trials at a moderate intensity at a velocity equivalent to 1mmol above lactate threshold (LT). The two trials were completed within one week of each other with a minimum of 48 hours recovery. The study used a 'within-subject' design where subjects act as their own controls. In the first trial subjects breathe naturally without interference and in the second trial they are asked to breathe as deeply as is comfortable.

1.5 Study 2: 'To measure the effect of deep breathing on running performance in male endurance athletes'

The objective of this study was to show the effects a deep breathing pattern has on endurance running performance. A three-minute incremental vVO_2peak protocol was selected as it was identified to be the most valid and reliable lab-based endurance running performance test, to assess if deep breathing could improve endurance running performance in male endurance athletes.

Subjects initially underwent pulmonary function testing to assess respiratory health. The study used a 'within-subject', random cross-over design where subjects act as their own controls and randomly were assigned to either spontaneous breathing first or deep breathing first. Subjects then completed two vVO_2peak tests on two occasions separated by at least 72 hours and no more than 10 days, one breathing spontaneously and the other adopting a deep breathing pattern. Performance was assessed by calculating the vVO_2peak for each breathing condition. Secondary to this primary outcome, gas-exchange and locomotor parameters were also analysed.

1.6 Study 3 - 'To measure the effect of deep breathing on high intensity interval exercise performance in endurance athletes'

The objective of this study was to show the effects a deep breathing pattern has on the ability to perform high intensity interval exercise (HIIE). HIIE provides a potent training stimulus and if the ability to perform a greater number of repetitions or increase to time accumulated close to 100% VO_2peak can be achieved it may improve subsequent performance.

Subjects initially underwent pulmonary function testing to assess respiratory health followed by a vVO_2peak test to establish the intensity for the subsequent HIIE tests. The HIIE protocol consisted of alternating work and recovery intervals of 60-second duration at 100% vVO_2peak and 50% vVO_2peak respectively. The study used a 'within-subject', random cross-over design where subjects act as their own controls and were randomly assigned to either spontaneous breathing first or deep breathing first. Subjects then completed two HIIE tests to volitional fatigue, on two occasions separated by at least 72 hours and no more than 10 days, one breathing spontaneously and the other adopting a deep breathing pattern. Performance was assessed by the number of repetitions completed and time accumulated above various percentages of vVO_2peak. Secondary to this primary outcome, gas-exchange and locomotor parameters were also analysed.

1.7 Delimitations

Testing was laboratory based and assessed treadmill running performance and not actual outdoor running performance. Running performance was evaluated indirectly and not by actual running performance. Subjects were aged between 18-35yrs (except for Study 1 where subjects were 18-50yrs), healthy (no history of respiratory disease) and were endurance trained runners (except for Study 1). Subjects did not have a history of respiratory muscle training.

1.8 Limitations

The studies were restricted to laboratory based testing and used simulated performance tests which did not directly assess field based conditions or actual competitive performance. The studies did not directly measure 'over ground' running performance. The heterogeneity of breathing pattern, as well as musculoskeletal restriction that may limit thoracic expansion may have inhibited some subjects from adopting the proposed breathing pattern, which we did not assess or control for. Subjects could not be blinded to

the treatment as they were required to consciously adopt the altered breathing pattern and a placebo affect could not be tested.

2. Literature Review

2.1 Introduction

This chapter presents an overview of respiratory physiology, focusing initially on respiratory mechanics, control, ventilation and flow rates. The area of respiratory limitation to exercise is then explored and possible limiting mechanisms identified from the current literature which will be briefly explained, followed by a focus on the physiological responses to deep breathing respiratory patterns. Justification for the focus and application of deep breathing stems from the facts, that exercise can be limited by the respiratory system, respiratory system plasticity and adaptations that have shown improvements in exercise performance in the respiratory patterns of athletes, adaptations to respiratory muscle training and the physiological response to deep breathing evidenced in meditation research. A review of cardiopulmonary exercise testing considerations pertinent to this study will also be presented, focusing on current trends directing the design of test protocols, namely, maximal aerobic capacity and lactate threshold testing.

2.2 Respiratory Physiology Overview

2.2.1 Respiratory function

The main function of the respiratory system is the exchange of gases, the provision of O_2 and removal of CO_2, to and from metabolically active tissues, which are under ever increasing demands with exercise. This is achieved via the process of respiration which can be subdivided into distinct sub-processes concerning the movement of air and the exchange of gases. Pulmonary ventilation occurs between the atmosphere and the lung, external respiration between the lung and the blood, transportation of gases in the blood, internal respiration between blood and active tissue, and cellular respiration within the active tissue. Changes to pulmonary ventilation via ventilatory pattern manipulation may have a positive influence on the subsequent processes of respiration. It is evident from research that the respiratory system may be incapable of achieving its primary role of gas exchange during intense exercise, resulting in exercise induced arterial hypoxemia (EIAH) (Dempsey et al., 2008b). This may result in a reduction in O_2 availability in exercising muscle and may play a considerable role in the development of fatigue and therefore performance limitation. Respiration attempts to tightly regulate PO_2, PCO_2 and pH within close limits by altering ventilation (V_E) in response to perturbations in these variables.

Ventilation is changed autonomically by altering both tidal volume (V_T) and respiratory rate (RR) which dictates the ventilatory pattern and is regulated by complex autonomic, neuro-humoral control. It is affected principally by PCO_2 and pH centrally, and PO_2, PCO_2 and pH peripherally, but also by mechanical and psychological factors. The degree of response may be influenced by chemosensitivity (Feldman et al., 2003b), cardio-respiratory (BuSha, 2010) and locomotor-respiratory rhythms (Fabre et al., 2007).

2.2.2 **Respiratory control**

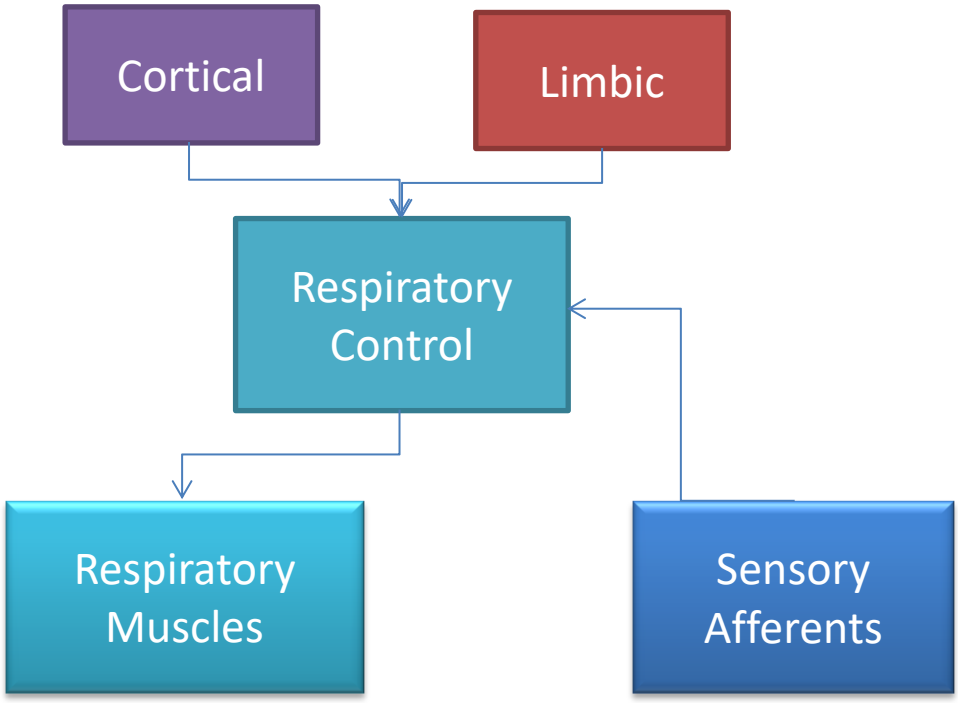


Figure 2-1 Overview of respiratory control showing inputs and outputs

The mechanisms explaining the control of respiratory responses to exercise are still debated and further investigation into this 'fundamental question in respiratory physiology' has been called for (Haouzi, 2012). The autonomic nature of ventilation has been accepted to date and investigation into optimisation of this process which has an effect on performance is absent. This process has implications on metabolic cost, local fatigue of respiratory muscles and fatigue of peripheral muscles due to metaboreflex mediated competition for blood flow, all of which limit exercise capacity. If this process may be altered to reduce, metabolic cost, RM fatigue and/or blood flow competition, and positively affect acid base disturbance, fatigue and/or perception of dyspnoea, it may result in performance improvements.

Respiration is primarily an autonomic process controlled by a central controller in the brain which affects respiration via the respiratory musculature, based on sensory information received from central and peripheral chemo-receptors, various lung receptors and other sensors throughout the body (West, 2008). An important aspect of respiratory control is that it can be voluntarily overridden by conscious control via the cerebral cortex, (West, 2008). This allows us to hold our breath, deepen our breath or slow our breath if we choose. We can do so quite easily without fighting against autonomic control mechanisms unless we alter the pattern to such a degree that powerful chemoreflexes, governing CO_2 in particular, begin to exert powerful control. The ability to alter breathing pattern is utilised in breath-holding activities such as breath-hold diving (Ferretti, 2001) and in meditation based practices such as yoga, tai chi and mindfulness, and has been shown to have powerful physiological and psychological benefits (Brown and Gerbarg, 2005, Balaji et al., 2012, Brown and Gerbarg, 2009, Li et al., 2001).

The primary function of respiration is the maintenance of homeostasis in the face of metabolic and environmental disturbances (Homma and Masaoka, 2008). Our understanding of respiratory control, especially exercise hyperpnea is still evolving and while not completely understood, recent technological advances have significantly advanced our understanding (Ikeda et al., 2017, Braegelmann et al., 2017). Breathing pattern is the result of respiratory pattern generation output and is thought to have genetic origins but also to be sensitive to change during specific developmental periods (Besleaga et al., 2016, Mitchell and Johnson, 2003). The ventilatory response to exercise is strictly controlled, increasing dramatically with exercise intensity and eventually breathlessness occurs (Faull et al., 2018). Respiratory control is functionally different in the athletic brain, and the very high ventilation rates of athletes makes them susceptible to breathlessness-anxiety which may impair performance but these ventilation perception pathways could be the target for performance improvements (Faull et al., 2016, Faull et al., 2018).

2.2.2.1 Neuro-physiological basis of control

Components of respiratory control include medullary and pontine respiratory circuits that control rhythm generation, pattern formation in the brain stem and spinal cord premotor neurons which are neuro-modulatory neurons that project to the other control neurons and sensory neurons in the central nervous system (CNS), and the peripheral nervous system (PNS), lungs, vasculature and muscle which respond to mechanical and chemical signals which are relayed back to the brain stem (Mitchell and Johnson, 2003, Dutschmann and

Dick, 2012). Medullary rhythm generation controlling inspiration and expiration is the result of interaction between two sites, the parafacial respiratory group (pFRG) and the pre-Botzinger complex (preBotC), the latter containing inspiratory pacemaker neurons (Ikeda et al., 2017). Numerous excitatory and inhibitory neuromodulatory systems impact on breathing pattern through the release of neuromodulators with the pons described as the 'adaptive breathing centre' (Dutschmann and Dick, 2012) controlling exercise hyperpnea (Mitchell and Babb, 2006).

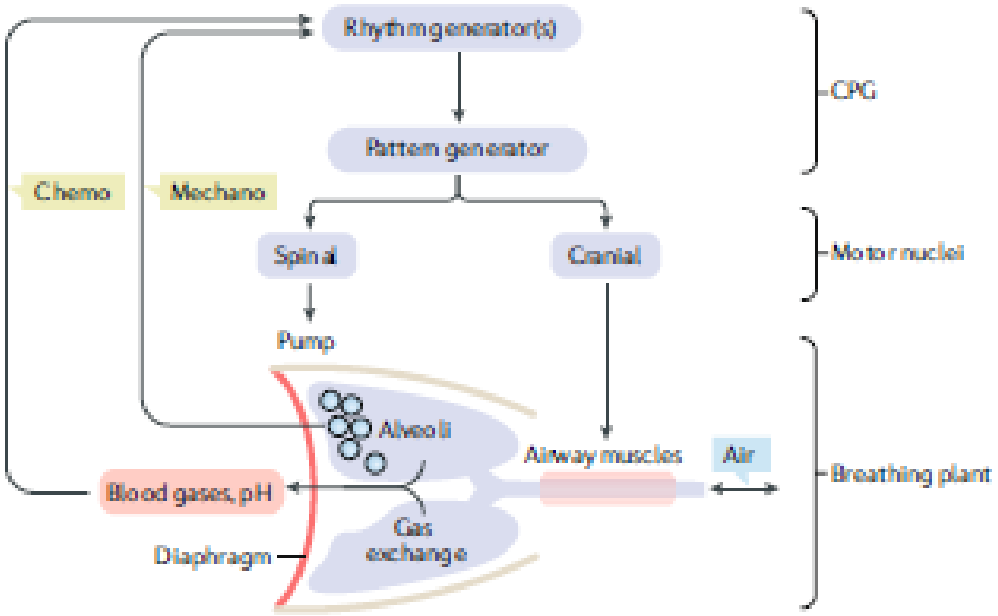


Figure 2-2 Overview of neuro-physiological basis of respiratory control

Several putative mechanisms are thought to interact to control the response to mild and moderate exercise, namely feedforward, feedback and adaptive layers of control (Mitchell and Babb, 2006, Faull et al., 2018). Breathing is produced by rhythm generators in the medulla, modulated by the adaptive centre within the pons to produce efferent signals generated by premotor neurons in the brainstem and spinal cord, and is dynamically able to adjust to varying external and internal homeostatic disruptions (Ikeda et al., 2017, Dutschmann and Dick, 2012, Braegelmann et al., 2017). Integration of chemical and mechanical sensory feedback from central and peripheral chemoreceptors (Figure 2-2), respiratory muscles and locomotion is under continued modulation adaptive control that can lead to long-term plastic responses in respiratory control which influence the rhythmicity, chemosensitivity and plasticity of the system (Mitchell and Babb, 2006, Mitchell and Johnson, 2003, Babb et al., 2010, Feldman et al., 2003b).

2.2.2.2 Metabolic inputs

CO_2 is often overlooked in favour of O_2 (Jones, 2008) but its importance cannot be overstated, and if not factored in or controlled for, incorrect conclusions may be drawn (Farra et al., 2016). It is a powerful vasodilator affecting cerebral perfusion, it impacts on acid-base balance and through stimulation of central and peripheral chemoreceptors alters cardiopulmonary parameters (Farra et al., 2016). Training results in a reduction in CO_2 production for the same absolute intensity, mediated by a reduction in glycolysis. Metabolic CO_2 production increases with increased exercise intensity resulting in higher PCO_2 and lower pH which affect contractile properties and rate limiting enzymes of muscle metabolism and CO_2 transport effects acid-base balance (Jones, 2008). Jones (2008) suggests that that the transport of CO_2 needs to be considered as a possible exercise limiting factor and the increased ventilatory demands to eliminate CO_2 also increase the sense of dyspnoea that may also limit exercise performance.

2.2.2.2.1 Acid-base balance

It has been proposed that an individual critical limit of peripheral metabolic disturbances exists which cannot be voluntarily surpassed (Amann, 2011a). During intense exercise when metabolic disruption occurs, it is detected and relayed to the CNS via metabosensitive afferent neural pathways, Group III and IV afferents, inhibiting central motor drive (CMD) when this threshold is reached, leading to fatigue and ultimately reducing exercise intensity and/or resulting in exercise termination (Amann, 2011a) (see Figure 2-3). These afferent pathways also provide feedback which regulate ventilatory and cardiovascular responses to exercise (Amann, 2011a).

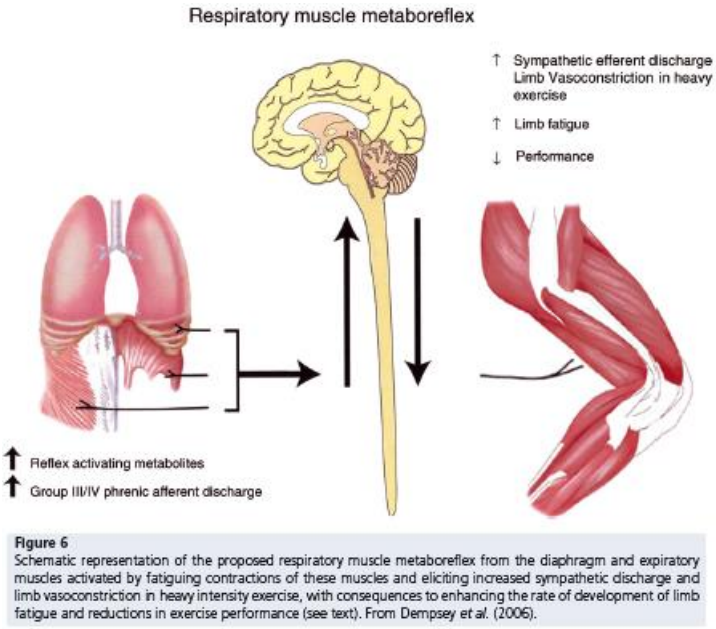


Figure 6
Schematic representation of the proposed respiratory muscle metaboreflex from the diaphragm and expiratory muscles activated by fatiguing contractions of these muscles and eliciting increased sympathetic discharge and limb vasoconstriction in heavy intensity exercise, with consequences to enhancing the rate of development of limb fatigue and reductions in exercise performance (see text). From Dempsey *et al.* (2006).

Figure 2-3 Respiratory muscle metaboreflex – taken from Dempsey et. al (2006)

Hydrogen ions are one such metabolite, which disrupt acid-base balance, and intramuscular levels are related to metabolic CO_2 accumulation. Therefore the elimination of CO_2 plays a key role in the regulation and maintenance of acid-base balance (Robergs et al., 2005). Disruption to acid-base balance or the metabolic cost of ventilation incurred to meet these metabolic requirements may both be factors in the development of fatigue and the limitation of exercise performance. PCO_2, directly and indirectly by way of its effects on pH, is thought to be the primary afferent stimulus for increasing ventilation during exercise, however this has been questioned (Haouzi, 2012) and consensus as to the underlying mechanisms regulating exercise hyperpnea remains elusive.

In the absence of arterial blood gases, end-tidal CO_2 ($P_{ET}CO_2$) is a non-invasive, surrogate marker of arterial PCO_2 (P_aCO_2) and is a product of metabolic CO_2 production, RR and chemoreceptor set point (Bussotti et al., 2008). A high $P_{ET}CO_2$ is traditionally interpreted as a sign of inadequate ventilation and is thought to occur due to mechanical constraints being reached or reduced chemosensitivity. However, Bussotti (2008) suggests that an elevated $P_{ET}CO_2$ as a result of a blunted hyperventilatory response and associated blood acidosis (shifting the HbO_2 saturation curve to the right and increasing O_2 delivery) could be beneficial, improving performance by reducing the cost of breathing and allowing for greater perfusion of the exercising muscles and delivery of O_2. Indeed, Bussotti (2008) has shown that performance in well-trained subjects is related to $P_{ET}CO_2$, those with the lowest $P_{ET}CO_2$ exhibiting a breathing pattern characterised by high V_E due to high RR and a lower

performance. Subjects that performed best had higher VCO_2 and $P_{ET}CO_2$ levels at peak exercise possibly resulting from a beneficial blunted hyperventilatory response. Bussotti (2008) also demonstrated that the disparity in VCO_2 was not an artefact of the breathing pattern and the algorithm used in the Sensor Medics Vmax system, and therefore not the cause of the VCO_2 differences observed. A key function and characteristic of the respiratory response is P_aCO_2 homeostasis. To achieve this, alveolar ventilation (V_A) must be increased to match increased metabolic CO_2 production (Babb et al., 2010) which is accomplished by increasing V_E through some combination of increased V_T and RR.

A ventilatory pattern that may be more effective and efficient in CO_2 elimination may decrease this afferent stimulus which may be responsible for driving an inefficient pattern and thereby reduce the metabolic cost and/or delay acid-base disturbance, delaying fatigue onset and improving exercise performance. In theory, if a reduced metabolite accumulation could be achieved, a higher intensity of exercise may be achievable before the critical limiting threshold is reached and fatigue initiated, allowing for improved endurance exercise performance.

2.2.3 **Locomotor inputs: Locomotor-respiratory coupling**

Alterations in respiration may also have indirect effects on exercise performance by producing changes in locomotion which may affect mechanical efficiency of movement. Autonomic control is responsible for locomotor-respiratory coupling (LRC), a relationship between breathing rates and step frequency. LRC describes the synchronisation of the two cyclical processes of locomotion and respiration and exists in mammals at various walking and running step frequencies, however, respiration can be voluntarily overridden by conscious control via the cerebral cortex, therefore de-coupling the two mechanisms (Bramble and Carrier, 1983; Lafortuna et al., 1996; Siegmund et al., 1999; Rabler and Kohl, 2000). It was initially thought that respiration was subordinate to locomotion and the pattern of respiration was entrained as a result of the chemical and neuro-mechanical implications of locomotion (Fabre et al. 2007). For example, the vertical oscillating pattern of locomotion causes the visceral contents to act as a so called ‘visceral piston’ within the abdominal cavity as they move up and down, acting on the diaphragm to influence respiration (Bramble and Carrier, 1983; Bramble and Jenkins, 1993).

Various LRC ratios exist between stride rate (SR) and RR and an integer ratio is thought to suggest a tight coupling (Bramble and Carrier, 1983; Rabler and Kohl, 2000). Unlike other

mammals (quadrupeds) who seem to be confined to a strict 1:1 ratio, humans exhibit a much greater range of distinct coupling ratios including 1:1, 2:1, 3:2, 3:1, 4:1 and 5:2 (Bramble and Carrier, 1983), with the 2:1 ratio being predominant (Bernasconi and Kohl, 1993). Transitions between various coupling ratios occur seamlessly (4:1 to 2:1) and are not sensed by individuals during steady state running (Bramble and Carrier, 1983) or with increasing speeds (McDermott et al. 2003). McDermott et al. (2003) have also noted that concomitant changes in stride and breathing frequency in response to increased mechanical and metabolic demand of increasing speed and increased training would appear to signify greater adaptation in both respiratory and locomotor systems to stabilise a dominant ratio.

The degree of coordination may be affected by the mode of exercise due to variations in mechanical loading, posture and muscles utilised, the intensity of exercise and training state (Fabre et al. 2007; Bernasconi and Kohl, 1993; Bramble and Carrier, 1983). While Bramble and Carrier (1983) have suggested a higher degree of coordination in highly trained athletes, and the ability of well-trained marathon runners to achieve LRC with four to five strides, McDermott et al. (2003) found contrary to these findings and believe that this may be a product of the less sensitive methods used by Bramble and Carrier (1983) to measure LRC. Increasing intensity has been shown to increase levels of coordination (Rabler and Kohl, 1996; Bernasconi et al., 1995) while others suggest that at increased intensities, chemical stimuli may be more influential and adversely affect this phenomenon (Fabre et al. 2007). Rabler and Kohl (1996) showed an increased coupling when walking at higher speeds, although McDermott et al. (2003) did not. It has been shown that contrary to previous beliefs, an increased LRC variability actually reduces oxygen consumption which is of particular interest due to possible improvements that deep breathing may have on LRC (O'Halloran et al., 2012).

While much research has focused on the existence of LRC, the tightness of the coupling and the affect training state, locomotor pattern and intensity have on the respiratory pattern, few have attempted to manipulate the respiratory side of the coupling (Bramble and Carrier, 1983; Bramble and Jenkins, 1993; Bernasconi and Kohl, 1993; Bernasconi et al., 1995; Rabler and Kohl, 1996; Benchetrit, 2000; McDermott et al. 2003; Fabre et al. 2007). Two studies were identified that used paced breathing, and therefore only minimally altered the respiratory pattern. Rabler and Kohl (2000) utilised paced breathing to attempt to improve coordination and identified that LRC may not be purely unidirectional, that in fact a mutual attraction exists between the two cyclical processes. Bernasconi and Kohl

(1993) also attempted to increase coordination with paced breathing and while some subjects showed decreased coordination and annoyance with paced breathing, they demonstrated a decrease in VO_2 with increased coordination without changes in V_T, RR or V_E, possibly due to a lowering of metabolic rate due to reduced sympathetic tone. Device-guided slow breathing, decreasing RR and increasing V_T and expiratory time, has however been shown to decrease sympathetically mediated vascular tone to reduce blood pressure (Anderson et al., 2009). No studies investigating the effect of gross manipulation of respiratory pattern on LRC were identified.

2.2.3.1 Population difference in control

Faull (2018) has identified fundamental differences in the functioning of athletic and sedentary brains in higher brain regions such as the thalamus, insula and primary sensorimotor cortices. There areas produce opposing anticipatory perception of impending dyspnoea resulting in more positive and accurate anticipation in athletes. It is unknown if these differences are a result of training or part of the selection process for participation but other evidence of modulation and plasticity within the neural control mechanism would suggest an affect for training (Salazar-Martinez et al., 2016, di Paco et al., 2017).

There are also gender differences termed ‘sexual dimorphism’ in the control of respiration and neuroendocrine mechanisms influenced differentially by sex hormones (Kinkead and Schlenker, 2017).

2.2.4 Breathing pattern

Breathing pattern is determined by neural output which is influenced by the various inputs to neural control including lung volume and associated inflation reflexes, chemo-reflex stimulation, temporal variability of neural mechanisms and rhythmic movement during exercise (Tipton et al., 2017). Breathing also responds to various biochemical, biomechanical, physiological, pathophysiological, psychological and/or unknown stimuli with changes in ventilation (V_E) via changes in tidal volume (V_T) and/or respiratory rate (RR), the combination of which may have important physiological consequences (Tipton et al., 2017, Chapman et al., 2016). Environmental stressors such as cold, heat and hypoxia affect respiration but less well known are the affective components, psycho-emotional effects on neural control of breathing pattern that may limit performance (Faull et al., 2016). Limbic, hypothalamic, cortical and forebrain structures associated with thoughts and emotions input into respiratory control and may be influenced by external sensory

inputs or information, or internal cognitive origins such as fear and anxiety and perception of breathlessness (Faull et al., 2018, Tipton et al., 2017, Homma and Masaoka, 2008).

Cognitive and emotional states can interfere with cardiopulmonary and cardiac sympathetic control (Mortola et al., 2016). Emotions cause autonomic, behavioural and physiological changes throughout the body, and respiration is but one system known to be affected (Homma and Masaoka, 2008). Numerous studies by Masoaka and Homma (2001, 2004, 2004, 2008) have investigated the effects of emotions such as fear and anxiety and identified the amygdala as playing a vital role in ventilatory response to emotions, and personality characteristics such as higher trait anxiety scores correlated with higher RR.

Performance of dual tasks such as a motor task and a cognitive task (e.g. consciously deep breathing) can results in a decrement in the performance of either or both (Schott and Klotzbier, 2018, Grassmann et al., 2016). The effects are referred to as dual task effects (DTE) and are the results of competition for our limited attentional resources. Grassmann et al. (2016) examined the alterations in breathing associated with cognitive load, concluding that cognitive load caused overbreathing, resulting in decreased end-tidal CO_2 ($P_{ET}CO_2$) and increased VO_2 and VCO_2. It is therefore possible that the interaction between the consciously deep breathing may have a negative impact or at least represent a confounding factor in our results.

2.2.4.1 Individuality of breathing pattern

Large individual variation in breathing pattern exists with individual airflow profiles during spontaneous and volitional breathing (Mortola et al., 2016). Many factors influence breathing pattern including genetic, anatomical, physiological and psychological factors. Deep breathing is a form of volitional breathing, supressing the spontaneous rhythm and is thought to occur via cortical inputs into the medullary rhythmic control centre or by acting directly on the phrenic motor nucleus, although consensus is lacking as to the mechanisms by which this is achieved (Besleaga et al., 2016). Evidence suggests that even when breathing pattern is altered volitionally, key characteristics such duty cycle are maintained.

Breathing pattern is the output of the respiratory control system which can be affected during sensitive developmental phases resulting in altered adult respiratory control (Mitchell and Johnson, 2003). Cognitive and emotional states can impact breathing pattern and cardiopulmonary function; simply thinking about breathing causes changes in

respiratory sinus arrhythmia (RSA) (Mortola et al., 2016). Mortola (2016) highlights differential response to changes in breathing pattern depending on whether V_T or RR increases. RSA may play a role in mitigating the imbalance between intermittent air flow and continuous blood flow, playing a role in improving V_A/Q matching. It is established that increased RR results in reduced RSA while it requires V_T to at least double to have a significant effect on RSA. Therefore, increased RR versus increased V_T as a method of increasing ventilation would have negative effects of V_A/Q (Mortola et al., 2016).

2.2.4.2 Deep breathing evidence

Deep breathing which increases V_T provides more effective alveolar ventilation (V_A) than a similar increase in ventilation via increased respiratory rate (RR) (McArdle et al., 2007). Inadequacies in alveolar ventilation relative to pulmonary perfusion (V_A/Q inequality) have been observed in athletes at intensities as low as 40% VO_2max, resulting in exercise-induced arterial hypoxemia (McArdle et al., 2007). Other pulmonary limitations have been identified with regard to fatigue of the respiratory musculature and numerous studies have shown an increase in performance following respiratory muscle training (McConnell and Sharpe, 2005), (Gething, Williams and Davies, 2004). Deep slow frequency breathing, the breathing pattern itself, initial lung volume and rate of volume change enhances the sympathetic modulatory effect on muscle sympathetic nerve activity (MSNA) in skeletal muscle (Seals et al., 1990). The depth of breathing greatly influences this effect and maximal influence may occur at moderate lung volumes, suggesting an optimal V_T for this modulatory effect which may contribute to improvements in efficiency.

2.2.4.3 Meditation and respiratory sinus arrhythmia

The physiological effects of altered respiration patterns are clearly demonstrated in meditation and meditation like activities. Physiological effects of an altered breathing pattern include cardiovascular changes such heart rate modulation, known as respiratory sinus arrhythmia (RSA), changes in heart rate variability, blood pressure, and arterial oxygen saturation (S_aO_2). When changes in breathing pattern are effected through meditation and RR is decreased, tidal volume is adjusted to prevent hypoventilation (Cysarz and Bussing, 2005). Mediation and yoga induced changes in cardio-respiratory parameters include increases in expiratory reserve contribution, and respiratory muscle relaxation has also been suggested (Vyas and Dikshit, 2002; Harinath et al., 2004).

Most forms of meditation utilise a low RR (as low as 6bpm-8bpm), resulting in various physiological effects, specifically RSA which attenuates heart rate with increases in V_T and

constant RR (Cysarz and Büssing, 2005). They suggest that the increase in V_T may improve gas exchange through RSA and therefore avoiding hypercapnia. RSA has been linked with improved pulmonary gas exchange via improved V_A/Q matching. Slow, deep breathing which increases RSA has been shown to improve gas exchange possibly through a change in the ratio of physiological dead space (V_D) to V_T (V_D:V_T) (Giardino et al. 2003; Hayano et al., 1996). Other responses include increased arterial saturation (S_aO_2) and increased arterial baroreflex sensitivity as a result of increased V_T increasing parasympathetic vagal activity suppression of the sympathetic nervous system (Bernardi et al., 2002). V_T greater than one liter stimulates the Hering-Breuer inflation reflex, activating stretch receptors in the lung and slowing RR (Bernardi et al., 2002; West, 2008).

2.2.4.4 Hypoventilation

Exercise with voluntary hypoventilation using the 'exhale hold technique' results in decreased P_aO_2 (87%) and increased P_aO_2 and P_ACO_2 irrespective of lung volume and has been used as a form of intermittent hypoxic training and been shown to improve swim performance in well-trained triathletes (Woorons et al., 2016). Woorons (2016) suggests that the combination of hypoxemia and hypercapnia together increases glycolytic reliance and that the improved glycolytic pathway plays a role in improved performance but does not fully consider the effect of alteration to chemosensitivity, despite highlighting previous work that showed increased PCO_2 with reduced RR, possibly due to lower CO_2 sensitivity.

2.2.4.5 Breathing pattern disorders

Breathing pattern disorders (BPDs) represent dysfunctional patterns including paradoxical breathing and are estimated to affect at least one in ten people and by implication suggest that functional breathing patterns exist, namely diaphragmatic breathing patterns (Chapman et al., 2016, Kadambande et al., 2006). BPDs include paradoxical breathing which is inefficient, relies heavily on accessory muscle of respiration and may result in inadequate gas exchange and resultant respiratory distress, and metabolic disturbance, hyperventilation syndrome and tachypnoea which may cause respiratory alkalosis and a myriad of resultant symptoms (Chapman et al., 2016). Breathing is a mechanical process requiring coordinated muscle contraction and posture-related issues, muscle dysfunction or imbalance may have negative consequences (Chapman et al., 2016).

2.2.5 **Respiratory neuroplasticity**

Breathing must demonstrate considerable flexibility and be able to rapidly adapt to various external environmental conditions and changing internal metabolic demands to enable us to deal with the constantly changing requirements of life and exercise. There is evidence for considerable neuroplasticity in respiratory motor control involving structural and/or functional adaptations (Mitchell and Johnson, 2003). Adaptations may occur in the ventilatory response to exercise through modulation, meta-modulation, plasticity and meta-plasticity in respiratory control including the exercise ventilatory response (Babb et al., 2010, Mitchell and Johnson, 2003). Modulation is the modification of the respiratory control neural network induced by neurochemical changes in synaptic strength or cellular properties and is typically reversed but can serve as a stimulus for plastic adaptations in structure and/or function of the network (Mitchell and Johnson, 2003, Babb et al., 2010). Synaptic strength may adapt to previous activity at the synaptic junction and/or neuromodulators such as serotonin, brain derived neurotrophic factor (BDNF) or norepinephrine (NE) which function as part of numerous excitatory and inhibitory neuromodulatory systems. The neuromodulatory system may adapt through changes in existing neurons, the growth of new synapses increasing synaptic connectivity in the existing network, shifts in the balance between excitatory and inhibitory systems and/or changes in neural network dynamics such as synchronisation (Mitchell and Johnson, 2003)
.

2.2.6 **Ventilation**

Total ventilation (V_E), the amount of air breathed each minute, is a product of tidal volume (V_T) and respiratory rate (RR). V_T at rest is approximately 500ml of which 150ml comprises the anatomic dead space (V_D), air which remains in the conducting zone of the respiratory system and does not reach the respiratory zone for gas exchange, the remainder, the volume of alveolar gas (V_A), reaches the alveoli for gas exchange. Benchetrit (2000) describes the highly individual nature of resting breathing patterns, with large variations in V_T and RR combinations to achieve the same V_E. Individuals can exhibit either regular or irregular reproducible patterns of respiration, but tend to move to more similar patterns at higher intensities. At rest, the RR can vary from 6 to 31 breaths per minute and V_T can range from 442ml to 1549ml, independent of height, vital capacity (VC) and forced expiratory volume in one second (FEV1) (Benchetrit, 2000). However, the mechanisms underlying this individual variability and the influence, if any, they may have on

respiratory parameters at higher intensities are unknown (Benchetrit, 2000). Exercise modality also affects breathing patterns with cycling utilising a greater V_T compared to running (Elliott and Grace, 2010).

The ratio of anatomic dead space to tidal volume (V_D:V_T) is approximately 35% at rest but during exercise an increase in V_T decreases the percentage of anatomic dead space per breath and therefore reduces V_T:V_T to 5%-25%, increasing the amount of available gas for gas exchange and also ventilatory efficiency. With exercise there is a sizable, largely unexplained, increase in ventilation (West, 2008), with ventilation rates increasing quickly from a resting RR of 10-15bpm (breaths per minute) to 40-50bpm (as high as 60-70bpm in elite athletes) during maximal exercise (McKardle et al., 2007). There is a dramatic increase in gas exchange, due to an increase in oxygen (O_2) uptake (O_2 demands are 10-20 times that at rest) and carbon dioxide (CO_2) production (West, 2008). V_E is thought to be primarily increased in response to increasing CO_2 levels, maintaining arterial PO_2 (P_aO_2) at 40mmHg, (Hughes and Pride, 2000). There is a linear increase in V_E to increasing work rate, until the ventilatory threshold point when a marked increase occurs. V_E continues to increase until it reaches the ventilatory ceiling, ($V_E$max). Increases in V_T are responsible for increases in V_E up until 60% to 70% of vital capacity (VC), after which increased RR dominates (Wagner, 1996). V_T plateaus and decreases as RR continues to increase with ventilation referred to as the tachypnoeic shift (McClaran et al., 1999; Lucia et al., 2001). It is widely accepted that a breathing reserve above $V_E$max exists with a theoretical upper limit set by the maximum voluntary ventilation (MVV), a voluntary hyperventilatory manoeuvre performed for 12-15 seconds. In normal healthy individuals the ratio of V_E:MVV is 60%-80%, suggesting an adequate reserve and therefore supporting the belief that the respiratory system does not limit exercise capacity. It may however reach 85%-90% in some individuals and pose a possible respiratory limitation to exercise (Kift and Williams, 2008). Kift and Williams (2008) suggest the use of MVV is limited and is mostly a theoretical limit that is not applicable to the exercise domain. MVV is based on voluntary control, higher lung volumes, lower V_T, much higher RR and a very short time course (12-15sec) than occurs during exercise. The dramatic differences with breathing pattern, metabolic conditions and involuntary control during exercise calls into question the application of this theoretical limit and therefore the commonly held assumption that a considerable breathing reserve exists in normal, healthy individuals. Kift and Williams (2008) also believe that no reserve exists and during maximal exercise the respiratory system is stressed to full capacity.

While V_E is measured at the mouth it does not necessarily reflect alveolar ventilation, V_A, which is critically where gas exchange takes place. V_E is the product of V_E and RR, the depth and rate of breathing, and while an increase in either will result in increased V_E, there will be different physiological effects depending on the ventilatory efficiency of the pattern (Tipton et al., 2017). Another important aspect is dead space ventilation, the V_D:$V_{T,}$ which will have a direct effect on the efficiency and overall cost of breathing. While breathing pattern can be analysed by looking at V_T and RR, it can also be analysed as a product of two more informative components, the ratio of inspiratory time (T_i) to total breath time (T_{tot}) referred to as the duty cycle (T_i/T_{tot}) which also reflects the relationship between inspiration and expiration and central inspiratory activity, the ratio of V_T/T_i (Salazar-Martinez et al., 2016). Increases in rate alone or in conjunction with decreases in V_T results in greater dead space ventilation and an inefficient breathing pattern while increases primarily in V_T result in a more efficient pattern. It is efficient for V_T to be increased initially until mechanical constraints such as lung compliance and respiratory muscle length have a negative effect.

The regulation of CO_2 plays a significant role in the neurohumoral control of ventilation and in normal healthy subjects rising CO_2 levels drive hyperpnea (increase in RR). At maximal exercise this hyperventilatory response would appear to be attenuated when tidal expiratory flow rates reach the maximum expiratory flow volume (MEFV) envelope (McClaran et al., 1999). McClaran et al., (1999) further suggest that mechanical constraints such as the increased work of breathing needed to overcome lung elastic properties during expiration may play a role in the plateau of V_T. EILV reached 85% of total lung capacity (TLC) before expiratory flow limitation (EFL) was a factor, and the disproportionate increase in breathing frequency (tachypnoeic shift) with increasing exercise intensity is a more efficient method to increase ventilation. McClaran et al. (1999) also suggests that decrease in V_T sometimes observed at maximal exercise may be due to an increase in EELV due to EFL and mechanical constraints rendering increases in EILV to maintain V_T unavailable. In highly trained subjects exercising at maximal capacity, inspiratory muscle may work at 80-90% of their maximal capacity to overcome an increased elastic resistive load due to increased EELV and compensatory reduction in EILV to maintain V_T and increase V_T (McClaran et al., 1999). Contrary to these findings, Dominelli et al. (2011) found that females who didn't exhibit EFL breathed at a higher EELV taking advantage of the higher flow rates achievable with such volumes to avoid EFL.

It would appear that mechanical factors are crucial to the control of ventilation and limitation thereof, affecting pattern of breathing, lung volumes and flow rates (McClaran et al., 1999). Increased ventilation leads to increased, lung volumes and flow rates which can produce EFL which leads to dynamic lung hyperinflation and increases respiratory work, further limiting the ventilatory response. Ventilatory response may be regulated by lung volumes which are limited by the elastic properties of lung tissue, the rib cage and respiratory musculature, and the bi-directional interaction with flow rates that may lead to limitation in an attempt to increase ventilation in response to increasing exercise demands (McClaran et al., 1999, Mota et al., 1999a, Aliverti, 2008). In the presence of EFL, resistance and limits to increasing lung volumes may affect breathing pattern and ventilatory response to exercise (McClaran et al., 1999). The resultant attenuated hyperventilatory response is linked to the development of exercise induced arterial hypoxemia (EIAH) which may further limit exercise capacity (McClaran et al., 1999). McClaran et al. (1999) propose a mechanical mediated feedback mechanism caused by EFL. Ventilation shows signs of constraint before absolute EFL occurs (MEFV curve not reached), and they suggest that EFL may have a graded response inhibiting efferent respiratory motor drive as flow limitation is approached to stop expiration and begin inspiration which is in turn inhibited by lung stretch receptors as EILV increases (Hering-Breur reflex). They also reference the attenuation of the hyperventilatory response despite increasing chemical stimuli to support the role of some inhibitory reflex pathways in ventilatory control. There is evidence for an upper limit to V_T and an increased cost on nearing this limit which may negatively impact overall performance.

2.2.7 **Ventilatory efficiency**

The ratio of V_E to VCO_2, the volume of air ventilated to remove a volume of CO_2, determines ventilatory efficiency for a given metabolic rate, V_E/VCO_2 and the slope of this relationship (V_E/VCO_2 slope or DeltaCO_2) is used during incremental exercise and is an indicator of global ventilatory efficiency and sensitivity (Salazar-Martinez et al., 2016). The V_E/VCO_2 slope requires the identification of VT2 which is not always possible, especially with longer stage incremental protocols (Meyer et al., 2005). When assessing ventilatory efficiency in world class cyclists, Salazar-Martinez et al. (2016) showed no change in V_E/VCO_2 slope or breathing pattern over a three-year period. The ventilatory response profile (V_T/V_E inflection points 1 and 2) are individually assessed by manual means from Hey plots and have also been used to assess training adaptations, showing

considerable change over an eight-month training program in elite soccer players (di Paco et al., 2017). Di Paco's (2017) results are interesting in that they show a reduced ventilatory demand and changes in efficiency from pre- to end of competitive season. Unfortunately, an assessment after the offseason was not carried out, so it is unknown if these changes reverse during the offseason. Neither is it possible to compare with the longitudinal study of Salazar-Martinez et al. (2016) if any season to season changes occur.

2.2.8 **Mechanics of respiration**

The mechanics of respiration not only involve the complex, synchronised coordination of respiratory musculature but are also affected by muscle and tissue elastic properties, the dynamic responses of a collapsible airway and the physics of fluid mechanics which ultimately govern airflow, all of which may aid or hinder movement of the ventilatory apparatus and airflow. Therefore, any intervention that may alter any of these components has the potential to effect a change in respiratory performance and consequently on exercise performance. The process of respiration incurs an energy cost and limitation within the system, namely respiratory muscle fatigue has been consistently shown to negatively affect exercising limbs and therefore whole body performance. An overview of the key aspects of respiratory function provides a basis for understanding the limitations which may ensue and also possible mechanisms for improvement. Lung volumes, particularly the effects of volume change and the limitations and possible improvements which may or may not occur, provide the rationale for this project.

Lung volumes are constrained physically by the size of the lungs and thoracic cavity but also functionally by the dynamic interaction of respiratory musculature and the opposing elastic properties of the tissues comprising the respiratory system (muscle, lung, airway, rib cage). The total lung volume (TLV) and residual volume (RV) refer to the maximum and minimum operational volumes respectively. They are determined by the balance of these opposing factors. For example, to achieve TLC, the inspiratory muscles must contract maximally to overcome the resistance of the elastic forces of the rib cage as the shortening muscle moves away from optimal length and these elastic forces are increasing with stretch until ultimately the maximum limits imposed by the physical size of both rib cage and lung tissue are reached (Aliverti, 2008). It has been suggested that TLC may increase slightly during exercise but other evidence supports its stability up to maximal exercise (Aliverti, 2008).

Increases in ventilation are mainly achieved by increases in tidal volume (V_T) via an increase in end inspiratory lung volume (EILV) and reduction in end expiratory lung volume (EELV) (up to 50% of expiratory reserve volume (ERV)) (Aliverti, 2008). With increasing exercise intensity expiration becomes active with the recruitment of expiratory muscles to reduce EELV below functional residual capacity (FRC), thereby increasing V_T but also increasing intra-abdominal and intrathoracic pressures which if greater than transpulmonary pressure may cause airway collapse and a resultant increase in EELV as occurs with expiratory flow limitation (EFL)(McClaran et al., 1999).

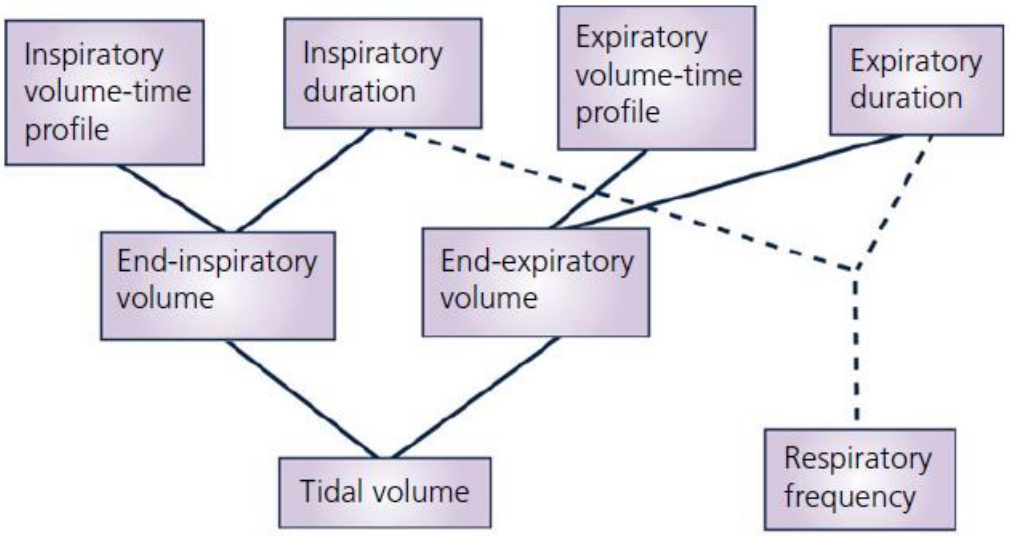


Figure 2-4 Factors influencing tidal volume and respiratory rate - taken from (Tipton et al., 2017)

The respiratory muscles must work to overcome the elastic recoil of the lung tissue and chest wall, and compression and expansion of gas and airway resistance (Aliverti, 2008). The complex coordination is not always apparent. For example, during quiet breathing at rest it would appear that the diaphragm is the only muscle during inspiration at work because the rib cage doesn't move. However, to achieve this and prevent the inward compression of the chest cavity as intrathoracic pressure is reduced as the contracting diaphragm descends and increase thoracic volume, the inspiratory muscle acting on the rib cage must act in concert with the elastic resistance of tissues opposing rib cage compression (Aliverti, 2008).

Ventilation is achieved via the flow of air from the atmosphere in and out of the lungs due to a negative pressure gradient in the direction of flow, established by increasing or decreasing thoracic volume which expands/contracts the lung tissue by way of the pleura. Aliverti (2008) describes the combination of diaphragm (inspiratory muscle), abdominals (expiratory muscle) and the rib cage (inspiratory and expiratory) as the 'ventilatory pump',

the coordinated action of which, under neurohumoral control, facilitates respiration (Aliverti, 2008). Aliverti (2008) describes the mechanics of breathing as a compartmental model based on the actions of three distinct muscle groups and the different compartments that they act upon, in terms of expansion of abdominal cavity and thoracic cavity and how changes in lung volumes are not uniformly distributed between cavities and in fact vary depending on inspiratory or expiratory phase, as opposed to grouping them under function corresponding to inhalation and exhalation. Effective and synchronised contraction/relaxation of the abdominal muscles allows compression/expansion of the abdominal cavity to create the necessary thoraco-abdominal pressure gradients, minimise inefficient rib cage movements and reduce the load the diaphragm must overcome during inspiration, and pre-stretch the diaphragm storing elastic energy, the elastic recoil aiding in expiration (Aliverti, 2008).

The abdominal muscles are the main expiratory muscles but also aid inspiration by reducing EELV (increasing V_T) and placing the diaphragm muscle at optimal length to increase efficiency. They must also relax in synchronisation with inspiration so as not to oppose inspiration and increase the work of breathing (Hopkinson et al., 2010). Expiratory muscles are activated with increasing demands of exercise and serve to expand V_T (~40%) by decreasing EELV (200-400ml), almost solely through compression of the abdominal cavity and not rib cage compression, and placing the inspiratory muscles, namely the diaphragm, at optimal length for efficient inspiration, during which synchronised relaxation is required so as not to inhibit it, while expansion into EILV is achieved via rib cage expansion (Aliverti, 2008). The pre-inspiratory length of the diaphragm is dictated by EELV, and is subject to the force-length relationship, having an optimal length for respiratory efficiency, while end-inspiratory length must not be excessively short, as would happen with hyperinflation, to optimise diaphragm efficiency (Aliverti, 2008, Dempsey et al., 2008). During inspiration, the inspiratory muscles acting on the rib cage operate most efficiently if pre-inspiratory length is not excessively long, during which synchronised relaxation is required so as not to inhibit it, while expansion into EILV is achieved via rib cage expansion (Aliverti, 2008). Expiratory muscle fatigue is exacerbated in the presence of EFL as the muscles contract but fail to reduce EELV and this may play a role in exercise limitation indirectly through competition for locomotor blood flow (Hopkinson et al., 2010).

Alveolar gas volume (V_A), the amount of air that participates in gas exchange, may differ from volumes measured at the mouth and vary with each breathing cycle due to variations in, the quantity of air entering and leaving the alveoli, gas exchange across alveolar-capillary membrane (i.e. the volume of gas entering the capillary blood may not equal the volume of gas leaving the blood, due to differences in partial pressure gradients between the alveoli (A) and arterial (a) blood ($P[A\text{-}a]O_2$ and $P[a\text{-}A]CO_2$) and difference in gas properties), gas compression or expansion within the lung (Aliverti, 2008).

During exercise when ventilatory demands are high, the alternating negative and positive pleural pressures which switch between exerting an expanding and contracting force on the lungs are complex and not uniform over the surface of the lung. The costal surface differs from the diaphragm surface, and the different respiratory muscles will therefore exert expanding/contracting forces only on the compartment which they act upon (the accessory muscles acting on the rib cage will exert a force on the lung tissue adjacent to the costal surface) (Aliverti, 2008).

Breathing requires the coordination of respiratory musculature to achieve a synchronised movement of abdominal and thoracic compartments, thus avoiding paradoxical breathing patterns, (Aliverti, 2008a, Hopkinson et al., 2010, Hammer and Newth, 2009). During inspiration, the external intercostals must resist thoracic compression due to reduced intrathoracic pressure accompanying diaphragm contraction. This increases intra-abdominal pressure and has an expanding effect on the lower rib cage which surrounds the upper abdomen. The diaphragm also increases the transverse diameter of the chest by pulling the lower rib cage upwards and outwards (Hammer and Newth, 2009).

2.2.9 **Flow rates**

Flow rates are another potentially limiting factor to respiration during exercise and are a product of volume (V_T) and time (breath time). Total breath time (T_{tot}) is the sum of the inspiratory time (T_E) and expiratory time (T_E), ($T_{tot} = T_i + T_E$). Flow rates can be divided into mean inspiratory flow rate (V_T/T_i) and mean expiratory flow (V_T/T_E), and described by the duty cycle which is the T_i:T_{tot} ratio (Hughes and Pride, 2000). Reduction in T_E and T_i at high ventilatory rates can result in a flow limitation, a mechanical limitation seen in normal healthy subjects (sedentary and trained). It has been shown in one study to be absent in elite athletes, namely professional cyclists, and thought to be due to the altered breathing pattern and respiratory kinetics (Lucia et al., 2001). These professional cyclists

continue to increase V_T with increasing V_E showing an absence of the tachypnoeic shift and also a prolonged T_E ($T_E > T_i$), possibly unique to cyclists. A breathing pattern utilising a greater V_T and longer T_E may therefore be advantageous, especially during exercise, possibly avoiding the flow limitation described above. At rest, different combinations of T_i to T_E ratios ($T_i<TT_E$ is maintained) exist, therefore demonstrating further diversity and individuality in respiratory patterns (Benchetrit, 2000).

Increases to V_T without a concurrent increase in T_i and T_E will produce increased flow rates. While an increase in inspiratory flow rates may not be problematic, with expiration the potential high flow rate may exacerbate flow mediated limitation as seen in EFL. Therefore, the potential benefits of an increased V_T may not be evident unless appropriate flow rates can accompany the changes. It is therefore important to analyse the temporal variables of breathing pattern as these changes may impact the effectiveness of using a higher V_T in deep breathing.

2.3 Respiratory limitation

The respiratory system was traditionally believed to have adequate reserve and therefore was not considered to pose a limiting factor to exercise, except possibly in limited circumstances with highly trained athletes, pathological conditions or abnormal environmental conditions. This view has changed and a growing body of evidence has shown that the respiratory system may limit exercise performance due to a variety of factors. Of particular note are inherent structural differences in the female population which may predispose them to limitation.

2.3.1 Respiratory limitation in exercise

A definitive picture of respiratory limitation is lacking, however, various possible mechanisms have been suggested including, gas exchange inefficiency, metaboreflex mediated blood flow limitation and expiratory flow limitation. Gas exchange inefficiency exists in normal subjects during exercise due to ventilation-perfusion (V_A/Q) inequality, a mismatching between pulmonary blood flow and V_E. The mechanisms aren't clearly understood but it is thought to be the result of alveolar-capillary diffusion limitation, with increased V_T, among the many other possible causes (Wagner, 1992). Evidence that respiratory muscle fatigue metaboreflex limits blood flow to the working locomotor muscles in the legs has been shown by Dempsey et al., (2006) and Sheel et al., (2002). Dempsey et al., (2006) suggests that at high intensities the diaphragm must compete with

the locomotor muscles for available blood flow and that reductions in respiratory muscle work improves endurance exercise performance. This has been shown to improve blood flow and O_2 transport, decreasing muscle fatigue and dyspnoea. Guenette and Sheel (2007) suggest that the functional capacity of the respiratory system in healthy subjects exceeds the demands placed on it during exercise. Exercise performance can however be diminished as a result of respiratory limitation due to expiratory flow limitation (EFL) and diaphragm fatigue which affects blood flow competition between locomotor and respiratory musculature. It has also been suggested that high ventilatory flows may trigger bronchoconstriction (Holm et al. 2004; Spengler et al., 1999). Guenette et al. (2007) provide evidence that EFL may be more common in females and they may experience greater increases in EELV and EILV at maximal exercise relative to males, utilising a higher proportion of breathing reserve as a result of smaller lung volumes and smaller diameter airways, constituting a higher cost of breathing. Indeed it has been shown that gender differences exist mainly due to hormonal and structural differences (Harms, 2005) and in particular the smaller lungs and higher maximal flow rates in females are responsible for expiratory flow limitation, particularly in highly fit females (McClaran et al., 1998). Even in moderate exercise, significant expiratory flow limitation have been observed in highly fit healthy subjects, as a result of increased EELV with a resultant increase in V_D and a blunted hyperventilatory response usually seen in normal subjects (McClaran et al., 1999). Harms (2005) supports the challenge to traditional thinking with regard to the robust capacity of the pulmonary system, and points to the increasing evidence that exercise tolerance may be limited by the pulmonary system, especially in the case of females.

2.3.2 **Dyspnoea**

Dyspnoea is the subjective perception of the effort required to breathe and may be a limiting factor to exercise (Sheel et al., 2011). The causes underlying the perception of breathlessness, dyspnoea, are not clearly understood but the complex neural control of respiration is linked to its origins (Dominelli et al., 2011). Respiratory feedback is disassociated from efferent signals despite an increased central respiratory drive and dyspnoea has been linked with the increased work of breathing associated with a breathing pattern with dynamic hyperinflation of the lungs that can occur with EFL (Dominelli et al., 2011).

Physiological underpinnings to dyspnoea include EFL and dynamic lung hyperinflation as seen in patients with chronic obstructive pulmonary disease (COPD) with excessive inspiratory muscle loading implicated (O'Donnell, 2006). Respiratory patterns that may reduce EFL or avoid hyperinflation may prove beneficial in minimising dyspnoea but it is also possible that attempting to increase V_T above what is tolerable may increase dyspnoea and limit exercise tolerance. Recently, a psychological component has been suggested and that psychological or emotional components may be involved in a heightened sense of dyspnoea (Sanchez et al., 2012). As mentioned previously, deep breathing techniques as evidenced in meditation provide psychological and emotional benefits and therefore may alleviate dyspnoea.

Perceived breathlessness is higher with cycling than running at similar V_E as a result of different breathing patterns (Kalsas and Thorsen, 2009). The relationship between V_E and V_T is influenced by the visco-elastic properties of the lungs and chest wall, airway resistance and mode of exercise. The V_E/V_T relationship has three distinct phases: Phase I is characterised by a linear relationship, Phase II exhibits an increase in V_E mainly due to RR with a disproportionate increase in V_T, and Phase III is mediated solely by increases in RR with a possible decline in V_T (Kalsas and Thorsen, 2009).

The perceived intensity of breathlessness increases with age, due in part to higher ventilatory requirements, progressive inspiratory muscle weakness and impaired V_T expansion due to mechanical constraints which restrict inspiratory capacity (Ofir et al., 2008).

2.3.2.1 Breathlessness-anxiety

Breathlessness-anxiety, linked with perception and anticipatory networks in higher brain regions, has been shown to negatively modify exercise behaviour in COPD patients and may also pose performance limitations in highly trained athletes (Faull et al., 2016). Faull (2016) suggested that some athletes may be more susceptible to breathing anxiety, either due to lower respiratory muscle endurance or higher ventilatory sensitivity, and may be at increased risk for performance limitation and consequently benefit from psychophysiological interventions.

2.3.3 Respiratory muscle fatigue

The primary muscle of inspiration is the diaphragm, a specialised fatigue-resistant muscle which affects depth and rate of inspiration but will fatigue and compete for blood with

locomotor muscles when heavy intensity (>80% max) exercise is sustained (Dempsey et al., 2008). Respiratory muscle fatigue has been identified as a cause of exercise limitation (Voliantis et al., 2001; Spengler et al., 1999). Edwards et al. (2004) suggests diaphragmatic fatigue and the resultant competition for blood flow may be a possible cause. Normal endurance training fails to provide adequate training stimulus for the respiratory musculature (Romer et al., 2002) and specific respiratory muscle training (RMT) is needed to overload and induce adaptation in the respiratory musculature to counteract fatigue induced limitation to exercise.

2.3.4 **Expiratory flow limitation**

Expiratory flow limitation (EFL) is a mechanical limitation to the flow of gas through the airways that occurs during exhalation when expiratory muscles increase pleural pressure (Ppl) and the pressure gradient (intrathoracic vs. intra-pulmonary) is such that the airway collapses and restricts the passage of air (Aliverti, 2008). Put simply, it is the obstruction to airflow in the intrathoracic airways (Koulouris and Hardavella, 2011). This alters lung volumes, increasing EELV resulting in hyperinflation of the lung with an increased EILV. This negatively affects respiratory mechanics, efficiency and gas exchange (Aliverti, 2008). Female athletes in particular are affected due to their smaller lung volumes and relatively smaller airway diameters, as are older athletes who experience reduced ventilatory capacity due to an age related loss in elastic recoil (Dempsey et al., 2008, Harms, 1998, Harms, 2006).The occurrence of EFL, an exercise limiting factor, has been linked to exercise mode, breathing pattern and pulmonary anatomy (Koulouris and Hardavella, 2011, Dominelli et al., 2011). EFL is more likely to occur in treadmill exercise than when using a cycle ergometer due to higher maximal ventilations and oxygen requirements, when breathing pattern is altered when maximal ventilatory capacity is reached and in females due to dysanapsis, a mismatch in size between lungs and airways (Dominelli et al., 2011). With EFL, expiratory flow will not increase with an increase in transpulmonary pressure (Koulouris and Hardavella, 2011, Dominelli et al., 2011). It is also associated with a tachypnoeic respiratory pattern combined with increased lung volumes occurring over the middle part of the MEFV curve (Dominelli et al., 2011).

There are two main techniques for assessing EFL: 1) superimposition of FVL on MEFVL and 2) negative expiratory pressure (NEP) techniques, the former having some limitations (Milic-Emili, 2000, Mota et al., 1999a, Koulouris and Hardavella, 2011). This method compares tidal and maximal expiratory flow-volume curves (Milic-Emili, 2000) but is

unreliable and may falsely diagnose EFL due to pressure, volume and time-dependant changes affecting lung and gas behaviour (Mota et al., 1999a, Milic-Emili, 2000, Koulouris and Hardavella, 2011). The NEP method addresses these concerns and works by applying negative pressure at the mouth and comparing the resulting flow-volume curve with the previous one and has been found to be a better method to evaluate dyspnoea (Milic-Emili, 2000, Koulouris and Hardavella, 2011). Differences in methodologies to assess EFL has led to conflicting results as to its existence with factors such as exercise mode during test and subject's sport identified as confounding (Mota et al., 1999a). Respiratory muscles also have postural roles and different positions and arm involvement with varying sports can affect respiratory muscles' efficiency (Mota et al., 1999a). In trained cyclists the EELV may decrease at first but can then increase above resting levels in some cases in the absence of EFL while EILV may increases to TLC (Mota et al., 1999a, McClaran et al., 1999). EFL and the increased EELV create a sub-optimal breathing pattern with a reduced V_T that may inhibit the hyper-ventilatory drive needed at maximal and near maximal exercise (McClaran et al., 1999).

The gradual increase in EILV combined with reduced T_E also promotes increased EELV. A decrease in EELV places inspiratory muscles at a more optimal length to function efficiently and increase elastic work during expiration (Mota et al., 1999a). Mechanical limitations impose a maximal expiratory flow through intrathoracic airways which is determined by a critical expiratory pressure (the pressure at which maximal flow is achieved and above which increases in flow cannot be achieved due to effect on increased intrathoracic expiratory pressure exceeding transpulmonary pressure of the collapsible airway), above which EFL may manifest with a 'paradoxical' decrease in flow rendering the increases in expiratory muscle work inefficient and ineffective (Mota et al., 1999a). If limited, maximum ventilation ($V_E$max) may affect maximal flow rates which are in turn determined by maximal expiratory pressure above which EFL may occur (Milic-Emili, 2000, Mota et al., 1999b, Mota et al., 1999a).

In patients with COPD, EFL has been linked to premature activation of abdominal muscle for expiration, increases the cost of breathing and expiratory muscle fatigue while also being associated with dynamic lung hyperinflation which increases inspiratory muscle work and may negatively affect hemodynamics and dyspnoea (Hopkinson et al., 2010, Milic-Emili, 2000). Ottenheijm et al. (2008) have outlined four factors which determine the diaphragm's ability to generate force - "central drive, phrenic nerve conductance,

neuromuscular transmission and excitation-contraction coupling" - and diaphragm weakness in COPD is linked to increased dyspnoea. EFL is rarely seen in healthy subjects even when exercising maximally (Milic-Emili, 2000). However there is evidence that expiratory muscle fatigue can develop in healthy subjects during heavy intensity exercise affecting exercise capacity (Hopkinson et al., 2010). Hyperinflation places the diaphragm at shortened sub-optimal length which is mechanically disadvantageous (Ottenheijm et al., 2008), negatively affecting inspiration.

Airways respond to increased ventilation by reducing airway resistance and altering lung volumes to minimise respiratory muscle work and achieve more effective ventilation. They do so by relaxing bronchial smooth muscle, synchronising dilation of airways with inspiratory muscles, increasing EILV and recruiting expiratory muscles to reduce EELV. As a result, lung volumes increases fivefold and flow rates can exceed 10 times resting values during exercise. However, at high ventilation rates expiratory flow may encroach on the expiratory section of maximum flow-volume envelope causing flow limitation, and preventing ventilation from increasing as desired. As a result respiratory limitation can occur which in turn, inhibits exercise capacity (Dempsey et al., 2008, Aliverti, 2008).

EFL occurs in highly trained endurance athletes who have high maximal ventilation rates and in the elderly, possibly due to loss or lung elastic recoil (Aliverti, 2008). EFL may occur in both the intrathoracic and extrathoracic airways which narrow and restrict air flow and limit ventilation either as a result of an abnormal, malresponsive airway or as a consequence of excessive ventilatory demands of exercise in the presence of a normal airway and flow-volume envelope (Dempsey et al., 2008). EFL can manifest in endurance athletes in a number of ways, in those with abnormal airways, either extrathoracic or intrathoracic, or those with normal airways affecting only intrathoracic airways (Dempsey et al., 2008).

Abnormal airways:

Intrathoracic airways: Exercise Induced Asthma (EIA) affects the intrathoracic airways and is characterised by reduced airway diameter but mostly occurs on cessation of heavy-intensity exercise. The reduced airway compliance increases airway resistance, negatively affecting respiratory mechanics and ventilation, resulting in an increased $P[A\text{-}a]O_2$ manifesting in EIAH (Dempsey et al., 2008).

Extrathoracic airways: Vocal Cord Dysfunction (VCD) can affect athletes during severe-intensity exercise when flow rates are high and can be distinguished from EIAH by relief on cessation of exercise and decrease in flow rate and is described as "sudden-onset paradoxical narrowing of the glottis aperture" (p.615 (Dempsey et al., 2008)) followed by immediate flow limitation and EIAH.

Normal airways – EFL in normal intrathoracic airway

EFL in this instance results from the considerable ventilation and maximal flow rates during heavy exercise. The airway narrowing which results causes lung hyperinflation (increased EELV and increased EILV) which creates an inefficient ventilatory pattern increasing the elastic work of breathing (Aliverti, 2008, Dempsey et al., 2008). The negative effects on performance can be outlined as follows:

- reduced dynamic lung compliance increases the work of breathing and limits hyperventilatory response to exercise leading to EIAH and dyspnoea
- increased positive expiratory intrapleural pressure can exceed critical closing pressure of airway and increase cardiac afterload which reduces stroke volume and cardiac output
- tachypnoeic shift occurs at lower V_E
- increased respiratory muscle fatigue due to increased work as muscle work at increased velocities and inefficient lengths (Dempsey et al., 2008).

Hyperinflation negatively affects, respiratory muscle mechanics and control, CO_2 elimination and therefore acid-base balance and CO_2 mediated respiratory control, circulation through its effect on CO_2 due to raised intrathoracic pressure, and O_2 competition of inefficient respiratory musculature (increasing the cost of breathing) with locomotor muscle (Aliverti, 2008).

EFL has been demonstrated in athletes. Mota et al. (1999a) demonstrated that with 10 competitive cyclists (mean VO_2max = 72ml/kg/min) performing an incremental maximal exercise test, EILV increased to ~97% of TLC (two subjects reaching TLC) while EELV decreased up to 75% of maximal exercise after which it increased, reaching resting levels at maximal exercise. McClaran et al. (1999) showed similar changes in breathing pattern with progressive exercise with cyclists but EILV plateaued at 89-91% TLC. McClaran et

al. (1999) demonstrated that MEFV can be increased using a low density gas, HeO_2 ($26\%O_2$ – HeO_2 balance), allowing greater flow rates and reducing EFL. It allowed for V_T to reach and maintain higher volumes because EELV did not increase as it does in EFL and EILV remained lower (not hyperinflation) and facilitated higher maximal ventilation rates ($V_E$max). Indeed they suggest that even at lower lung volumes where flow rates may periodically reach maximal rates during part of the V_T, it may be involved in regulating ventilation and EELV (McClaran et al., 1999). Therefore, the presence of EFL may limit ventilation through its effect on EELV and V_T, and this attenuated hyperventilatory response in highly trained endurance athletes may lead to EIAH because of a failure to increase V_E to compensate for a widened P[A-a] O_2 (McClaran et al., 1999).

2.3.4.1 EFL in females

Females appear to be more susceptible to respiratory limitation than their male counterparts, manifesting in EFL and EIAH (Dominelli et al., 2011). It has been suggested that the demand placed on the respiratory system, determined by aerobic fitness, can be greater than the capacity thus limiting performance and that a high level of aerobic fitness is necessary for EFL to occur (Dominelli et al., 2011). This may me more important in female athletes who possess a greater capacity and pulmonary anatomy may be the principal determinant of EFL due to dysanapsis, a mismatch in size between lungs and airways that is most prevalent in females with smaller lung volumes and expiratory flows, affecting the larger airways in particular (Dominelli et al., 2011). When these airway are reduced in size airflow may move from laminar to turbulent flow increasing airflow resistance and consequently EFL as intrapulmonary pressure is increased to overcome resistance (Dominelli et al., 2011). It is also suggested that those females with larger lung volumes and expiratory flows are less likely to experience EFL than those who approach their maximal capacity and tend to adopt an altered breathing pattern.

2.3.5 Exercise induced arterial hypoxemia

EIAH refers to the reduction in arterial oxygen saturation (S_aO_2) from normal values of approximately 98% (in healthy individuals under normal conditions) and can occur to varying degrees described as mild, moderate or severe (<88%). This can negatively affect performance by decreasing VO_2max and increasing peripheral muscle fatigue. The underlying cause is inefficient gas exchange resulting in a widening of the alveolar-arterial PO_2 difference (P[A-a]O_2) that results in EIAH but other possible putative mechanism include cardiac and pulmonary shunts, Va/Q inequalities and EFL. In those with high

VO_2max values when exercising at heavy intensities and especially when running this effect is exaggerated (Dempsey et al., 2008). Factors contributing to increased $P[A\text{-}a]O_2$ are diffusion limitation due to interstitial pulmonary oedema, VA/Q mismatching, intra-cardiac and intrapulmonary shunts.

2.4 Respiratory System Plasticity

Evidence of respiratory system plasticity, namely adaptation to respiratory muscle training (RMT), has shown enhancements to exercise performance (Bailey et al., 2010, Illi et al., 2012, Edwards, 2013, Wilson et al., 2014, Feldman et al., 2003a). However, to date the research targeting respiratory system improvements has focused primarily on respiratory muscle function and training (Wilson et al., 2014, Illi et al., 2012) and ventilatory pattern has not been manipulated. There is a general consensus that RMT improves exercise performance (McConnell, 2012) which is supported by a recent meta-analysis (Illi et al., 2012), however this has been questioned by some (Patel et al., 2012). Improved respiratory muscle function and fatigue resistance with RMT has shown significant improvements in exercise performance ranging between 3% and 16% in running (Tong et al., 2008), cycling (Gething, 2004, Johnson et al., 2007) and rowing performance (Volianitis et al., 2000). Ventilatory pattern plasticity is evident from the apparent pattern adaptations uniquely seen in elite athletes, most notably professional cyclists, who have been seen to increase tidal volume (V_T) without exhibiting the expected tachypnoeic shift, the plateau of V_T and disproportional increase of RR (Lucia et al., 2001). This observation supports the investigation into ventilatory pattern effects on endurance performance.

2.4.1.1 Respiratory muscle training

Inspiratory muscle training (IMT) has been shown to improve exercise performance (Gigliotti et al., 2006) and evidence that RMT elicits performance improvement exists in many sports, particularly in swimming (Kilding et al., 2009), rowing (Voliantis et al., 2001), cycling (McConnell and Romer, 2004; Holm et al., 2004; Romer et al., 2002; Spengler et al., 1999) and running (Tong et al., 2008; Edwards et al., 2004). Despite considerable research and evidence supporting the benefits of RMT, the mechanisms responsible for improvements are unclear (Edwards et al., 2004). Research suggests that expiratory muscle training (EMT) has no apparent benefits and devices which combine IMT and EMT may negatively affect IMT training via induced fatigue resulting from the expiratory work (Griffiths and McConnell, 2007). Voliantis et al. (2001) suggest that the decreased demand for blood flow from the inspiratory muscles following IMT improves

blood distribution to exercising muscles. A common finding of IMT is an increase in maximum inspiratory pressure (MIP) (Griffiths and McConnell, 2007; Romer et al., 2002) although Gething et al. (2004a) suggest that a concomitant increase in performance is not always evident. Griffiths and McConnell (2007) suggest that an increase in MIP greater than 25% is needed to result in a performance improvement and that the increase in MIP reduces the relative workload on the respiratory musculature therefore increasing efficiency. RMT exists in three main forms (McConnell and Romer, 2004), voluntary isocapnic hyperpnea (VIH) which trains inspiratory muscle endurance, inspiratory flow resistive loading (IFRL) which involves sustained maximal static load, and inspiratory pressure threshold loading (IPTL) which involves inspiring from residual volume (RV) overcoming a set pressure threshold. IPTL has been shown to increase MIP and inspiratory muscle strength (Edwards et al, 2004), attenuate inspiratory muscle fatigue (Romer et al, 2002), reduce dyspnoea which can limit exercise and improve tolerance to high intensity intermittent running (Tong et al, 2008), decrease RPE (Kilding et al., 2009; Tong et al., 2008; Griffiths and McConnell, 2007), decrease tachypnoeic shift and significantly improve 20km and 40km TT in cyclists (Romer et al., 2002; Voliantis et al., 2001), decrease peak lactate (Griffiths and McConnell, 2007; Edwards et al., 2004; Voliantis et al., 2001), attenuate heart rate (Griffiths and McConnell, 2007), and increase 6 minute all out and 5000m rowing performance (Voliantis et al., 2001). The primary inspiratory muscle is the diaphragm which generates half of its energy requirements from carbohydrate metabolism mainly from lactate, and Spengler et al. (1999) suggest that training of respiratory musculature aids in increased lactate clearance, an observation also pointed to by Edwards and Cooke (2004). This may be glycogen sparing and therefore delay the onset of respiratory muscle fatigue (Spengler et al., 1999).

2.4.1.2 Respiratory adaptation to exercise training

With regard to the control of breathing, the brain of athletes is functionally different from non-athletes with altered connectivity in the neural control network, demonstrating improved psychophysical matching, specifically ventilatory perceptive accuracy, compared to sedentary subjects (Faull et al., 2018). Experience and familiarity with exercise induced breathlessness can alter anticipatory and predictive cognitive processing of breathlessness and affect athletes' perception of such events which may influence performance and it is suggested that improved ability to process internal homeostatic disturbance may be a feature of the athletes' improved 'interoception'(Faull et al., 2018).

The ventilatory response to exercise is strictly controlled, increasing dramatically with exercise intensity until eventually dyspnoea occurs (Faull et al., 2018). Exercise training is known to change the ventilatory profile of athletes and locomotor muscle training can reduce metaboreceptor and mechanoreceptor stimulation of ventilatory demand (di Paco et al., 2017). Faull (2016) suggested that some athletes may be more susceptible to breathing anxiety either due to lower respiratory muscle endurance or higher ventilatory sensitivity and at increased risk for performance limitation and could benefit from psychophysiological interventions. Improvements in ventilatory efficiency in world class cyclists may be the result of changes in breathing pattern and breathing control (Salazar-Martinez et al., 2016).

Depending on the parameters assessed and the time course of observation, mixed findings have emerged in relation to respiratory adaptations to exercise. Salazar-Martinez et al. (2016) showed no change in V_E/VCO_2 slope or breathing pattern over a three-year period in elite cyclists. Conversely, (di Paco et al., 2017) found that the ventilatory response profile in elite soccer players showed considerable change over a 8 month training program. They had a reduced ventilatory demand, changes in efficiency, a modified ventilatory profile, and an increase in athletes' ventilatory ceiling achieved via an increase in FEV1, VT and RR.

2.5 Cardiopulmonary exercise testing

Cardiopulmonary exercise testing has many functions depending on the specific test adopted. Clear aims as to the specific outcome measure should inform the choice of test, in addition to a critical understanding of the validity, reliability, sensitivity and limitations of each test. Despite the ubiquitous use of certain tests without question, it has become apparent that they may have methodological issues that call into question their use. A critique of the tests used in the three studies is presented with this in mind. We approach this by first presenting some general considerations for all tests and then the individual tests are dealt with.

2.5.1 General protocol considerations

2.5.1.1 Gradient

A 1% gradient has been shown to compensate for the lack of air resistance in the laboratory, therefore resulting in an energy expenditure equivalent to outdoor running (Jones and Doust, 1996). Inclined protocols result in increased 'type II muscle fibre'

recruitment and therefore increased anaerobic contribution leading to increased lactate levels as well as increased respiratory parameters (Miller et al. 2007; Kang et al. 2001). Protocols using excessive gradients (20%) recruit more type II fibres and large increments in workloads incur a greater anaerobic contribution which may underestimate VO_2max (Miller et al., 2007; Kang et al,. 2001). According to Midgley et al. (2008), tests should not exceed 15% incline.

2.5.1.2 Warm up

A warm up of five minutes at a velocity of 8km/h and 10km/h for female and male trained subjects respectively was used by Kuipers et al. (2003) when testing competitive, well-trained (60-150km/week) middle distance runners. Midgley et al. (2008) suggest that the initial stages of longer protocols provide a sufficient warm up, while shorter more intense ramp protocols require a separate warm up period.

2.5.1.3 Test duration

Midgley et al. (2007) studied male distance runners who produced VO_2max values that were not significantly different between one, two and three-minute incremental tests despite a duration range between 10 and 30 minutes. Midgley et al. (2008) suggest that in spite of significant evidence to the contrary and the limited nature of a single study performed by Buchfuhrer et al. (1983) that VO_2max should be elicited between 8 and 12 minutes, a dogmatic approach exists in relation to test duration. In view of the evidence produced by Midgley et al. (2008), it is suggested that VO_2max assessment using continuous protocols assessed by treadmill which last between 5-26 minutes should elicit valid VO_2max values in both trained and untrained subjects, provided short tests are preceded by an adequate warm up and that treadmill grades do not exceed 15%.

2.5.1.4 Stage duration: effects on specific parameters

VO_2max and lactate threshold (LT) can be assessed using different protocols: short ramp protocols for VO_2max and longer increments for LT, or a single test using stages greater than three minutes to assess both (Bentley et al., 2007). Despite the widespread use of such protocols, some have suggested serious inaccuracies in the use of stage duration shorter than six minutes for lactate measurement (Kuipers et al., 2003). Midgley et al. (2008) also highlight the advantage of longer protocols enabling secondary measures to be evaluated such as LT, advocating the use of short rest periods between stages to attenuate cumulative fatigue which may adversely affect the outcome of prolonged tests. Bentley et al. (2007) found that VO_2max was not affected by variations in stage length of three and four minutes

but five-minute stages resulted in lower values in cyclists. In contrast Kuipers et al. (2003) found no significant difference between stages of 60 seconds up to six minutes.

2.5.2 VO_2max/VO_2peak

Currently a 'true' VO_2max is accepted when either primary (plateau in VO_2) or secondary criteria (RER > 1.1, Blood Lactate > 8mmol/L, HR>90% HRmax) are met (Midgley et al., 2009). It has been suggested by both Midgley et al., (2009) and Poole et al., (2008) that traditional criteria are invalid and recommend that they should be abandoned with alternative criteria presented by Midgley et al. (2009). The occurrence of a plateau in VO_2 values is not always demonstrated, in particular with the adoption of continuous maximal incremental or ramp protocols over the more traditional discontinuous constant work-rate tests (Poole et al., 2008). Different criteria values have also been used (100ml-280ml) to verify its achievement which is in fact dependant on protocol and VO_2-workrate slope which are dictated by the size of workload increment (Midgley et al., 2009). The use of secondary criteria for the acceptance of VO_2max can underestimate the true value by up to 27% and should therefore be abandoned (Poole et al., 2008). Secondary criteria criticism is based on RER and lactate variability due to population (runners vs. cyclists), exercise type, protocol or a combination of all of the above. Continuous protocols result in lower RER and lactate concentrations than discontinuous protocols and longer protocols result in lower RER. Blood lactate demonstrated huge variability at peak levels, as well as variability in the blood medium used, so threshold cut-offs are inapplicable and heart rate (HR) should not be used as end-point to test. Also with regard to the traditional use of a verification phase, while it may reinforce the value achieved during the previous incremental trial, it does not validate if a true VO_2max was reached (Midgley et al., 2009).

Levine (2008) provides a review on VO_2max, identifying that a plateau phenomenon does not always occur in incremental VO_2max tests and instead proposed the use of repeated discontinuous tests to satisfy an alternative criteria. Midgley et al. (2009) looked at both runners and cyclists and suggest using 'task specific measures of motivational components' to assess subjects' psychological preparedness to exercise to maximal effort and recommend the use of a multi-stage verification trial and more robust criteria:

1. VO_2 plateau: difference between modelled (regression slope) and actual VO_2 is greater than 50%

2. VO_2max verification: greater than 50% difference between modelled (regression slope of linear VO_2 – work-rate relationship) and actual VO_2verif. Non-significant mean VO_2max – VO_2verif difference (applied on individual basis)

3. HRmax verification criteria: HRmax – HRverif <4bpm.

Midgley et al. accept that these new criteria may have limitations but they provide a more objective and valid criterion for true VO_2max acceptance. They recommend that incremental protocols should use a verification bout of supra-maximal square wave exercise following a rest period to volitional exhaustion (Midgley et al., 2007; Midgley et al., 2008). They conclude that insufficient research exists to recommend duration limits for discontinuous protocols. Due to the rest periods between stages which attenuate cumulative fatigue it may be suggested that a longer duration is acceptable in these protocols. Discontinuous protocols with short rest between stages and duration of approximately 22 minutes elicit a 'true' VO_2max as well as allowing for lactate measurement (Midgley et al. 2007), provided the test is discontinuous and rest periods between stages are relatively short. These rest periods are conducive to lactate sampling at the end of each stage. In untrained individuals, VO_2max attainment was independent of protocol and tests as short as five minutes elicited valid measurements. However in trained individuals, a protocol-dependant result is evident and protocols with smaller increments and a lower final gradient produce a higher VO_2max (Kang et al., 2001). Kang et al. (2001) also demonstrated that untrained subjects can achieve valid VO_2max values using aggressive incremental protocols (Costill/Fox protocol) previously thought to be too aggressive, despite test times of less than six minutes.

2.5.3 **Lactate threshold**

The 'lactic acid hypothesis' theorises that the accumulation of lactic acid is a causal factor in the onset of fatigue but the validity of this assumption has been questioned and a possible performance enhancing effect of lactic acid production suggested (Cairns, 2006). Acidosis due to hydrogen ion (H^+) accumulation associated with lactate production is thought to inhibit muscle contraction and induce fatigue. Blood lactate (BLa^-) concentration is an important marker of exercise intensity and training adaptation (Cairns, 2006). The lactate threshold (LT) is an important performance determinant of endurance performance and can be used to establish training intensities for endurance events and in particular for monitoring training improvements (Plato et al., 2008). Plato et al. (2008)

highlight the importance of LT measurement in elite athletes as this parameter continues to improve with training and provides a sensitive marker of adaptation to training. Debate also exists as to the use of the term threshold, with arguments against the existence of definitive breakpoint and suggestions of a continuous increasing lactate response and transition proposed as a better descriptor reflecting metabolic events (Faude et al., 2009).

Definitions of LT vary and confuse as the same term is used to identify physiologically different states, namely the first increase in lactate concentration above resting levels and alternatively 0.2mmol/L, 0.5mmol/L and 1mmol/L above resting levels (Bentley et al., 2007; Faude et al., 2009). This is confounded by alternative and subjective estimation of the threshold by visual inspection, logarithmic transformations, DMax, the use of fixed blood lactate concentrations (2mmol/L and 4mmol/L) and individual anaerobic thresholds (IAT) (Bentley et al., 2007). This is further confounded by considerable daily variation in these parameters which occurs in highly trained athletes (Bentley et al., 2007; Faude et al., 2009).

Considerable variation exists in the use of blood lactate concentration to establish a critical threshold reflecting metabolic conditions from which performance can be inferred (Plato et al., 2008). There is a lack of consensus leading to differences in terminology, blood concentration and direct measurement of lactate via blood or indirect inference from respiratory parameters. Each coincides with a distinct and different physiological state and exercise intensity so clarification is required between anaerobic threshold (AT), LT of 1mmol/L above resting, a curvilinear increase in lactate, and fixed blood lactate concentration of 4mmol termed the onset of blood lactate accumulation (OBLA). Methodological variations in blood medium, modelling to estimate thresholds, stage duration and workload increments create variation in the results obtained and currently no standardisation exists (Bentley et al., 2007). It is therefore essential to standardise testing protocols so that valid comparisons can be made between tests.

The first rise in blood lactate (BLa^-) is the intensity at which BLa^- begins to rise above baseline levels (Faude et al., 2009). Historically, before the advent of blood lactate measurement, it was assessed from ventilatory parameters and thought to correspond to V_T but controversy surrounds this issue. No consensus, standardisation or agreement exist as to which method should prevail, however visual determination is inappropriate due to inter-observer variability, and the use of arbitrary though objective values (0.2/0.5/1.0 mmol/L) above either resting or low intensity values are questionable. This is because each

one would identify a unique and different intensity, therefore questioning if any truly represents the first rise in BLa^- and therefore accurately reflects the distinct metabolic condition it attempts to measure. Consequently, the use of log-log transformation may provide an objective method to identify the first rise in BLa^- which is indeed reflective of a distinct change in metabolic conditions.

Stage duration must be considered for all lactate thresholds assessments (Kuipers et al., 2003). Bentley et al. (2007) suggest that despite the advantages in longer duration stages for lactate equilibrium, an incremental test comprising of three-minute work increments provides the most reliable and valid measures of endurance performance in trained subjects. However, Kuipers et al. (2003) suggest that stages less than five minutes do not allow for equilibrium between muscle and blood and therefore do not yield steady-state lactate results. A minimum stage length of 5-6 minutes is required. Blood lactate response is affected by both ramp slope and stage duration. Kuipers et al. (2003) cite a study by Foxdal et al. (1995) which found that at least eight-minute stages were need for lactate equilibrium to occur during running. There is a rightward shift of the lactate curve with three-minute stages compared to six-minute stages, possibly overestimating all lactate threshold values with significant differences between threshold velocities, on average 1.5km/h lower with six-minute stages (Kuipers et al. 2003).

Kuipers et al. (2003) also make reference to the maximal lactate steady state (MLSS) velocity which they found to coincide approximately with the velocity corresponding to a blood lactate concentration of 4mmol/L (OBLA) when using six-minute stages. Jones and Doust (1998) identified the MLSS as the upper limit to steady state exercise and used a 30-minute constant exercise bout to verify, while Kuipers et al. (2003) used a 15-minute constant intensity treadmill run to verify MLSS. These findings suggested that if lactate steady state was not achieved between 10 and 15 minutes it would not be achieved in a 30-minute bout. Despite these findings, Kuiper et al (2003) concluded that six minutes is insufficient to attain a true lactate steady state and for true assessment a follow up trial with 15-minute stages at velocities of (+/-) 0.5km/h relative to v-OBLA should be used on a different day to confirm exact MLSS. Stockhausen et al. (1997) found that in cyclists the time needed for lactate equilibrium, what is termed quasi-steady-state (QSS), was dependant on increment size. As a result, indirect measurement of MLSS, a performance determinant in endurance athletes, from incremental tests is error prone due to mismatches in work increment and stage duration. This results in threshold and endurance capacity

overestimation with more rapid workload increments. Stage durations of three minutes and four minutes for 20W and 30W respectively are suggested to achieve QSS despite reference to other studies suggesting 6-10 minutes (Kuipers et al., 2003).

Tanner et al. (2010) in a comparison of three handheld lactate analysers found the Lactate ProTM (Arkray KDK, Japan) to have good reliability and is strongly correlated (r=0.9988) with the enzymatic method (Arkray KDK, Japan). It is designed for use with whole blood and contamination of samples with perspiration or alcohol and delay in sampling with prolonged exposure of blood drop to air may interfere with accuracy.

2.5.4 **Economy of movement**

Economy of movement in the context of this thesis refers to locomotor economy, specifically, running economy and walking economy. It is determined principally by both physiological and biomechanical factors and improves with various training methods, including endurance, resistance and altitude, and also nutritional strategies such as dietary nitrate supplementation (Barnes and Kilding, 2015). Running economy has been identified as a performance determining factor in runners, those with better economy/efficiency performing better (Saunders et al., 2004, Lacour and Bourdin, 2015). The variation can be as high as 20% amongst heterogeneous populations but decreases to ~4% between top level athletes (Lacour and Bourdin, 2015). The measurement of economy is reliable with slight difference depending on metric used (Helgerud et al., 2010).

Economy has moved from the measurement of O_2 cost (O_C) to more sensitive methods like energy cost (E_C) which attempt to account for substrate utilisation (Fletcher et al., 2009). These are more accurately referred to as gross unit costs as resting metabolic rate (RMR) is not subtracted due to uncertainty in the validity of doing so (Fletcher, 2009). O_C can be measured as a rate in ml/kg/min but this doesn't reflect difference in speed. Alternatively, O_2 unit cost is the cost to cover a given distance measured in ml/kg/km thereby allowing comparisons across speeds. Fletcher (2009) concluded that despite this improvement over O_2 rate, $E_{C,}$ the energy unit cost measured in kcal/kg/min, utilises the respiratory exchange rate (RER) to calculate the calorific equivalent for a given VO_2 and is thought to be a superior and more sensitive metric that accounts for difference in substrate utilisation across speeds. Both Fletcher (2009) and Shaw et al. (2014) demonstrated that O_C was insensitive to increases in speed while E_C increased with increasing speed, therefore giving

a true reflection on the cost of movement. Shaw et al. (2014) also suggest the use of an appropriate scaling for body mass.

Gas exchange measurements of VO_2 and VCO_2 can be used in conjunction with metabolic equations to calculate equations to calculate E_C (Jeukendrup and Wallis, 2005). While numerous equations exist that make this that make this calculation based on estimated fat and carbohydrate utilisation inferred from the VO_2 and VCO_2 the VO_2 and VCO_2 Jeukendrup and Wallis (2005) have proposed equations for different exercise intensities. exercise intensities.

Figure 2-5 presents the equation for moderate to high intensity exercise. Using this equation one can easily calculate the E_C in kcal/kg/km.

Proposed equation for **moderate to high intensity exercise** (50 – 75 % $\dot{V}O_{2max}$)	
Carbohydrate oxidation (g/min) =	$4.210 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2 - 0.40 \cdot n$
Fat oxidation (g/min) =	$1.695 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2 - 1.77 \cdot n$
Energy from 1 g of carbohydrate (20% glucose; 80% glycogen)	4.07 kcal
Energy from 1 g of fat	9.75 kcal
Energy expenditure (kcal/min)	$0.550 \cdot \dot{V}CO_2 - 4.471 \cdot \dot{V}O_2$

Calculations of energy expenditure assume negligible contribution of protein oxidation

Figure 2-5 Metabolic equation used in calculation of E_C - Adapted from (Jeukendrup and Wallis, 2005)
The figure presents the equation and calorific equivalents for fat and carbohydrate that are used in the calculation.

2.5.5 Constant work rate (CWR) exercise tests

CWR is based on the principle of steady state assessed by gas exchange parameters which reflect muscle energetics. Steady state implies that a constant rate of metabolic energy liberation (metabolic power) and the three main metabolic pathways contribute in a constant manner (Ferretti et al., 2017). At the onset of exercise, muscle energetics must respond immediately in a 'square-wave' manner to meet the external work requirements but neither the cardiopulmonary response or the contribution of the three metabolic pathways are 'square-wave' in nature (Poole et al., 2008). Measurement of the VO_2 response to CWR exercise reveals inertia in these systems and a delay in achieving the expected square-wave response. The kinetics of this response (VO_2 kinetics) follows a particular pattern depending on intensity domain of the exercise (Jones et al., 2011).

In the moderate intensity domain (below LT/VT1) there are three phases: Phase I, the initial immediate rise with exercise onset, Phase II or primary component, the rapid exponential rise in VO_2 until Phase III, when a steady state is reached. The heavy intensity

domain (LT/VT1< Heavy <MLSS/VT2) differs with the presence of a VO_2 'slow-component' superimposed on the primary component with a delay in steady state being achieved, usually within 10-15 minutes depending on position at the lower or upper end of the intensity domain. In the severe intensity domain (>MLSS/VT2/CS) steady state is not achievable and there is a continued rise until VO_2max is reached or fatigue terminates exercise (Jones et al., 2011, Poole et al., 2008, Ferretti et al., 2017). Once the VO_2 kinetics plateau it signifies steady state based on the assumption that measurement of stable VO_2 at the mouth reflects equilibrium at all levels of the respiratory system from mouth to muscle and therefore muscle energetics can be reliably calculated allowing for calculation of the energy cost of locomotion. While this is the basis of open-circuit spirometry it is know that fluctuation occurs at all levels, from muscle to mouth (Ferretti et al., 2017, Shephard, 2017, Shephard, 1957).

VO_2 during both heavy and severe exercise changes as a function of work rate and time and therefore must be factored into the design of CWR protocols in the heavy intensity domain, allowing sufficient time for steady state VO_2 to be achieved if gas exchange measurement and energy cost are to calculated and evaluated.

2.5.6 **Running performance tests**

A principal aim of research in the area of sports performance is to determine the effect of a specific intervention on sports performance. However, the assessment of this is often problematic due to issues with reliability and validity of tests used (Hopkins and Hewson, 2001). Actual performance is rarely, if ever, assessed. Instead, simulated endurance performance measures (Currell and Jeukendrup, 2008) or physiological performance parameters are assessed (Jacobs et al., 2011), despite the imprecise relationship to actual performance (Hopkins and Hewson, 2001). Indirect physiological measures include maximum oxygen uptake (VO_2max), lactate threshold (LT), exercise efficiency, peak running speed (Vpeak), critical speed (CS) (Buchheit et al., 2008, Galbraith et al., 2014), time trials (TTs), time to exhaustion (TTE) (Jacobs et al., 2011, Machado et al., 2013b, Currell and Jeukendrup, 2008).

Indeed, the whole area of performance assessment in sports physiology, in particular the rigour of the methods used has been questioned and improvements recommended in the validation of these measures include validation against criterion method, use of coefficients of variation (CVs) with confidence intervals (CIs) reported, and also standard error

measurement which should be less than the smallest worthwhile change (Impellizzeri & Marcora, 2009; Atkinson et al. 2012). The variation in elite athlete performance needs to be considered when quantifying the 'minimal worthwhile improvement', the variation between events (within-athlete variation) measured as the coefficient of variation (CV) and between athletes (between-athlete variation) and intra-class correlation coefficient (ICC) to assess relative reliability (Hopkins and Hewson, 2001). A performance enhancement greater than the CV needs to be seen if a possible performance improvement is to be expected and the between-subject variation also needs to be considered. If it is much greater than the within-subject variation an improvement based on this measure alone may not have any impact on actual performance (finishing position). Hopkins and Hewson (2001) recommend CVs in predictive tests for running performance (CV < 2.5% for half and full marathon (CV < 1.5% for shorter distance). Time trials (TTs) have better predictive, validity, reliability and possibly sensitivity over time to exhaustion (TTE) tests, with a CV for running of less than 5% (60min run CV = 2.7%) (Currell and Jeukendrup, 2008). However these CVs exceed those recommended by Hopkins and Hewson so an alternative method was chosen to evaluate endurance running performance in our study.

2.5.6.1 vVO_2peak

Peak running speed during an incremental test (Vpeak) or velocity at VO_2peak (vVO_2peak) is the best predictor of running performance (McLaughlin et al., 2010). Different definitions are used to assess Vpeak/vVO_2peak including the highest speed maintained for 60 seconds ($V_{peak\text{-}60}$), the speed of the last completed stage ($V_{peak\text{-}C}$) or the speed and duration in seconds of the last incomplete stage ($V_{peak\text{-}P}$) calculated using Vpeak-P = Vpeak-C + (t/T) * speed increment Equation 1

$V_{peak\text{-}P} = V_{peak\text{-}C} + (t/T) * \text{speed increment}$ **Equation 1**

in which *t* is the number of seconds completed in the final stage and *T* is the number of seconds per stage (i.e. 180sec) (Machado et al., 2013b). Vpeak has been found to be highly correlated with both 5K and 10K TT running performance but is affected by stage duration: three minutes the recommended duration (Machado et al., 2013b). Machado et al. (2013a) compared three commonly used Vpeak protocols: the one-minute ($V_{peak\_1\_min}$), two-minute ($V_{peak\_2\_min}$) and three-minute stages ($V_{peak\_3\_min}$). The stage duration has an effect on peak lactate (BLa^-_{peak}), peak heart rate (HR_{peak}), V_{peak} and TT performance prediction. $V_{peak\_3\_min}$ produces significantly lower V_{peak} and BLa^-_{peak} compared to the other

two protocols. The lower vVO_2peak for the three-minute protocol is also supported by the work of Midgley (2007c). The $V_{peak\_3\_min}$ protocol using $V_{peak\text{-}P}$ has the highest predictive scores and the lowest standard error of the estimates (SEE) for 5K (r^2 = 0.92; SEE = 0.8 min) and 10K (r^2 = 0.83; SEE = 2.5 min) performance and the recommended standard for 5K and 10K running performance prediction by Machado (2013b). Mclaughlin et al. (2010) have shown $V_{peak\_3\_min}$ to be the best predictor of 16km performance (r^2=0.94) explaining 94% of the variance in performance and superior to velocity just below the onset of plasma blood lactate accumulation, V_OBLA (r^2=0.83) and running economy (RE) (r^2=0.66). These recommendations are further supported by the more recent work of Peserico et al. (2014), confirming a three-minute protocol used in conjunction with $V_{peak\text{-}P}$ to be the most reliable method ($1.5\% \leq CV \geq 1.8\%$; SEM = 0.3; ICC = 0.9; Highly Reliable). They also noted the effect of increment, 0.5km/h more reliable than 1 km/h which was more reliable than 2km/h. The high coefficients of determination (r^2) and use of standard error or measurement (SEM), CIs and ICCs supports the predictive capacity of this metric to meet the requirements for test validation set out by Impellizzeri and Marcora (2009). With this in mind, the vVO_2peak protocol chosen for Study 2 and Study 3 used three-minute stages with 1km/h increment (Midgley et al., 2007c) with an initial speed of 10km/h in endurance trained male athletes (Thevenet et al., 2008) and $V_{peak\text{-}P}$ as our vVO_2peak calculation method.

2.5.7 **High intensity interval exercise (HIIE) tests**

High intensity interval exercise has existed in various forms for over 100 years and is considered one of the most effective training methods to promote greater physiological adaptations (Tschakert and Hofmann, 2013, Buchheit and Laursen, 2013b, Billat, 2001a, Billat, 2001b, Buchheit and Laursen, 2013a).

While athletes regularly engage in heavy and severe intensity exercise, it is less common in the non-athletic population. High intensity interval exercise (HIIE) has moved from the almost exclusive realm of the trained athlete to the domain of the recreational athlete, physically active adolescents and adults, and clinical populations, although with some safety concerns (Costigan et al., 2015, Gosselin et al., 2012, De Nardi et al., 2018). A possible barrier to exercise adherence, and in particular HIIE, is perceived exertion and the resultant affective feelings of motivation, mood state, arousal and exercise enjoyment. This may be mitigated by choosing intervals not exceeding 60 seconds with a 1:1 work-to-rest ratio which may minimize negative feelings and promote better continued adherence while

maintaining a high cardio-metabolic stimulus (Kilpatrick et al., 2015, Seiler and Sjursen, 2004).

HIEE protocols are diverse, with variations in intensity and duration of both the work and recovery phases with at least nine variables that can be manipulated (Buchheit and Laursen, 2013b). The intensity is above maximum lactate steady state MLLSS and critical speed (CS) and below the maximum exercise intensity, the maximum sprint speed (MSS) which characterises the severe and extreme intensity domains (Jones et al., 2011). HIIE generally elicits a RPE ≥ 6 on the Borg CR-10 scale and ≥ 15 on the standard Borg scale. HIEE has been shown to be a powerful stimulus for improving endurance performance using a different signalling pathway to high volume lower intensity training to signal oxidative fibres. It promotes various physiological adaptations including muscle remodelling, mitochondrial biogenesis, increased fat oxidative capacity and increased GLUT4, MCT 1and 4 and glycogen content (Laursen, 2010, Gibala, 2009, Kohn et al., 2011, Perry et al., 2008).

HIIE can be categorised into very short (3 to 7 second) repeated sprint training (RST) in the 120-170% vVO_2max intensity range, short all-out effort(~30sec) sprint interval training (SIT) in the >160%vVO_2max to MSS range, short (<60sec) intervals (HIT short) in the 100-120% vVO_2max range, and long (>60sec) intervals (HIT long) in the 90-100% vVO_2max range (> MLSS/CS) (Buchheit and Laursen, 2013b).Depending on the method adopted the physiological stimulus challenges cardiopulmonary, metabolic, neuromuscular, autonomic nervous system (ANS) and musculoskeletal systems to different extents and therefore elicits different physiological adaptions. Importantly, the differing physiological stresses of such protocols can result in different stresses on the ANS which play a vital role in both adaptation and recovery (Seiler et al., 2007). There is no consensus on the dose-response to HIIE and not enough evidence to link specific protocols with specific adaptation, however, some global recommendations can be made. Metabolic stress will vary, placing higher or lower emphasis on oxidative and glycolytic fibres and energy pathways and HIEE protocols can be programmed based on specific loading of ATP/PCr, glycolytic and oxidative pathways (Buchheit and Laursen, 2013b, Tschakert and Hofmann, 2013).

Cardiopulmonary stress can be assessed by quantifying the time spent at or near VO_2peak and it has been suggested that time accumulated at high intensities (>T90%) are necessary to attain maximal or near-maximal cardiac output and optimally signal cardiac and

oxidative muscle fiber adaptation (Buchheit and Laursen, 2013b). HIIE protocols are commonly used to elicit VO_2max and suggested to be an optimal training stimulus for improving VO_2max and have been assessed by calculating the accumulated time above 90% VO_2max (T90%), 95%VO_2max (T95%) and 100%VO_2max (T100%/T VO_2max) (Midgley et al., 2007c, Turnes et al., 2016, Buchheit and Laursen, 2013b). Also recovery intensity will affect overall T90% with recovery intensities of 50% vVO_2max shown to elicit greater T90% and greater total VO_2 than either 67% or 84% (Thevenet et al., 2008). The quantification is based on the valid measurement of VO_2max and Kuiper et al. (2003) have shown no significant difference between protocols ranging from one to six minutes in stage length but a significant difference can occur with different time-averaging calculation of VO_2max. Indeed, the large inter-breath variability with breath-by-breath gas exchange analysis can result in an inverse relationship between VO_2max and rolling average duration, giving higher estimations of VO_2max with smaller rolling averages (Hill et al., 2003). VO_2max has been reported to have day-to-day variation as high as 5.6% and therefore the less stringent T95% is recommended for intermittent running (Midgley et al., 2007c). These metrics have however been shown to have poor reproducibility with high coefficients of variation (CV), T90% (CV = 24.5%) and T95% (CV= 34.5%) (Midgley et al., 2007b). In the absence of a more reliable and valid measure these metrics were used to tentatively assess HIIE performance in Study 3. They were extended to include time above 80% VO_2max (T80%) and 95%VO_2max (T85%) and used in conjunction with the number of completed repetitions to assess overall HIIE performance. While we could find no CVs for lower metrics such as time above 80% (T80%) and time above 85% (T80%), it was decided to include these metrics post hoc as many subjects failed to record any time above T90% and T95%.

One protocol used by endurance runners is 60-second intervals with a 1:1 work rest ratio, completing approximately 24 work intervals (Seiler and Sjursen, 2004, Kilpatrick et al., 2015). This specific session when self-paced resulted in a lower work VO_2peak, higher VO_2 in recovery but a similar average over the entire session when compared to two, four and six-minute protocols. When the interval session is broken into sets the T@VO_2peak is reduced. Intervals not exceeding 60 seconds may minimize negative feelings and promote better continued adherence and exercise enjoyment (Kilpatrick et al., 2015, Seiler and Sjursen, 2004).

2.6 Summary

While the human respiratory system has a robust capacity to deal with most eventualities, limitations and inefficiencies do exist, in particular at high intensities of exercise in elite athletes. Significant adaptations in athletes exist, who exhibit an enhanced respiratory efficiency and improved performance. Training of the respiratory musculature has also been shown to improve performance. Considerable individual breathing pattern diversity exists that can be either functional or dysfunctional. These patterns are influenced genetically, developmentally and by multiple psychophysiological mechanisms. Advances in neuroscience have identified the potential for respiratory neuroplasticity and its bi-directionality, and also meditation style activities incorporating deep breathing showing functional and structural changes in neural structures and circuitry. Differences between athletes and non-athletes, and males and females in both pattern and response to exercise exist. Little research has focused on manipulation of respiratory pattern which can potentially influence physiological and psychological factors in fatigue mechanisms. Much of the research has identified how changes in tidal volume and respiratory rate have modulatory effects on the autonomic nervous system and can improve efficiency and performance. While the mechanisms behind these limitations and improvements may be unclear it is apparent that improvement in efficiency and performance are possible and necessitate further investigation.

Based on evidence supporting the beneficial effects of deep breathing, an investigation into its application to exercise is merited. If the adoption of this pattern can elicit an improvement in performance, it may also provide valuable information as to the underlying mechanisms because the effects can be directly attributed to the altered breathing pattern as opposed to underlying physiological adaptation that may accompany changes in respiratory patterns witnessed in elite athletes. A review of cardiopulmonary exercise testing has identified a changing landscape in what is considered best practice for the measurement of maximal aerobic capacity (VO_2max) and lactate threshold. A number of protocol-specific considerations were identified with regard to the assessment of performance measures and HIIE and the design of protocols which have informed our choice and design of protocols for each of our studies.

3. Study 1

'The effect of deep breathing on economy of locomotion during heavy intensity exercise in trained and untrained male and female subjects '

3.1 Introduction

Exercise is pursued by healthy, clinical and athletic populations and unquestionably, it plays a pivotal role in health and wellbeing, the treatment and prevention of numerous pathophysiological conditions and the advancement of athletic performance. Exercise has been extensively researched showing many physical and psychological benefits, physically active lifestyles improving cardiopulmonary and metabolic health, and reducing the risk of chronic disease (Penedo and Dahn, 2005, Kilpatrick et al., 2015). Physical activity recommendations have traditionally focused on moderate intensity exercise, however, increased benefits are possible with exercise in the heavy and severe intensity domains (Kilpatrick et al., 2015).

However, the ability to exercise, especially at higher intensities is compromised by fatigue-limiting symptoms, both physiological and psychological, the mechanisms underpinning which are not completely understood. It is therefore important to understand what factors may prevent engagement in heavy intensity exercise and if any strategies can ameliorate these barriers to participation. While athletes regularly engage in heavy intensity exercise, it is less common in the non-athletic population. Recent advances have been made in our understanding of fatigue mechanisms including the identification of respiratory system limitations and the potential for respiratory adaptation (Dempsey et al., 2008b, Dempsey et al., 2008a, Amann, 2011b). Another possible barrier to exercise adherence is perceived exertion and the resultant affective feelings of motivation, mood state, arousal and exercise enjoyment (Kilpatrick et al., 2015, Seiler and Sjursen, 2004). The increased demands on

the respiratory system and resultant hyperpnea, increases subjective sensations of breathlessness with negative performance outcomes (Faull et al., 2018).

Research has sought to train and optimise various physiological systems to improve cardiovascular, metabolic and neuromuscular function to elicit improvements in performance (Joyner and Coyle, 2007, Midgley et al., 2007a). The respiratory system been only been recently added as a possible avenue of investigation and it's role is far more complex and pervasive than previously thought (McKenzie, 2012). The traditional consensus that the respiratory system did not limit performance has changed amidst growing evidence that it may limit exercise performance, especially in elite athletes, but more importantly, that it may be trained to improve performance (Dempsey et al., 2008a, Dempsey et al., 2008b, Dempsey et al., 2006, Romer and Dempsey, 2006, Tong et al., 2008, Tong et al., 2004, Guenette and Sheel, 2007b, Amann, 2011b, Gigliotti et al., 2006, McKenzie, 2012). Also, new developments in our understanding of fatigue mechanisms and the role peripheral metabolite accumulation, which the respiratory system may influence also highlights the need to take a deeper look at this often overlooked physiological system (Amann, 2011a).

The role of the respiratory system in many of the physiological and psychological factors contributing to the development of fatigue and ultimately to the limitation of exercise performance has been largely ignored. In light of a growing body of research challenging this view, emerging evidence suggests the respiratory system may fail to meet the demands imposed during exercise and therefore play a role in the development of fatigue, both locally and systematically, limiting exercise performance (Dempsey et al., 2008a, Dempsey et al., 2008b, McKenzie, 2012, Romer and Polkey, 2008, Amann, 2011b, Harms et al., 1997), especially in athletes (Guenette and Sheel, 2007a, Romer and Polkey, 2008) of which female athletes may be at even greater risk (Dominelli et al., 2011, Guenette et al., 2009, Guenette et al., 2007, Hopkins et al., 1998, Harms and Rosenkranz, 2008b). Ventilation patterns may have a considerable influence on ventilatory efficiency, the effectiveness of gas exchange, the development of respiratory limitation, the mechanics and therefore the metabolic cost of breathing, and also the mechanics of locomotion (Aliverti, 2008b, Koulouris and Hardavella, 2011, Dominelli et al., 2011). The control of respiration is still debated and not fully understood (Haouzi, 2012). This changing research landscape recognises the respiratory system as a contributing factor to fatigue, posing a

limiting factor to exercise performance. It is proposed that an altered breathing pattern, specifically a deep breathing pattern (DB) may improve athletic performance via moderation or amelioration of respiratory limiting factors.

The autonomic ventilatory pattern adopted during exercise may fail to meet the imposed functional demands placed upon the respiratory system leading to respiratory limitation of exercise. This may be due to impaired ventilation perfusion matching (VA/Q), impaired gas exchange, expiratory flow limitation (EFL) and/or exercise induced arterial hypoxemia (EIAH) (Wagner, 1992, McClaran et al., 1999, Dempsey et al., 2008b, Dempsey et al., 2008a). Evidence of respiratory system plasticity has shown that respiratory adaptations via respiratory muscle training (RMT) can enhance exercise performance in running (Tong et al., 2008), cycling (Gething, 2004, Johnson et al., 2007) and rowing performance (Volianitis et al., 2000). Currently there is a lack of research in the area of ventilator pattern manipulation and how this may effect respiratory limitation, respiratory efficiency, acid-base balance and how these may influence the development of fatigue and/or exercise performance.

The ventilatory pattern adopted is consequential to the combined and proportional influences of afferent inputs on autonomic control centres. There is considerable heterogeneity in respiratory pattern both at rest and during exercise demonstrating that ventilatory requirements may be satisfied in varying ways and indeed some elite athletes exhibit unique ventilatory patterns during exercise (Benchetrit, 2000, Lucia et al., 2001). It is important to remember that while respiration is under autonomic control it can be consciously overridden allowing ventilatory pattern to be altered.

Ventilation pattern determines the mechanics and therefore the metabolic cost of breathing and also influences ventilatory efficiency. Its effects have implications both on the effectiveness in maintaining O_2, CO_2, and pH homeostasis and also the incurred cost in attempting to achieve this. An inefficient, sub-optimal ventilator pattern may result in an increased cost of breathing and the development of respiratory muscle fatigue which has been shown to result in competition for O_2 with locomotor muscles, negatively affecting exercise performance (Dempsey et al., 2006, Romer and Dempsey, 2006). In addition to these specific respiratory effects ventilation pattern may also affect the mechanics of locomotion (Baskurt, 2012, Bernasconi and Kohl, 1993, Rabler and Kohl, 2000) and therefore mechanical efficiency of exercise.

It has been proposed that an individual critical limit of peripheral metabolic disturbances exists which cannot be voluntarily surpassed (Amann, 2011a). During intense exercise when metabolic disruption is detected and relayed to the central nervous system (CNS) via metobosensitive afferent neural pathways inhibiting central motor drive (CMD), this threshold is reached leading to fatigue and ultimately reduced exercise intensity and/or exercise termination (Amann, 2011a). These afferent pathways also provide feedback which regulate ventilatory and cardiovascular responses to exercise (Amann, 2011a). Hydrogen ions (H^+) are one such metabolite which disrupts acid-base balance, and intramuscular levels of H^+ are related to metabolic CO_2 accumulation. Therefore the elimination of CO_2 plays a key role in the regulation and maintenance of 'acid-base' balance (Robergs et al., 2005). A ventilatory pattern that may be more effective and efficient in CO_2 elimination may decrease this afferent stimulus which may be responsible for driving an inefficient pattern. This may reduce the metabolic cost and/or delay acid-base disturbance, delay fatigue onset and improve exercise performance.

Deep breathing has been shown to affect the autonomic nervous system (ANS) causing sympathovagal modulation, affecting heart rate (HR) via heart rate variability (HRV), a phenomena called respiratory sinus arrhythmia (RSA), blood pressure, arterial oxygen saturation (S_aO_2), muscle sympathetic nerve activity (MSNA) in skeletal muscle and the peripheral microcirculation (Krasnikov et al., 2013, Yasuma and Hayano, 2004, Seals et al., 1990). It is suggested that deep breathing may improve gas exchange, ventilatory efficiency, reduce the cost of breathing and/or improving mechanical efficiency. This has the potential to decrease VO_2, delay acid-base disturbance and ultimately improve exercise performance.

The conscious overriding of autonomic respiratory control altering ventilatory pattern may positively affect exercise performance if it can reduce the effects of these exercise limiting factors without incurring other deleterious side effects such as exacerbating disruptions to homeostatic balances of blood gases and pH that occur in exercise and possibly exacerbating fatigue and reducing exercise tolerance.

3.2 Aims and Objectives

- **Hypothesis**

  'Deep breathing improves economy of locomotion during heavy intensity, CWR walking and running'

- ***Aims***
    - *To evaluate if deep breathing during exercise reduces the cost of locomotion*
    - *To explore what factors may underlie the reduced cost of locomotion*
- ***Objectives***
    - *Measure and calculate cost of locomotion in the heavy intensity domain, VO2, Oc and Ec, under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
    - *Measure other gas exchange parameters under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
    - *Measure overall and respiratory RPE under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
    - *Measure stride frequency and calculate locomotor respiratory coupling under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*

3.3 Methods

3.3.1 Subjects

Subjects were recruited by emailing athletics and triathlon clubs, and emailing staff and students in DCU advertising for research volunteers. Healthy males and females between the ages of 18 and 55years, either untrained but physically active individuals or endurance trained runners were selected. Subjects needed to be were injury free for the previous month and were excluded if they had any respiratory disease or musculoskeletal injury that could interfere with exercise testing. In total forty subjects (*n=40*) were recruited and tested. The study was approved by the Ethics Committee in Dublin City University.

3.3.2 Study Design

Figure 3-1 gives an overview of the structure of the study outlining the test sequence. The study used a within-subject design, participants completing the spontaneous breathing

(SB). Sample size was not calculated. Subject numbers were based on similar studies in the literature.

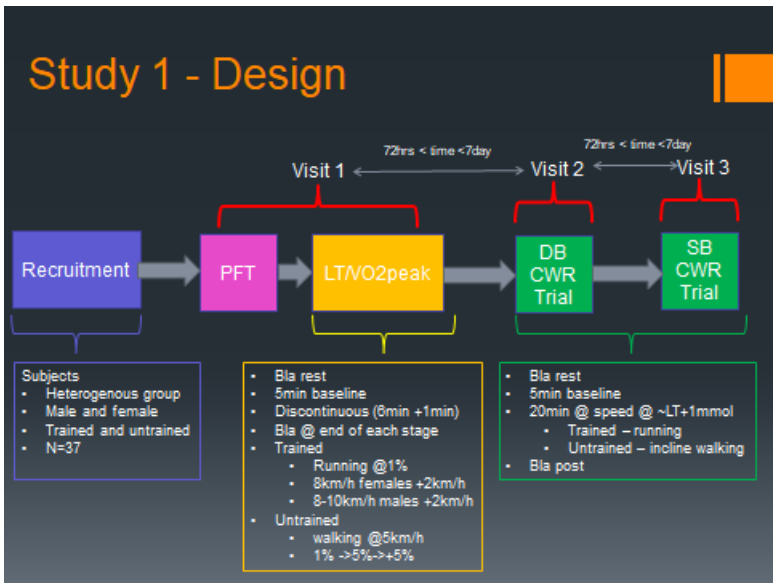


Figure 3-1 Study 1 – Design overview

All the subjects visited DCU Human Performance Laboratory in the School of Health and Human Performance for testing on three separate occasions. All three tests were conducted within 14 days, the two trials separated by at least 72 hours and no more than 7 days and ordered so that the Deep Breathing (DB) pattern was adopted in the final trial to minimize specific threats to internal validity, namely improvements in fitness and the effect of using altered breathing pattern may alter the normal pattern. On the first visit spirometry was assessed followed by a maximal incremental treadmill running test. Subjects were instructed to follow a similar diet and training regimen before all tests. This meant being well hydrated and abstaining from food and caffeine for 4 hours prior to testing, and performing no hard training in the 48 hours prior to testing. Every attempt was made to perform tests at a similar time and on the similar training day to control for diurnal changes, training fatigue and metabolic changes. Subjects had height, weight, resting heart rate, resting blood pressure and resting BLa^{-} measured prior to each test. Pulmonary function and maximal aerobic capacity and LT were assessed during the first visit.

3.3.3 **Pulmonary Function Testing**

Spirometry was carried out with an automated pulmonary function testing system (Viasys Vmax Encore 299; SensorMedics, Yorba Linda, CA) via indirect calorimetry using open-circuit spirometry. Tests were carried out in the standing posture following recommended

procedures. Pulmonary function measurements were expressed as absolute values and percentages of predicted values.

3.3.4 **Cardiopulmonary Exercise Testing**

Laboratory environment conditions were controlled at 18 degrees centigrade. Exercise testing was carried out on a Woodway Ergo ELG 55 motorised treadmill. Pulmonary data collected breath-by-breath throughout all exercise tests with the Viasys Vmax Encore 299 metabolic cart. The system was calibration in accordance with manufacturer guidelines prior to each test. Heart rate data was recorded with the Polar S610i heart rate monitor (Polar Electro, Inc., Kempele, Finland) using a 1 second sample rate and later downloaded for analysis. Perceived exertion was assessed on two scales, the standard Borg Rating of Perceived Exertion (RPE) scale for overall exertion which we shall refer to as RPE-O and the Borg CR-10 dyspnea scale. RPE was recorded at the end of each stage and on test termination, if during a stage. Subjects were verbally encouraged through the final stages to give maximum effort and all subjects exercised until volitional fatigue. VO_2peak was calculated from 1 minute rolling averages to obtain the highest value in the final stage.

Figure 3-1 presents an overview of the study design outlining test order and timeline. All the subjects visited DCU Human Performance Laboratory in the School of Health and Human Performance for testing on three separate occasions, separated by at least 72 hours and no more than 10 days between the two vVO_2peak tests. Following recruitment, on the initial visit subjects completed a medical health screening form and informed consent before a pulmonary function was tested to screen for respiratory disease. Subjects performed an initial graded exercise test to identify exercise intensity for subsequent constant work rate (CWR) trials. The order or the trial was fixed such that the SB trial was conducted first to prevent any learnt effect from DB affecting the SB trial. Subjects were instructed to follow a similar diet and training regimen before all tests. This meant being well hydrated and abstaining from food and caffeine for 4 hours prior to testing, and performing no hard training in the 48 hours prior to testing. Every attempt was made to perform tests at a similar time and on the similar training day to control for diurnal changes, training fatigue and metabolic changes. Subjects had height, weight and resting heart rate measured prior to each test.

3.3.5 VO_2peak/Lactate Threshold Protocol

A combined lactate threshold (LT) and VO_2peak protocol was used to assess both the LT which was used to set the intensity for the two heavy intensity exercise trials and VO_2peak which was used to assess subject cardiopulmonary fitness. A discontinuous incremental treadmill protocol was adopted utilising an initial gradient of 1%, 6 minute stages interspersed by 30sec rest intervals allowing for blood lactate sampling after each stage. A 6 minute protocol was selected for accurate LT assessment as shorter stage duration compromise accuracy due to the slower nature of BLa^- kinetics compared to O_2 kinetics (van Hall, 2010, Messonnier et al., 2013, Barron et al., 2015, Kuipers et al., 2003). All tests began with 5 minutes standing still on the treadmill attached to the Vmax system to obtain baseline cardiopulmonary measurements. Protocols differed for trained and untrained subjects. The use of different protocols (walking vs. running) for the untrained and trained subjects was necessitated by the large disparity in fitness levels between groups and the need for subjects to maintain the exercise intensity for 20minutes. Pilot work suggested that running would place the untrained subjects above LT and therefore prohibit the identification of the LT. The primary aim of the test was to identify a specific metabolic intensity in the heavy intensity domain determined by the LT assessment with VO_2peak secondary. As detailed in the literature review, this require longer duration stages (6-minutes) and a number of stages below LT in order to calculate the LT accurately. Also explained, is the lack of evidence to support the recommended durations for VO2peak testing and the validity of longer duration tests.

3.3.5.1 Trained Protocol

Trained subjects performed a running protocol with only speed increasing with each stage, gradient remaining constant at 1%. Initial speed was individualised for each subject, 8 km/h or 10km/h for males and 8km/h for all females. Speed increased by 2km/h for each stage until test termination.

3.3.5.2 Untrained Protocol

Untrained subjects performed a walking protocol with speed fixed at 5 km/h for the duration of the test and only gradient increased. Initial gradient was set a 1% and increased to 5% for the second stage and 5% for each stage thereafter until test termination.

3.3.5.3 Blood Lactate Sampling

A Lactate Pro^{TM} (Arkray KDK, Japan) handheld blood lactate analyser was used to measure blood lactate (BLa^-) from capillary blood sampled from the left earlobe, the device requiring 5µL of blood sampled via capillary action with a coded reagent strip, calculating BLa^- amperometricallly (Tanner et al., 2010). The device was calibrated following manufacturer guidelines with a manufacturer supplied calibration strip and check strip specific to each box of sampling strips prior to each test. Results took 60 s to analyse. Following calibration, test strips were only removed from foil wrapping ~60 seconds prior to each sample and inserted into the analyser. The puncture site was cleaned with an alcohol pad prior to the initial puncture and prior to each sample, dried with sterile gauze, the first drop of blood obtained by applying pressure to the surrounding site was wiped away to remove any contaminants (alcohol or perspiration) and the second drop of blood touched to the tip of the test strip.

3.3.5.4 Lactate Threshold Calculation

The LT was defined as the speed corresponding to a 1mmol/L rise in BLa^- above baseline (Faude, 2009) and was calculated using Lactate-E software from which the corresponding speed and gradient from the test was calculated (Newell et al., 2007). The LT was calculated based on the log/log LT method which has been suggested to be the most objective (Faude, 2009). This intensity was used as the treadmill speed and gradient for the two subsequent heavy intensity, constant work rate exercise trials. Intensity was based on this calculation insure subjects were above LT and therefore in the heavy intensity domain.

3.3.6 Constant Work Rate (CWR) Trials

3.3.6.1 Intensity domain classification

Exercise intensity domains can be classified based on VO_2 kinetics, metabolic and/or ventilatory thresholds into moderate, heavy and severe intensity domains (Jones and Poole, 2005). The heavy intensity exercise domain occurs above the LT (or ventilatory threshold 1 - VT1) and below maximum lactate steady state (MLSS) (or critical speed (CS) or ventilator threshold 2 (VT2) (Smith and Jones, 2001, Pringle et al., 2002). The VO_2 kinetic response to CWR exercise in the heavy intensity domain exhibits a slow component superimposed on the primary component and a delay of 10-15min in achieving steady state in the extreme. BLa^- also exhibits a higher but stable level compared to moderate intensity exercise below the LT (Jones and Poole, 2005, Jones et al., 2011).

3.3.6.2 Protocol description

The heavy intensity trials consisted walking/running at a fixed individualised intensity in the heavy intensity domain for 20 minutes to allow for steady state conditions to occur (Jones and Poole, 2005). Treadmill speed and gradient were individualised, based on the LT assessment, to the speed and/or gradient corresponding to a 1 mmol/L rise in blood lactate above baseline. Pulmonary data was measured breath-by-breath throughout the exercise. Heart rate (HR), stride frequency (SF),RPE-O and RPE-R were recorded every 5 minutes.

Subjects were give clear instructions before each test and before the second DB trial they were given additional specific instructions regarding the breathing pattern to adopt (see 3.3.6.3). Prior to the exercise test subjects sat still for 5min minutes after which resting heart rate and baseline lactate was sampled. Subjects began with 5 minutes standing still on the treadmill attached to the metabolic system to obtain baseline cardiopulmonary measurements. For the DB trial, subjects were given the final 2 minutes to practice the DB technique. There was no warm-up and subjects began by stepping onto the treadmill at the calculated intensity. The exercised at this intensity continuously, following which, they were instructed to step off the treadmill by placing their feet on either side of the treadmill and a final BLa^- was measured. Subjects were then disconnected from the Vmax system and allowed to warm down at their chosen intensity.

3.3.6.3 Deep Breathing Instructions

The deep breathing (DB) pattern was self-paced by the subjects. Instructions were verbally conveyed to the subjects, in which they were instructed to breathe as deeply and slowly as the felt comfortable doing. During the test tidal volume (V_T) was monitored to ascertain if they maintained a DB pattern based on the V_T from the SB trial. Periodically during the test the instructions were repeated if the V_T was observed to be decreasing significantly to SB levels.

3.3.7 Stride Frequency (SF) measurement

SF was measured by counting the number of strides manually for 60 seconds. Strides were measured by counting the number of times the right foot contacted the treadmill in a second period. It was measured for minutes 4-5, 9-10, 14-15 and 19-20.

3.3.8 **Locomotor Respiratory Coupling (LRC) calculation**

LRC was calculated by dividing SF taken for the last minute (19-20min) by the respiratory rate (RR). The manual counting of SF imposes limitation in the accuracy of assessment and does not allow for phase coupling to be assessed however it is a method that allows global assessment of the coordination (McDermott et al., 2003) and has been used previously (Bramble and Carrier, 1983).

3.3.9 **Locomotor Efficiency**

Efficiency of locomotion was assessed by calculating the O_2 unit cost (O_c) expressed as ml/kg/km and energy unit cost (E_c) expressed as kcal/kg/km (Fletcher et al., 2009). E_C was calculated using the updated formula of Jeukendrup & Wallis (2005) for moderate to high intensity exercise (seeEC = (0.550 * VCO2 – 4.471 *VO2* (# min/km)) / Body Mass (kg) Equation 2).

E_C = (0.550 * VCO2 – 4.471 *VO2* (# min/km)) / Body Mass (kg) Equation 2

3.3.10 **Data processing and analysis**

All manually recorded data was entered into a Microsoft Excel spreadsheet. Due to limitations with the Vmax software version all data from the system was only downloadable as text files. Data for each test was in 10 second samples and exported in four separate files in order to get all parameters for analysis and spirometry data was exported separately. These files were parsed using a Python script to remove text headers and combine all the data for all subjects into Excel format. The Excel files were then imported to Microsoft Access for analysis and formatting for SPSS. Microsoft Access SQL queries were written to further analyse the data. One minute rolling averages were calculated on all data fields and combined with manually recorded data and spirometry data. Data was averaged over the last 5 minutes for analysis. Data was then exported in Excel format for import into SPSS for statistical analysis.

3.3.11 **Statistical Analysis**

SPSS was used for statistical analysis. All normally distributed quantitative variables were analysed using a three-way Repeated Measures ANOVA. Three-way interactions between breathing pattern, gender and training status and two-way interactions between breathing pattern and gender and breathing pattern and training status were analysed. Higher order results are presented first, if three-way or two-way interaction was significant, the main

effect of each factor was not presented. If no three-way or two-way effects exist the main effects are presented. Results are represented as mean with standard deviation (mean ± SD), mean difference (Mean Diff.) with 95% confidence intervals (95% CI) and Partial η2 and effect size (based on Partial η2) are also shown where appropriate. The level of significance was set at $p < 0.05$.

3.4 Results

Table 3-1 Participant characteristics –mean ± SD

	Female			male		
	trained	untrained	Total	trained	untrained	Total
N =	14	7	21	11	5	16
Age	25.9 ± 7.5	28 ± 10.7	26.6 ± 8.5	35.3 ± 10.2	33.2 ± 12.6	34.6 ± 10.6
Height	165.4 ± 4.2	163.1 ± 5.7	164.6 ± 4.7	173.9 ± 5.2	179.6 ± 2.3	175.7 ± 5.2
Weight	56.7 ± 4.5	58.2 ± 7.9	57.2 ± 5.7	68.2 ± 6.8	87.4 ± 10.8	74.2 ± 12.1
BMI	20.7 ± 1.6	21.9 ± 3	21.1 ± 2.2	22.6 ± 2.2	27.1 ± 3.7	24 ± 3.4
FVC	3.873 ± 0.413	4.084 ± 0.48	3.943 ± 0.437	5.048 ± 0.685	5.73 ± 0.539	5.261 ± 0.705
FVC %Ref	105 ± 7	113 ± 11	107 ± 9	107 ± 14	114 ± 13	109 ± 14
FEV1	3.293 ± 0.34	3.46 ± 0.436	3.349 ± 0.372	4.103 ± 0.851	4.53 ± 0.556	4.236 ± 0.779
FEV1 %Ref	102 ± 6	110 ± 9	105 ± 8	104 ± 21	109 ± 15	106 ± 19
FEV1/FVC	85 ± 6	85 ± 3	85 ± 5	81 ± 8	64 ± 32	76 ± 19
VO_2peak (L/min)	3.085 ± 0.281	2.501 ± 0.328	2.89 ± 0.404	4.112 ± 0.452	4.418 ± 0.761	4.208 ± 0.559
VO_2peak (ml/kg/min)	54.8 ± 6	43.1 ± 6	50.9 ± 8.1	60.6 ± 7.5	50.8 ± 8.5	57.5 ± 8.9

Summary of key subject characteristics (N=37) divided by gender, training status and total for each gender. BMI = Body Mass Index; FVC = Forced Vital Capacity; %Ref = % of reference value based on normative data; FEV1 = Forced Expiratory Volume in 1 second; VO_2peak = peak oxygen uptake; SD = Standard Deviation;

3.4.1 Subjects

40 subjects were recruited, 37 completed both trials and 3 subjects failed to complete the second DB trial as they were unable to return for second DB trial within two weeks of the first. **Error! Not a valid bookmark self-reference.** presents summary physical and physiological characteristics of the participants (n=37) in the study. Participants were

healthy males (n=16) and females (21) and either endurance trained (n=25) or untrained but physically active (n=12).

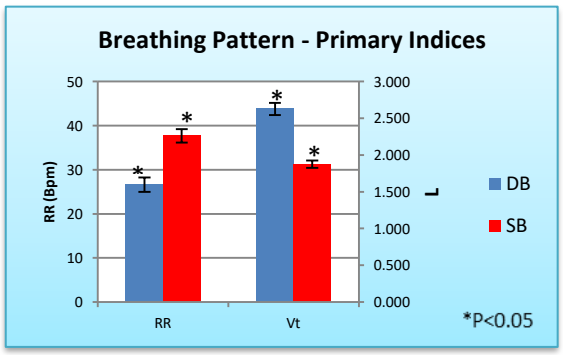
3.4.2 **Breathing Pattern Analysis**

Analysis was conducted on the average data from the last 5 minutes to investigate if a significantly different breathing pattern had been achieved with DB. Primary indices V_T, RR and V_E, and secondary indices T_{tot}, T_i, T_e, T_i/T_{tot} were analysed.

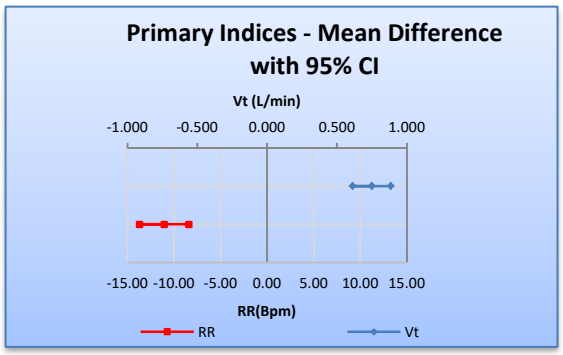
3.4.2.1 Primary Indices

There was a significant difference in RR and V_T and V_T normalised as a fraction of FVC (V_T/FVC) between SB and DB trials. Figure 3-2(A) presents the mean data comparing DB with SB and Figure 3-2(B) presents the mean difference with 95% CI. DB resulted in a significant increase in V_T and a significant decrease in RR.

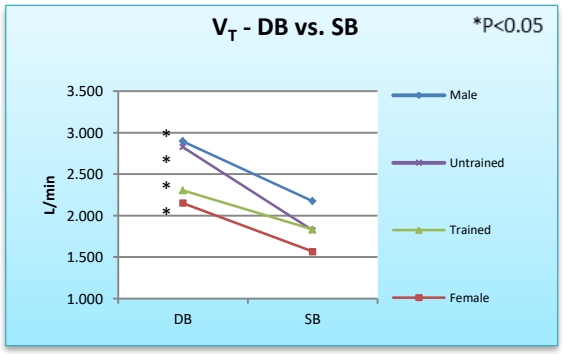
Analysis examined changes in V_T and V_T normalised as a fraction of FVC (V_T/FVC). No significant three-way interaction between breathing, gender and training were observed on V_T ($P = 0.756$) or V_T/FVC ($P = 0.362$). There was a significant interaction between breathing and training with a medium effect size, both on V_T ($P = 0.018$) and V_T/FVC ($P = 0.004$). Table 3-2 presents the results of these interactions.



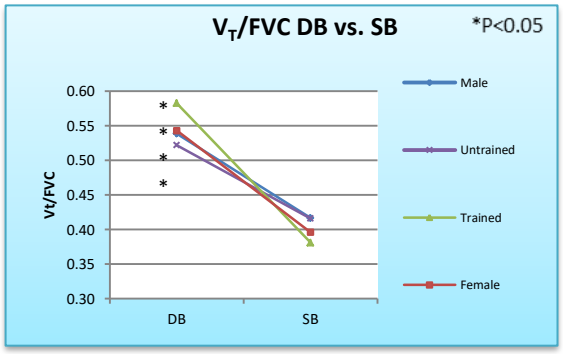
(A) Breathing Pattern – Primary Indices



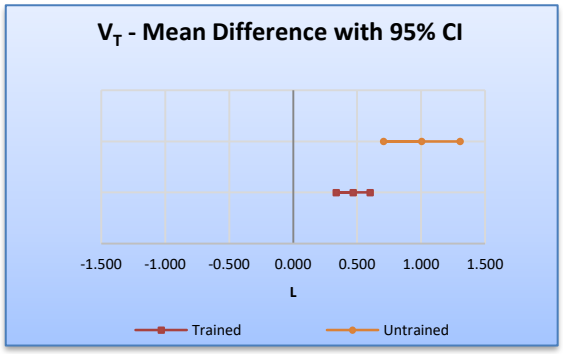
(B) Primary Indices – Mean diff. with 95% CI



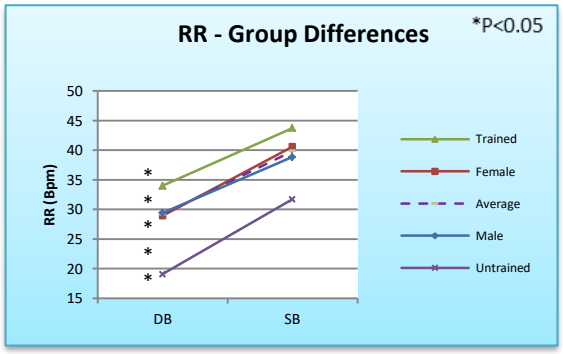
(C) V_T – DB vs. SB



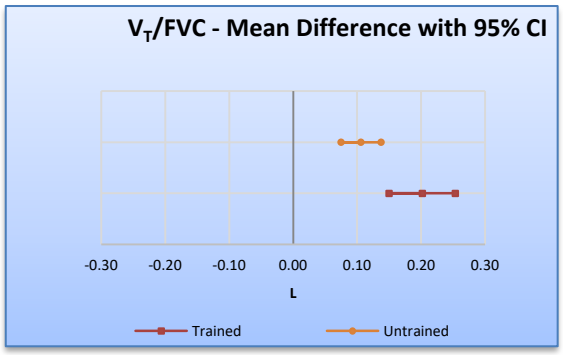
(E) V_T/FVC – DB vs. SB – Group Response



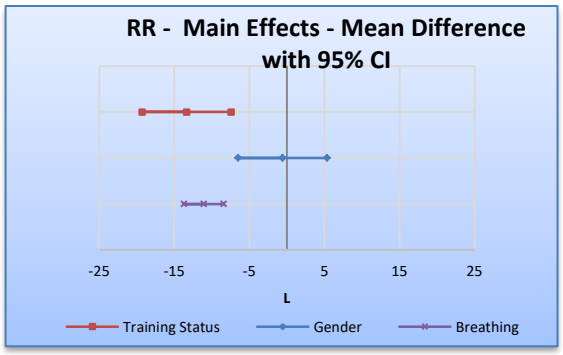
(D) V_T – Trained vs. Untrained response to DB



(G) RR – DB vs. SB



(F) V_T/FVC – Trained vs. Untrained



(H) RR – Training stays, Gender and Breathing

Figure 3-2 Breathing pattern changes between trials
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel A and B present the mean difference in tidal volume (V_T) and respiratory rate (RR). Panel C and E shows a significant decrease in absolute V_T and. The corresponding panels on the right, panels D and F, present the difference in response between trained and untrained for V_T and V_T/FVC. Panel G shows a significant increase in RR and panel H presents the difference for training status, gender and breathing pattern (SB vs. DB).

Table 3-2 V_T & V_T/FVC – Three-way and two-way interaction results

		P	Partial η2	ES
Breathing*Gender*Training	V_T	0.756	0.003	T
	V_T/FVC	0.362	0.027	S
Breathing *Training	V_T	**0.000****	**0.335**	**L**
	V_T/FVC	**0.004***	**0.238**	**M**
Breathing*Gender	V_T	0.230	0.044	S
	V_T/FVC	0.336	0.030	S

*Summary of statistical analysis presenting interactions. V_T = Tidal Volume; V_T/FVC = V_T normalised for Forced Vital Capacity; P<0.01; **P<0.001; ES = Effect Size; T = Trivial; S = Small; M = Medium; L= Large;*

This interaction was further analysed (see Table 3-3) revealing no difference between trained and untrained groups in the SB trial on V_T (P = 0.934) or V_T/FVC (P = 0.083) but during the DB trial the untrained group had a significantly higher V_T (P = 0.017) and

higher V_T/FVC (P = 0.010). The greater difference between groups under DB conditions was due to a greater significant increase in the untrained group compared to a smaller, but significant increase for the untrained group in V_T (P = 0.000) and V_T/FVC (P = 0.000).

*Table 3-3 V_T & V_T/FVC - Breathing*Training Interaction results*

			Untrained vs. Trained				DB vs. SB				
			P	Mean Diff	95% CI			P	Mean Diff	95% CI	
Breathing *Training	V_T	SB	0.934	0.012	-0.286	0.311	**UT**	**0.000****	**1.006**	**0.708**	**1.304**
		DB	**0.017***	**-0.524**	**0.209**	**-0.95**	**T**	**0.000****	**0.470**	**0.337**	**0.603**
	V_T /FVC	SB	0.083	0.03	0.00	0.07	**UT**	**0.000****	**0.20**	**0.15**	**0.25**
		DB	**0.010***	**-0.08**	**0.03**	**-0.13**	**T**	**0.000****	**0.11**	**0.08**	**0.14**

*V_T = Tidal Volume; V_T/FVC = V_T normalised for Forced Vital Capacity; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; P<0.01; *P<0.05; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained;*

There was no 2 way interaction between breathing and gender for V_T (P = 0.229) or for V_T/FVC (P = 0.336), however, the main effect for gender identified a significantly higher V_T in males with a large effect size (P = 0.000) but no difference when normalised as a fraction of FVC (P = 0.756). Results are presented below in Table 3-4.

Table 3-4 V_T & V_T/FVC - Summary results for main effect for Gender

		Main Effect			Gender Difference –Male vs. Female				
		P	Partial η2	ES	Female	Male	Mean Diff	95% CI	
Gender	V_T	**0.000***	**0.539**	**L**	**1.884**	**2.618**	**0.734**	**0.494**	**0.974**
	V_T/FVC	0.756	0.003	T	0.47	0.48	0.01	-0.04	0.05

*V_T = Tidal Volume; V_T/FVC = V_T normalised for Forced Vital Capacity; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; L= Large;*

Figure 3-2(C&E) shows the different responses for each group under SB and DB conditions for V_T and V_T/FVC respectively. Figure 3-2(D&F) show the mean difference with 95% CI for trained and untrained groups in response to DB for V_T and V_T/FVC respectively.

3.4.2.1.1 RR

Analysis of changes in RR showed no significant three-way interaction between breathing, gender and training status (P = 0.847). There was no significant interaction between breathing and training status (P = 0.293) or between breathing and gender (P = 0.373) and effect sizes were small. Table 3-5 presents the interaction results.

Table 3-5 RR – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.847	0.001	T
Breathing *Training	0.293	0.033	S
Breathing*Gender	0.373	0.024	S

Summary of statistical analysis presenting interactions. SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; S = Small;

There was a main effect for breathing with a large effect size (P = 0.000), RR decreasing significantly under DB conditions. There was no main effect for gender on RR (P = 0.853). There was a main effect for training, showing a significantly lower RR in the trained group, when compared to untrained group with a large effect size (P = 0.000). Table 3-6 presents details of the main effects.

Table 3-6 RR – Summary results for main effects

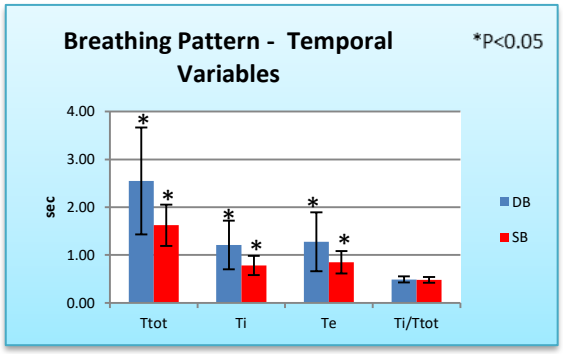
	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	**0.000***	**0.792**	**L**	**38**	**27**	**-11**	**-14**	**-8**
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	0.853	0.001	T	32	32	-1	-6	5
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000***	**0.389**	**L**	**39**	**25**	**13**	**7**	**19**

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; L= Large;*

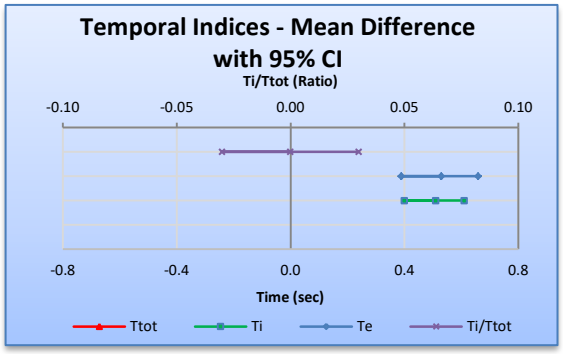
Figure 3-2(G) shows the different responses for each group under SB and DB conditions. Figure 3-2(H) shows the mean difference for trained and untrained groups.

3.4.2.2 Secondary indices

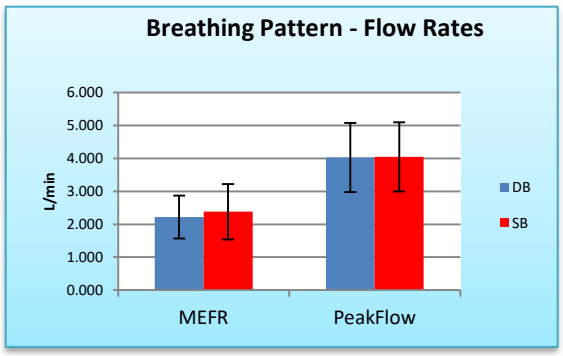
Analysis showed a significant increase in DB compared to SB in temporal variables T_{tot}, T_i and T_e but no significant increase in T_i/T_{tot}. Figure 3-3(A) presents mean data for each variable comparing SB and DB trials and Figure 3-3(B) presents the mean difference between SB and DB trials with 95% CI.



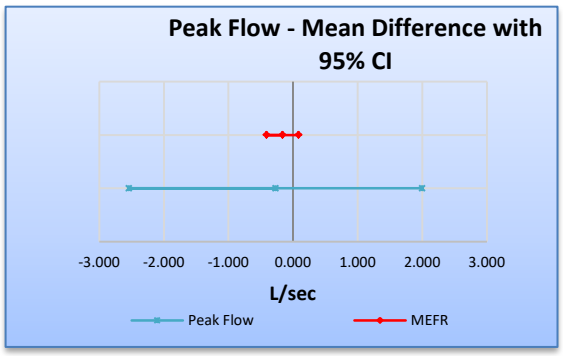
(A) Temporal Indices – DB vs. SB



(B) Temporal Indices –DB vs. SB



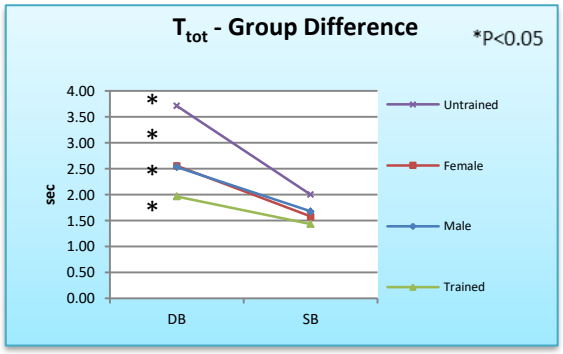
(C) Flow Rate Indices – DB vs. SB



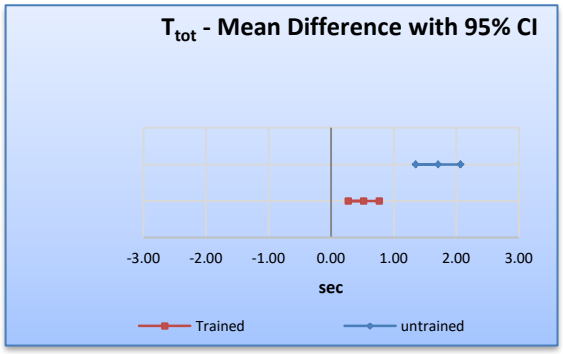
(D) Flow Rate Indices – DB vs. SB

Figure 3-3 Secondary indices of breathing pattern - changes between trials
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel A and B present the mean difference showing a significant increase in total breath time (T_{tot}), inspiratory time (T_i) and expiratory time (T_e) but no significant increase in duty cycle (T_i/T_{tot}). Panel C and D shows no significant difference in either maximum expiratory flow rate (MEFR) or peak flow.

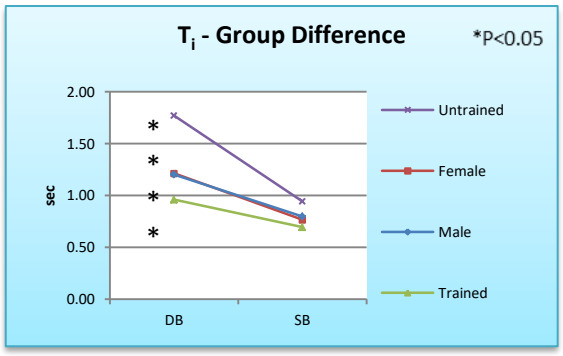
There was no significant difference in flow rate variables between SB and DB trials. Figure 3-3(C) presents mean data for each variable comparing SB and DB trials and Figure 3-3(D) presents the mean difference between trials with 95% CI. Each variable is dealt with individually in detail in sections **Error! Reference source not found.**to **Error! Reference source not found.**.



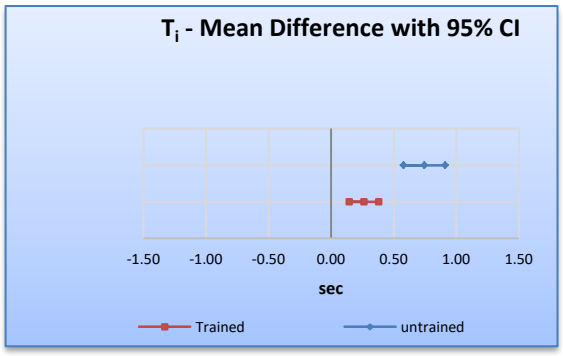
(A) T_{tot} – DB vs. SB



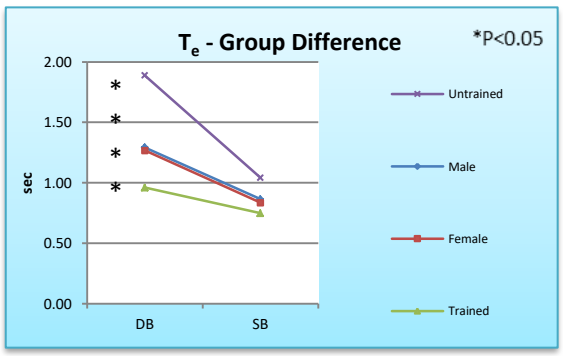
(B) T_{tot} – Mean difference with 95% CI



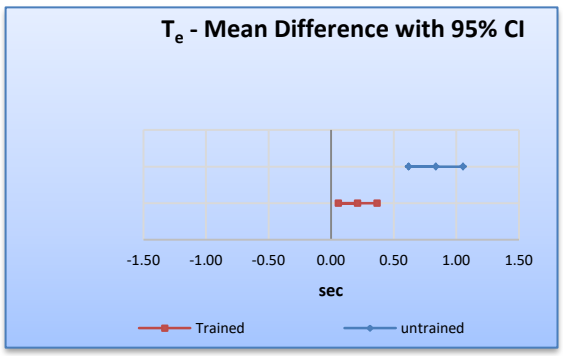
(C) T_i – DB vs. SB



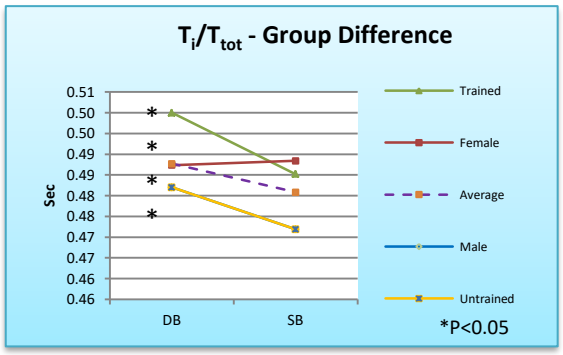
(D) T_i – Mean difference with 95% CI



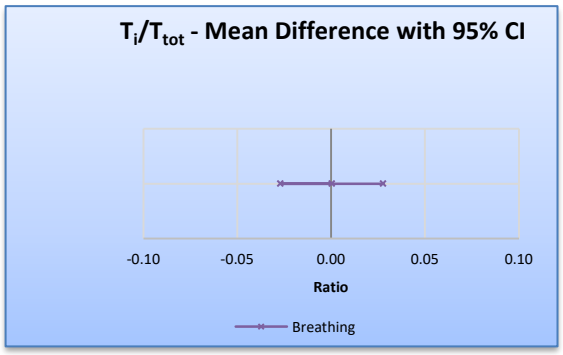
(E) T_e – Breathing*Training Interaction



(F) T_e – Breathing*Training Interaction



(G) T_i/T_{tot} – DB vs. SB



(H) T_i/T_{tot} – Mean difference with 95% CI

Figure 3-4 Temporal indices of breathing pattern – group changes between trials
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel A and B present the mean difference showing a significant increase in total breath time (T_{tot}),C and D inspiratory time (T_i), E and F expiratory time (T_e) and G and F show no significant increase in duty cycle (T_i/T_{tot}).

3.4.2.2.1 T_{tot}

No significant three-way interaction between breathing, gender and training status were observed (P = 0.731). There was a significant interaction between breathing and training status with a large effect size (P = 0.000). Table 3-7 presents the interaction results.

Table 3-7 T_{tot} – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.731	0.004	T
Breathing *Training	**0.000***	0.49	L
Breathing*Gender	0.800	0.002	T

*T_{tot} = total breath time; *P<0.001; ES = Effect Size; T = Trivial; L= Large;*

This interaction was further analysed (see Table 3-8) revealing a significantly longer T_{tot} for the untrained group in both the SB trial (P = 0.000) and the DB trial (P = 0.000), the difference greater under DB conditions. The greater difference between groups under DB conditions was due to a greater significant increase in T_{tot} in the untrained group (1.71 sec; P = 0.000), compared to a smaller, but significant increase for the trained group (0.53 sec; P = 0.000).

*Table 3-8 T_{tot} (sec) - Breathing*Training Interaction results*

	Untrained vs. Trained					DB vs. SB				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	SB	**0.000***	-0.56	-0.81	-0.31	T	**0.000***	0.53	0.28	0.78
	DB	**0.000***	-1.75	-2.32	-1.19	UT	**0.000***	1.71	1.36	2.07

*T_{tot} = total breath time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained;*

There was no significant two-way interaction between breathing and gender (P = 0.800), and there was a no significant main effect for gender (P = 0.729). Table 3-9 summarises the results.

Table 3-9 T_{tot} (sec) – Summary results for main effect for Gender

	Main Effect			Difference				
	P	Partial η2	ES	Female	Male	Mean Diff	95% CI	
Gender	0.729	0.004	T	2.31	2.25	0.06	-0.31	0.44

T_{tot} = total breath time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial;

Figure 3-4(A) presents mean data for each group in both trials and Figure 3-4 (B) presents the mean difference for trained and untrained groups.

3.4.2.2.2 T_i

No significant three-way interaction between breathing, gender and training status were observed (P = 0.421). There was a significant interaction between breathing and training status with a large effect size (P = 0.000). Table 3-10 presents the interaction results.

Table 3-10 T_i – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.421	0.021	S
Breathing *Training	**0.000***	0.431	L
Breathing*Gender	0.924	0.000	T

*T_i = inspiratory time; *P<0.001; ES = Effect Size; T = Trivial; S = Small; L= Large;*

This interaction was further analysed (see Table 3-11) revealing a significantly longer inspiratory time (T_i) for the untrained group in both the SB trial (P = 0.000) and the DB trial (P = 0.000), the difference greater under DB conditions. The greater difference between groups under DB conditions was due to a greater significant increase in T_i in the untrained group (P = 0.000), compared to a smaller, but significant increase for the trained group (P = 0.000).

*Table 3-11 T_i (sec) - Breathing*Training Interaction results*

	Untrained vs. Trained					DB vs. SB				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	SB	**0.000***	-0.25	-0.38	-0.13	T	**0.000***	0.26	0.15	0.38
	DB	**0.000***	-0.74	-1.02	-0.46	UT	**0.000***	0.75	0.58	0.91

*T_i = inspiratory time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained;*

There was no significant two-way interaction between breathing and gender (P = 0.924), and there was no significant main effect for gender (P = 0.574). Table 3-12 summarises the results.

Figure 3-4 (C) presents mean data for each group in both SB and DB trials and Figure 3-4 (D) presents the mean difference for trained and untrained groups.

Table 3-12 T_i (sec) – Summary results for main effect for Gender

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**Female**	**Male**	**Mean Diff**	**95% CI**	
Gender	0.574	0.010	T	1.33	0.82	0.05	-0.14	0.25

T_i = inspiratory time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial;

3.4.2.2.3 T_e

No significant three-way interaction between breathing, gender and training status were observed (P = 0.505). There was a significant interaction between breathing and training status with a large effect size (P = 0.000). Table 3-13 presents the interaction results.

Table 3-13 T_e – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.505	0.014	T
Breathing *Training	**0.000***	0.423	L
Breathing*Gender	0.991	0.000	T

*T_e = expiratory time; *P<0.001; ES = Effect Size; T = Trivial; L= Large;*

This interaction was further analysed (see Table 3-14) revealing a significantly longer expiratory time (T_e) for the untrained group in both the SB trial (P = 0.000) and the DB trial (P = 0.000), the difference greater under DB conditions. The greater difference between groups under DB conditions was due to a greater significant increase in T_e in the untrained group (P = 0.000), compared to a smaller, but significant increase for the trained group (P = 0.000).

*Table 3-14 T_e (sec) - Breathing*Training Interaction results*

	Untrained vs. Trained					**DB vs. SB**				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	**SB**	**0.000***	**-0.29**	**-0.43**	**-0.15**	**T**	**0.008***	**0.21**	**0.06**	**0.37**
	DB	**0.000***	**-0.91**	**-1.23**	**-0.59**	**UT**	**0.000***	**0.84**	**0.62**	**1.06**

*T_i = inspiratory time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; * P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained;*

There was no significant two-way interaction between breathing and gender (P = 0.991), and no significant main effect for gender (P = 0.815). Table 3-15 summarises the results. Figure 3-4(E) presents mean data for each group in both SB and DB trials. Figure 3-4(F) presents the mean difference for trained and untrained groups.

Table 3-15 T_e (sec) – Summary results for main effect for Gender

	Main Effect			Difference				
	P	Partial η2	ES	Female	Male	Mean Diff	95% CI	
Gender	0.815	0.002	T	1.42	0.89	0.02	-0.18	0.23

T_e = expiratory time; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial;

3.4.2.2.4 T_i/T_{tot}

There was no significant three-way interaction between breathing, gender and training status (P = 0.971) and no significant two-way interaction between breathing and gender (P = 0.685) or between breathing and training status (P = 0.289). Table 3-16 summarises the results.

Table 3-16 T_i/T_{tot} – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.971	0.000	T
Breathing *Training	0.289	0.036	S
Breathing*Gender	0.685	0.005	T

T_i/T_{tot} = ratio of inspiratory time to total breath time - breathing duty cycle; ES = Effect Size; T = Trivial; S = Small;

Table 3-17 T_i/T_{tot} – Summary results for main effects

	Main Effect			Difference				
	P	Partial η2	ES	SB	DB	Mean Diff	95% CI	
Breathing	0.971	0.000	T	0.48	0.48	0.00	-0.03	0.03
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	0.871	0.001	T	0.48	0.48	0.00	-0.04	0.03
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	0.145	0.067	S	0.49	0.47	0.02	-0.01	0.06

T_i/T_{tot} = ratio of inspiratory time to total breath time - breathing duty cycle; SB = Spontaneous Breathing; DB = Deep Breathing; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; ES = Effect Size; T = Trivial; S = Small;

There was no main effect for breathing (P = 0.971), gender (P = 0.871) or training status (P = 0.145) and effect sizes were trivial or small. Table 3-17 summarises the results.

Figure 3-4(G) presents mean data for each group in both trials. Figure 3-4(H) presents the mean difference for breathing pattern.

3.4.2.2.5 MEFR

There was no significant three-way interaction between breathing, gender and training status (P = 0.746) and no significant two-way interaction between breathing and gender (P = 0.866) or between breathing and training status (P = 0.914). Table 3-18 summarises the results.

Table 3-18 MEFR – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.746	0.003	T
Breathing *Training	0.914	0.000	T
Breathing*Gender	0.866	0.001	T

MEFR = Maximum Expiratory Flow Rate; ES = Effect Size; T = Trivial;

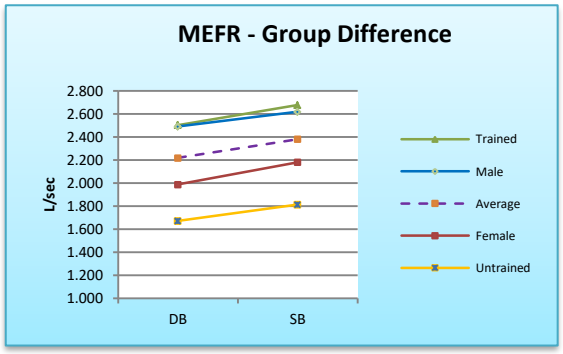
Table 3-19 MEFR (L/sec) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	0.203	0.052	S	2.124	2.282	-.158	-.406	.090
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	**0.007***	0.209	M	2.124	2.282	0.515	0.148	0.883
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000****	0.382	L	2.596	1.809	0.788	0.421	1.155

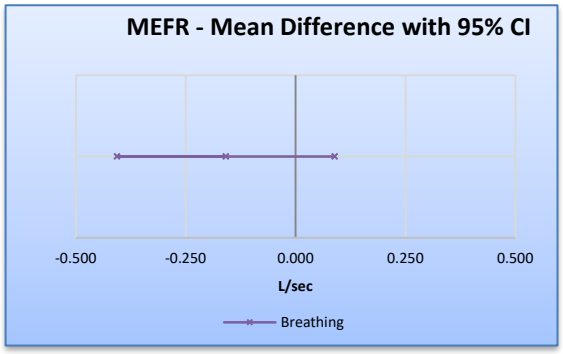
*MEFR = Maximum Expiratory Flow Rate; *P<0.01; **P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; ES = Effect Size; S = Small; M = Medium; L= Large;*

There was no main effect for breathing and effect size was small (P = 0.203). There was a main effect for gender with a medium effect size (P = 0.007), MEFR significantly higher in males. There was a main effect for training with a large effect size (P = 0.000), MEFR significantly higher in the trained. Table 3-19 presents the results for the main effects.

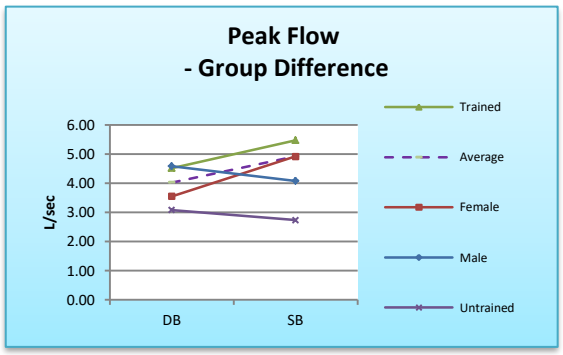
Figure 3-5(A) presents mean data for each group in both DB and SB trials. Figure 3-5(B) presents the mean difference for breathing pattern.



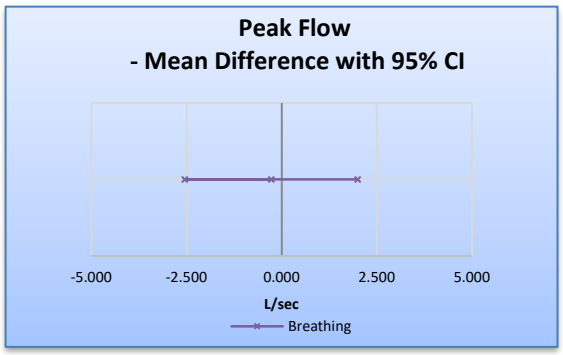
(A) MEFR – DB vs. SB



(B) MEFR – Mean difference with 95% CI



(C) Peak Flow – DB vs. SB



(D) Peak Flow – Mean difference with 95% CI

Figure 3-5 Differences in Flow Rates
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel A and B present the mean difference in maximum expiratory flow rate (MEFR) and C and D present the mean difference in peak flow, all showing no significant difference in groups.

3.4.2.2.6 Peak Flow

There was no significant three-way interaction between breathing, gender and training status (P = 0.560) and no significant two-way interaction between breathing and gender (P = 0.499) or between breathing and training status (P = 0.577). Table 3-20 summarises the interaction results.

Table 3-20 Peak Flow (L/sec) – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.560	0.011	T
Breathing *Training	0.577	0.010	T
Breathing*Gender	0.499	0.015	T

ES = Effect Size; T = Trivial;

There was no main effect for breathing (P = 0.813), gender (P = 0.786) or training status (P = 0.091) and effect sizes were trivial or small. Table 3-21 presents these results.

Table 3-21 Peak Flow (L/sec) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	0.813	0.002	T	3.866	4.132	-.266	-2.537	2.005
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	0.786	0.002	T	3.866	4.132	0.310	-1.991	2.611
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	0.091	0.089	S	4.982	3.016	1.966	-0.334	4.267

Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; S = Small;

Figure 3-5(C) presents mean data for each group in both DB and SB trials. Figure 3-5(D) presents the mean difference for breathing pattern.

3.4.3 **Primary Outcome measures**

Table 3-22 presents a summary of the primary outcome variables subdivided by gender, training status and overall average.

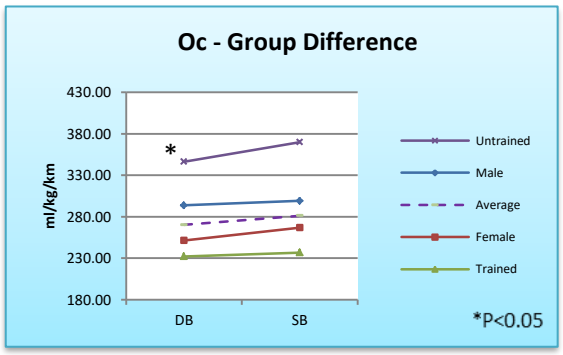
Table 3-22 Summary of all variables – Mean ± SD

		Male	**Female**	**Trained**	**Untrained**	**Average**
O_2 Cost	SB	299.1 ± 95.1	267.0 ± 83.1	236.9 ± 36.6	370.0 ± 100.0	281.3 ± 88.8
	DB	293.7 ± 86.9	251.6 ± 67.0	232.1 ± 38.7	346.6 ± 82.7	270.3 ± 78.3
Energy Cost	SB	1.18 ± 0.38	1.05 ± 0.34	0.93 ± 0.14	1.47 ± 0.39	1.11 ±0.36
	DB	1.16 ± 0.34	0.99 ± 0.26	0.91 ± 0.15	1.36 ± 0.32	1.06 ± 0.31
HR	SB	162 ± 12	161 ± 123	165 ± 9	154 ± 15	161 ± 12
	DB	159 ± 12	162 ± 112	165 ± 9	154 ± 13	161 ± 12
V_T	SB	2.174 ± 0.349	1.567 ± 0.219	1.833 ± 0.373	1.821 ± 0.503	1.829 ± 0.413
	DB	2.896 ± 0.638	2.151 ± 0.417	2.304 ± 0.53	2.828 ± 0.717	2.474 ± 0.637
RR	SB	39 ± 11	41 ± 10	44 ± 9	32 ± 7	40 ± 10
	DB	29 ± 11	29 ± 12	34 ± 10	19 ± 7	29 ± 11
V_E	SB	66.9 ± 13.9	51.2 ± 11.6	63.6 ± 11.9	46.2 ± 13.3	58.0 ± 14.7

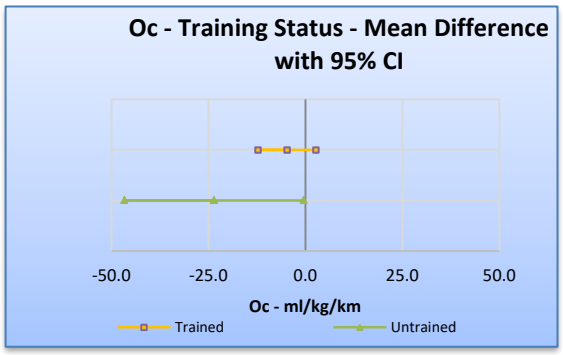
	DB	63.5 ± 14.2	46.3 ± 12.0	59.6 ± 13.0	41.5 ± 13.1	53.7 ± 15.4
V_A	SB	74.9 ± 15.3	56.2 ± 12.4	70.4 ± 13.8	51.6 ± 14.8	64.3 ± 16.5
	DB	71.6 ± 16.0	52.5 ± 14.0	67.6 ± 14.6	46.50 ± 14.4	60.7 ± 17.5
VO_2	SB	3.287 ± 0.584	2.266 ± 0.427	2.918 ± 0.609	2.268 ± 0.732	2.707 ± 0.712
	DB	3.261 ± 0.606	2.133 ± 0.408	2.855 ± 0.663	2.132 ± 0.714	2.62 ± 0.752
VCO_2	SB	3.067 ± 0.566	2.165 ± 0.44	2.783 ± 0.561	2.079 ± 0.639	2.555 ± 0.668
	DB	3.131 ± 0.603	2.137 ± 0.451	2.799 ± 0.614	2.082 ± 0.693	2.567 ± 0.717
RER	SB	0.94 ± 0.05	0.96 ± 0.06	0.96 ± 0.05	0.92 ± 0.07	0.95 ± 0.06
	DB	0.97 ± 0.05	1.02 ± 0.06	1.00 ± 0.06	0.98 ± 0.06	1.00 ± 0.06
V_E/VCO_2	SB	26 ± 3	29 ± 2	28 ± 3	27± 2	28 ± 3
	DB	25 ± 3	26± 4	26 ± 3	24 ± 3	26 ± 3
$P_{ET}CO_2$	SB	41.6 ± 3.4	38.4 ± 3.2	39.5 ± 3.8	40.3 ± 3.2	39.8 ± 3.6
	DB	44.7 ± 5.5	41.6 ± 5.9	42.1 ± 5.9	44.7 ± 5.5	42.9 ± 5.9
SF	SB	78 ± 14	77 ± 15	87 ± 5	58 ± 4	78 ± 14
	DB	77 ± 14	58 ± 2	86 ± 5	57 ± 4	77 ± 15
LRC	SB	2.1 ± 0.5	2.0 ± 0.4	2.1 ± 0.5	1.9 ± 0.3	2.0 ± 0.4
	DB	2.7 ± 0.7	3.1 ± 0.7	2.8 ± 0.7	3.1 ± 0.8	2.9 ± 0.7
RPE-O	SB	13 ± 2	13 ± 1	13± 1	14 ± 2	13 ± 2
	DB	13 ± 2	12 ± 2	12 ± 2	14 ± 2	13 ± 2.
RPE-R	SB	3 ± 1	3 ± 1	3 ± 1	3 ± 3	3 ± 1
	DB	3 ± 1	2 ± 1	2 ± 1	3 ± 1	3 ± 1
BLa^-	SB	2.5 ± 1.1	2.0 ± 0.6	2.3 ± 1.0	2.0 ± 0.5	2.2 ± 0.9
	DB	2.8 ± 1.4	2.3 ± 0.8	2.6 ± 1.2	2.3 ± 0.9	2.5 ± 1.1

Summary of primary outcome measures by gender and training status. Results are mean ± Standard Deviation (SD). SB = Spontaneous Breathing; DB = Deep Breathing;

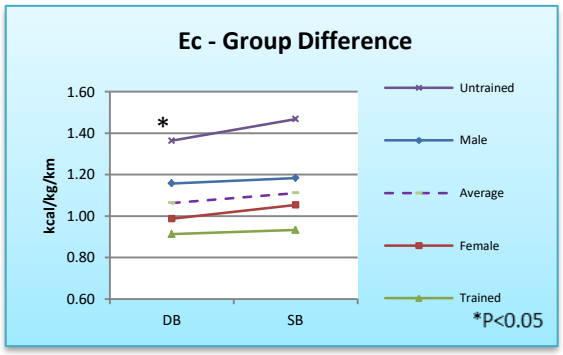
3.4.3.1 Efficiency of movement



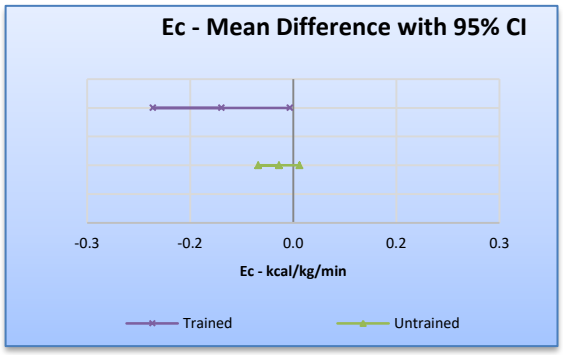
(A) O_c – DB vs. SB



(B) O_c – Mean difference with 95% CI



(C) E_c – DB vs. SB



(D) E_c – Mean difference with 95% CI

Figure 3-6 Differences in efficiency of movement
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel A and B present the mean difference in the oxygen cost (O_C) and panel C and D present the mean difference in the energy cost (E_C).There was a significant reduction (improvement) in both variable for the untrained group only.

3.4.3.1.1 O_2 Cost - O_C

No significant three-way interaction between breathing, gender and training status were observed (P = 0.818). There was a significant two-way interaction between breathing and training (P = 0.049). Table 3-23 presents the interaction results.

Table 3-23 O_2 Cost – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.818	0.002	T
Breathing *Training	**0.049***	0.116	S
Breathing*Gender	0.353	0.027	S

**P<0.05; ES = Effect Size; T = Trivial; S = Small;*

This interaction was further analysed (see Table 3-24) revealing a significantly lower O_C for the untrained group in both the SB trial (P = 0.000) and the DB trial (P = 0.000), the difference greater under DB conditions. The greater difference between groups under DB

conditions was due to a greater significant decrease in the untrained group (P = 0.047), compared to non-significant decrease for the trained group (P = 0.206).

*Table 3-24 O_2 Cost (ml/kg/km) - Breathing*Training Interaction results;*

	Untrained vs. Trained					DB vs. SB				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	SB	**0.000***	-133.1	-195.9	-70.3	Untrained	0.047	-23.5	-46.6	-0.4
	DB	**0.000***	-115.0	-169.0	-60.9	Trained	0.206	-4.7	-12.2	2.8

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained; Equal variances not assumed;*

There was no significant two-way interaction between breathing and gender (P = 0.353), but there was a significant main effect for gender (P = 0.026). Analysis identified a significantly higher Oc in males, when compared to females. Table 3-25 summarises the results.

Table 3-25 Summary results for main effect for Gender

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**Male**	**Female**	**Mean Diff**	**95% CI**	
Gender	**0.026****	0.146	M	322.9	276.3	46.7	6.1	322.9

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.05; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; M = Medium;*

Figure 3-6(A) presents mean data for each group in both DB and SB trials. Figure 3-6(B) presents the mean difference for trained versus untrained groups.

3.4.3.1.2 Energy Cost – E_C

No significant three-way interaction between breathing, gender and training status were observed (0.864). There was a significant two-way interaction between breathing and training (P = 0.039). Table 3-26 summarises the interaction results.

Table 3-26 Energy Cost (kcal/kg/km) – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.864	0.001	S
Breathing *Training	**0.039***	**0.126**	**S**
Breathing*Gender	0.381	0.024	M

**P<0.05; ES = Effect Size; S = Small; M = Medium;*

This interaction was further analysed (see Table 3-27) revealing a significantly lower E_C for the untrained group in both the SB trial (P = 0.001) and the DB trial (P = 0.000), the difference greater under SB conditions. The greater difference between groups under SB conditions was due to a greater significant decrease in the untrained group (P = 0.042), compared to non-significant decrease for the trained group (P = 0.173).

*Table 3-27 Energy Cost (kcal/kg/km) - Breathing*Training Interaction results;*

	Untrained vs. Trained					DB vs. SB				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	SB	**0.001****	-0.54	-0.79	-0.28	Untrained	**0.042***	-0.10	-0.20	0.00
	DB	**0.000*****	-0.45	-0.67	-0.24	Trained	0.173	-0.02	-0.05	0.01

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.05; **P<0.01; ***P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained; Equal variances not assumed;*

There was no significant two-way interaction between breathing and gender (P = 0.381). There was a significant main effect for gender with a medium effect size (P=0.024), males having a higher E_C than females. Table 3-28 summarises the results.

Table 3-28 Energy Cost (kcal/kg/km) – Summary results for main effect for Gender

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**Male**	**Female**	**Mean Diff**	**95% CI**	
Gender	**0.024***	0.150	M	1.28	1.09	0.19	0.03	0.35

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval;*P<0.05; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; M = Medium;*

Error! Reference source not found. (C) presents mean data for each group in both DB and SB trials. **Error! Reference source not found.** (D) presents the mean difference for trained versus untrained groups.

3.4.4 Secondary Outcome measures

3.4.5 Physiological measures

All physiological variables are presented individually in the following sections. Figure 3-7 summarises the responses.

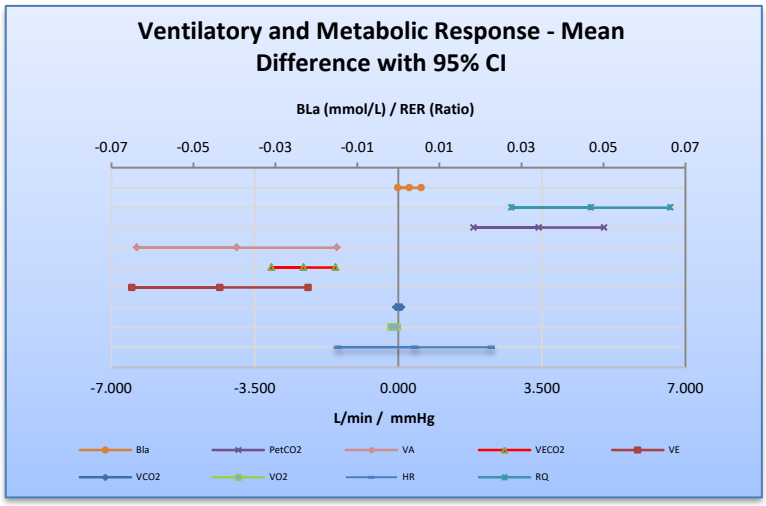


Figure 3-7 Summary of all physiological variables
The figure presents the difference observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 showing the mean difference with the 95% confidence interval (CI) for all physiological variables, blood lactate (BLa^-), minute ventilation (V_E), alveolar ventilation (V_A), O_2 consumption (VO_2), CO_2 production (VCO_2), respiratory quotient (RQ), ventilatory efficiency ($VECO_2$), end-tidal CO_2 ($P_{ET}CO_2$) and heart rate (HR). Significant differences are seen in V_E, V_A, VO_2, $VECO_2$ and $P_{ET}CO_2$ only.

3.4.5.1 VO_2

There was no significant three-way interaction between breathing, gender and training status (P = 0.380).There was no significant two-way interaction between breathing and training (P = 0.273) or between breathing and gender (P = 0.257). Table 3-29 summarises the interaction results.

Table 3-29 VO_2 – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.380	0.023	S
Breathing *Training	0.273	0.036	S
Breathing*Gender	0.257	0.039	S

VO_2 = volume of oxygen consumed; ES = Effect Size; S = Small;

There was a main effect for breathing (P = 0.012) with a medium effect size, VO_2 significantly lower in DB conditions. There was a main effect for gender (P = 0.000) with a Large effect size, males having a higher VO_2 than females. There was a main effect for

training status (P = 0.000) with a larger effect size, the trained group having a higher VO_2 than untrained. Table 3-30 summarises the results.

Table 3-30 VO_2 (L/min) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	**0.012***	0.175	M	2.666	2.572	-0.095	-0.167	-0.022
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	**0.000****	0.644	L	2.090	3.148	1.059	0.780	1.337
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000****	0.415	L	2.950	2.288	0.663	0.384	0.941

*Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.01; **P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; M = Medium; L= Large;*

Figure 3-8(A) presents mean data for each group in both DB and SB trials. Figure 3-8(B) presents the mean difference for breathing pattern.

3.4.5.2 VCO_2

There was no significant three-way interaction between breathing, gender and training status (P = 0.423) and no significant two-way interaction between breathing and training status (P = 0.769) or breathing and gender (P = 0.246). Table 3-31 summarises the results.

Table 3-31 VCO_2 – Three-way and two-way interaction results;

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.423	0.020	T
Breathing *Training	0.769	0.003	T
Breathing*Gender	0.246	0.041	S

VCO_2 = volume of expired carbon dioxide; ES = Effect Size; T = Trivial; S = Small;

There was no main effect for breathing (P = 0.650) and effect size was trivial. There was a main effects for gender (P = 0.000) with a large effect size, the male group having a greater VCO_2. There was a main effects for training status (P = 0.000) with a large effect size, the trained group having a greater VCO_2. Table 3-32 summarises the results.

Table 3-32 VCO_2 (L/min) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	0.650	0.006	T	2.496	2.511	.014	-.050	.079
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	**0.000***	0.585	L	2.033	2.975	0.942	0.661	1.223
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000***	0.427	L	2.161	2.847	0.686	0.405	0.967

*VCO_2 = volume of expired carbon dioxide; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; L= Large;*

Figure 3-8(C) presents mean data for each group in both DB and SB trials. Figure 3-8(D) presents the mean difference for breathing pattern.

3.4.5.3 RER

There was no significant three-way interaction between breathing, gender and training status (P = 0.923).There was no significant two-way interaction between breathing and training (P = 0.450) or between breathing and gender (P = 0.170). Table 3-33 summarises the interaction results.

Table 3-33 Table 3 33 RER – Three-way and two-way interaction results

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.923	0.000	T
Breathing *Training	0.450	0.017	T
Breathing*Gender	0.170	0.056	S

RER = Respiratory Exchange Ratio; ES = Effect Size; T = Trivial; S = Small;

There was a main effects for breathing (P = 0.000) with a large effect size, RER higher under DB conditions. There was no main effect for gender (P = 0.120) or training status (P = 0.130) and effect sizes were small. Table 3-34 summarises the results.

Table 3-34 RER – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	**0.000***	0.424	L	0.94	0.99	0.05	0.03	.07
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	0.120	0.072	S	0.98	0.95	-0.03	-0.06	0.01
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	0.130	0.068	S	0.98	0.95	0.03	-0.01	0.06

*RER = Respiratory Exchange Ratio; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; S = Small; L= Large;*

Figure 3-8(E) presents mean data for each group in both DB and SB trials. Figure 3-8(F) presents the mean difference for breathing pattern. These results show that DB resulted in a significant increase in RER across all groups irrespective of gender or training status.

3.4.5.4 V_E

There was no significant three-way interaction between breathing, gender and training status (P = 0.376). There was no significant two-way interaction between breathing and training (P = 0.619) or between breathing and gender (P = 0.701). Table 3-35 summarises the interaction results.

Table 3-35 V_E (L/min) - Three-way and two-way interaction results

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.376	0.024	S
Breathing *Training	0.619	0.008	T
Breathing*Gender	0.701	0.005	T

V_E = minute ventilation; ES = Effect Size; T = Trivial; S = Small;

There was a main effects for breathing (P = 0.000) with a large effect size, VE significantly lower under DB conditions. There was a main effects for gender (P = 0.000) with a large effect size, the female group having significantly lower VE. There was a main effects for training status (P = 0.000) with a large effect size, VE significantly higher in the trained group. Table 3-36 summarises the results.

Table 3-36 V_E (L/min) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	**0.000***	0.340	L	56.1	51.8	-4.4	-6.5	-2.2
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	**0.000***	0.423	L	45.7	62.2	16.6	9.7	23.4
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000***	0.441	L	62.5	45.3	17.2	10.3	24.0

*V_E = minute ventilation; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; L= Large;*

Figure 3-8(G) presents mean data for each group in both DB and SB trials. Figure 3-8(H) presents the mean difference for breathing pattern.

3.4.5.5 V_A

There was no significant three-way interaction between breathing, gender and training status (P = 0.874).There was no significant two-way interaction between breathing and training (P = 0.320) or between breathing and gender (P = 0.934). Table 3-37 summarises the interaction results.

Table 3-37 V_A (L/min)– Three-way and two-way interaction results

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.874	0.001	T
Breathing *Training	0.320	0.030	S
Breathing*Gender	0.934	0.000	T

V_A = alveolar ventilation; ES = Effect Size; T = Trivial; S = Small;

There was a main effects for breathing (P = 0.002) with a medium effect size, VA significantly lower under DB conditions. There was a main effects for gender (P = 0.000) with a large effect size, the female group having significantly lower VA. There was a main effects for training status (P = 0.000) with a large effect size, VA significantly higher in the trained group. Table 3-38 summarises the results.

Table 3-38 V_A (L/min) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	**0.002***	0.246	M	62.4	58.4	-3.9	-6.4	-1.5
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	**0.000****	0.442	L	50.8	70.0	19.2	11.5	26.8
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	**0.000****	0.444	L	70.0	50.8	19.2	11.6	26.8

*V_A = alveolar ventilation; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.01; **P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; M = Medium; L = Large;*

Figure 3-8(I) presents mean data for each group in both DB and SB trials. Figure 3-8(J) presents the mean difference for breathing pattern.

3.4.5.6 V_E/VCO_2

There was no significant three-way interaction between breathing, gender and training status (P = 0.780).There was no significant two-way interaction between breathing and training (P = 0.172) or between breathing and gender (P = 0.388). Table 3-39 summarises the interaction results.

Table 3-39 V_E/VCO_2 – Three-way and two-way interaction results

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.780	0.002	T
Breathing *Training	0.172	0.056	S
Breathing*Gender	0.388	0.023	S

V_E/VCO_2 = ventilatory equivalent for CO_2; ES = Effect Size; T = Trivial; S = Small;

There was a main effects for breathing (P = 0.000) with a large effect size, V_E/VCO_2 significantly lower under DB conditions. There was a main effects for gender (P = 0.044) with a small effect size, the female group having significantly higher V_E/VCO_2. There was no main effect for training status (P = 0.136) and effect size was small. Table 3-40 summarises the results.

Table 3-40 V_E/VCO_2 – Summary results for main effects

	Main Effect			Difference			
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**
Breathing	**0.000***	0.524	L	27	25	-2	-3 -2
				Female	**Male**	**Mean Diff**	**95% CI**
Gender	**0.044****	0.118	S	27	25	-2	-4 0
				Trained	**Untrained**	**Mean Diff**	**95% CI**
Training	0.136	0.066	S	27	26	2	-1 3

*V_E/VCO_2 = ventilatory equivalent for CO_2; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; **P<0.05; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; S = Small; L= Large;*

Figure 3-8(K) presents mean data for each group in both DB and SB trials. Figure 3-8(L) presents the mean for breathing pattern.

3.4.5.7 $P_{ET}CO_2$

There was no significant three-way interaction between breathing, gender and training status (P = 0.172).There was no significant two-way interaction between breathing and training (P = 0.312) or between breathing and gender (P = 0.631). Table 3-41 summarises the interaction results.

Table 3-41 $P_{ET}CO_2$ – Three-way and two-way interaction results

	P	**Partial η2**	**ES**
Breathing*Gender*Training	0.172	0.056	S
Breathing *Training	0.312	0.031	S
Breathing*Gender	0.631	0.007	T

$P_{ET}CO_2$ = end tidal CO2; ES = Effect Size; T = Trivial; S = Small;

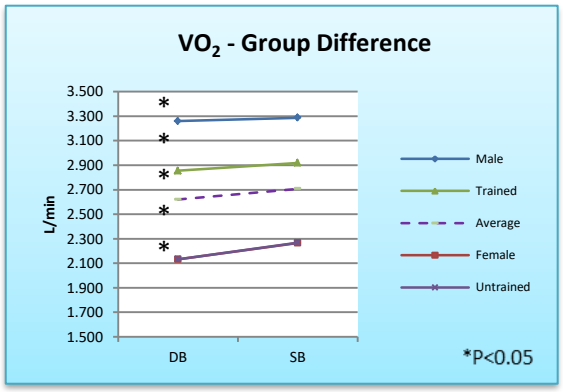
There was a main effects for breathing (P = 0.000) with a large effect size, $P_{ET}CO_2$ significantly higher under DB conditions. There was no main effect for gender (P = 0.077) or training status (P = 0.297) and effect sizes were small. Table 3-42 summarises the results.

Table 3-42 $P_{ET}CO_2$

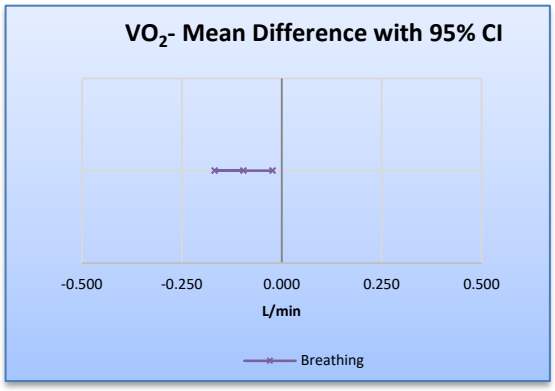
	Main Effect			Difference			
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**
Breathing	**0.000***	0.368	L	40	44	3	2 5
				Female	**Male**	**Mean Diff**	**95% CI**
Gender	0.077	0.091	S	40.5	43.1	2.6	-0.3 5.5
				Trained	**Untrained**	**Mean Diff**	**95% CI**
Training	0.297	0.033	S	41.1	42.6	-1.5	-4.4 1.4

*$P_{ET}CO_2$ = end tidal CO2; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; ES = Effect Size; S = Small; L = Large;*

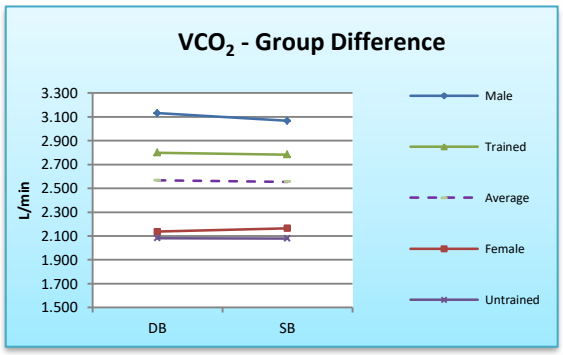
Figure 3-8(M) presents mean data for each group in both DB and SB trials. **Error! Reference source not found.**(N) presents the mean for breathing pattern.



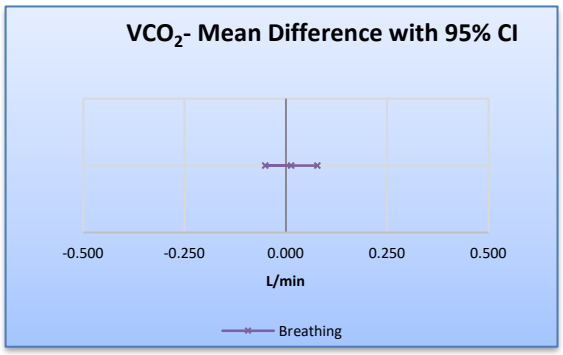
(A) VO_2 – DB vs. SB



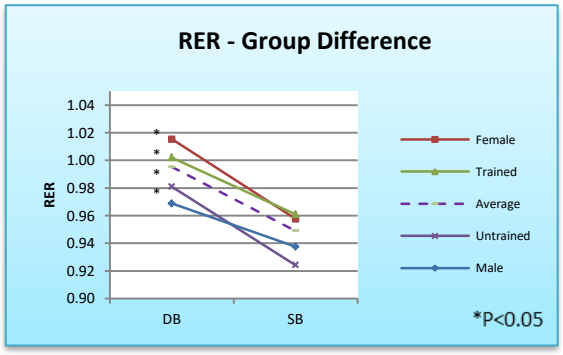
(B) VO_2 – breathing pattern



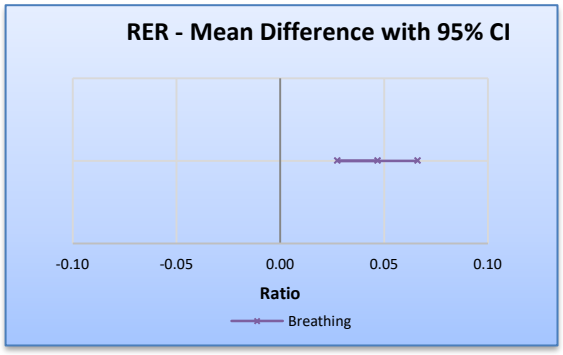
(C) VCO_2– DB vs. SB



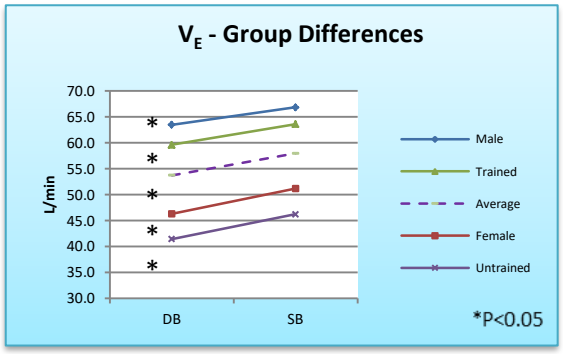
(D) VCO_2 – Mean difference with 95% CI



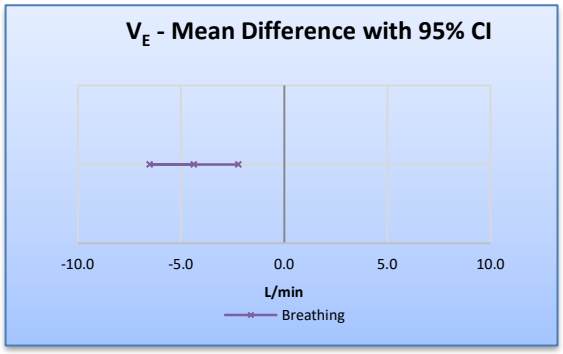
(E) RER – DB vs. SB



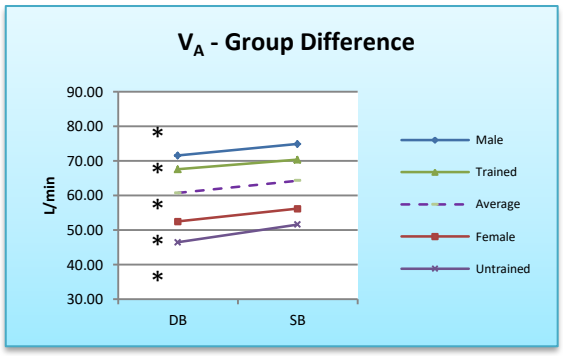
(F) RER– Mean difference with 95% CI



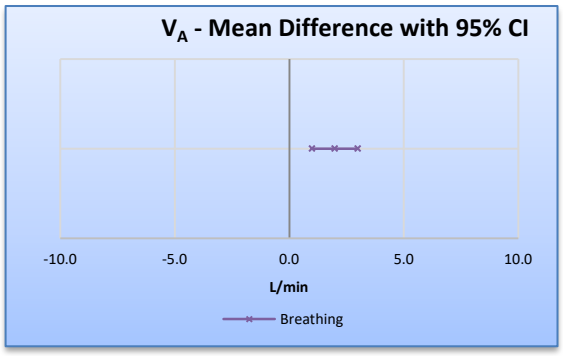
(G) V_E – DB vs. SB



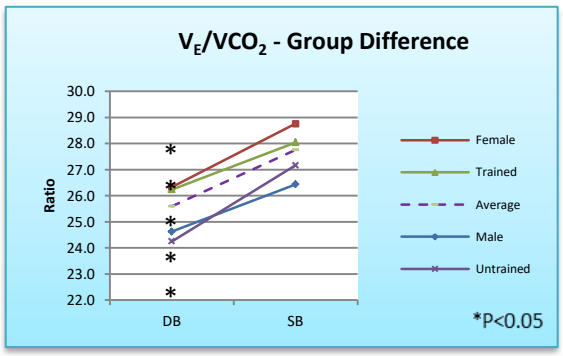
(H) V_E – Mean difference with 95% CI



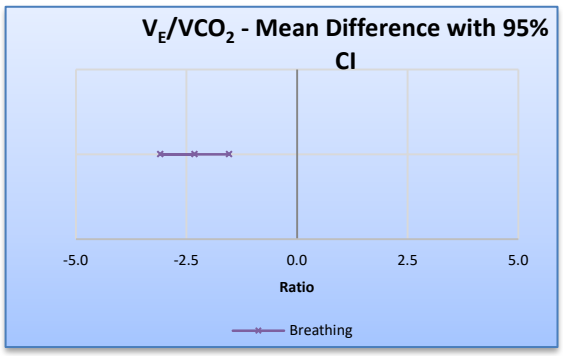
(I) VA – DB vs. SB



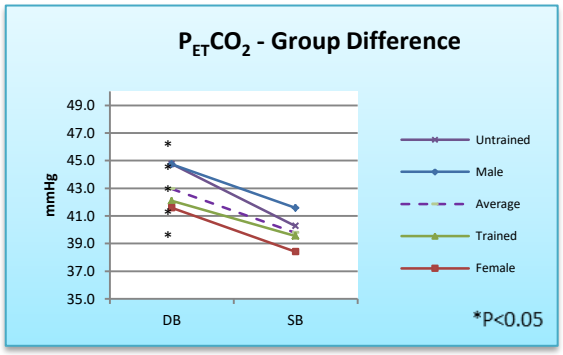
(J) VA – Mean difference with 95% CI



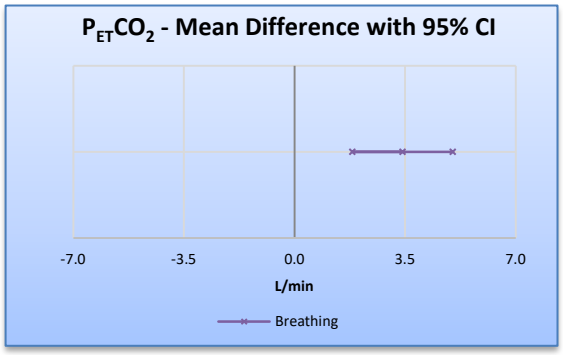
(K) V_ECO_2– DB vs. SB



(L) VECO2 – Mean difference with 95% CI



(M) $P_{et}CO_2$ – DB vs. SB



(N) PetCO2– Mean difference with 95% CI

Figure 3-8 Changes in ventilatory parameters between trials
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). There is a significant decrease in O_2 consumption (VO_2) for all groups during DB (A&B), no significant difference for CO_2 production (VCO_2) (C&D), a significant increase in respiratory exchange rate (RER) with DB (E&F), a significant decrease in minute ventilation (V_E) (G&H), alveolar ventilation (V_A) (I&J), ventilatory efficiency ($VECO_2$) (K&L), and a significant increase in end-tidal CO_2 ($P_{ET}CO_2$) (M&N).

3.4.5.8 Metabolic

3.4.5.8.1 BLa^{-1}

There was no significant three-way interaction between breathing, gender and training status (P = 0.150).There was no significant two-way interaction between breathing and training (P = 0.851) or between breathing and gender (P = 0.790). Table 3-43 summarises the interaction results.

Table 3-43 BLa^{-} – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.150	0.073	S
Breathing *Training	0.851	0.001	T
Breathing*Gender	0.790	0.003	T

ES = Effect Size; T = Trivial; S = Small;

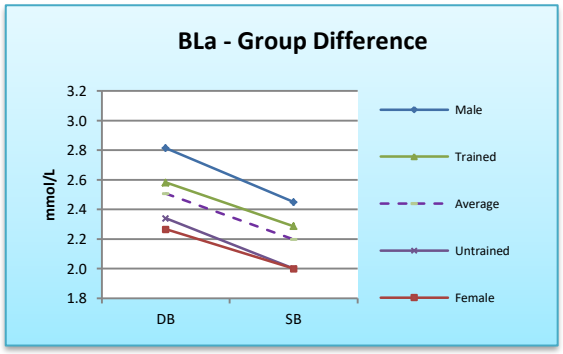
There was no main effect for breathing (P = 0.062), gender (P = 0.489) or training status (P = 0.427) and effect sizes were small or trivial. Table 3-44 summarises the results.

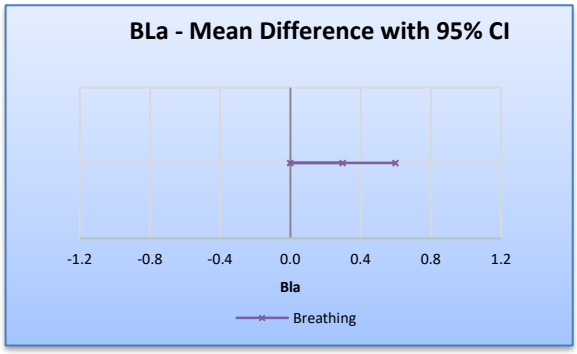
Table 3-44 BLa^{-} (mmol/L) – Summary results for main effects

	Main Effect			Difference				
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**	
Breathing	0.062	0.119	S	2.1	2.4	0.3	0.0	0.6
				Female	**Male**	**Mean Diff**	**95% CI**	
Gender	0.489	0.017	T	2.2	2.4	0.3	-0.5	1.0
				Trained	**Untrained**	**Mean Diff**	**95% CI**	
Training	0.427	0.023	S	2.4	2.1	0.3	-0.5	1.1

BLa^{-} = blood lactate concentration; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; S = Small;

Figure 3-9(A) presents mean data for each group in both DB and SB trials. Figure 3-9(B) presents the mean difference for breathing pattern.





(A) BLa^{-} – DB vs. SB **(B) BLa^{-} – Mean difference with 95% CI**

Figure 3-9 Blood Lactate (BLa^{-}) response
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). There is a non-significant increase in BLa^{-} with DB (A&B)

3.4.6 Ratings of perceived effort (RPE)

RPE was measured for both overall (RPE-O) and respiratory (RPE-R).

3.4.6.1 RPE-O

There was no significant three-way interaction between breathing, gender and training status (P = 0.462).There was no significant two-way interaction between breathing and training (P = 0.462). There was a significant two-way interaction between breathing and gender (P = 0.017). Table 3-45 summarises the interaction results.

Table 3-45 RPE-O – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.462	0.019	T
Breathing *Training	0.462	0.019	T
Breathing*Gender	**0.017***	**0.182**	**M**

*RPE-O = overall rating of perceived exertion; *P<0.05; ES = Effect Size; T = Trivial; S = Small; M = Medium; L= Large;*

This interaction was further analysed (see Table 3-46) revealing no significant difference in RPE-O for gender in the DB trial (P = 0.066) or the SB trial (P = 0.835). There was however a significant decrease in RPE-O in the female group (P = 0.032) in the DB trial but no significant difference for the male group (P = 0.313).

*Table 3-46 RPE-O - Breathing*Gender Interaction results*

	Male vs. Female					DB vs. SB				
		P	Mean DIff	95% CI			P	Mean Diff	95% CI	
Breathing *Gender	SB	0.835	0	-1	1	Female	**0.032***	-1	-2	0
	DB	0.066	1	0	3	Male	0.313	0	0	1

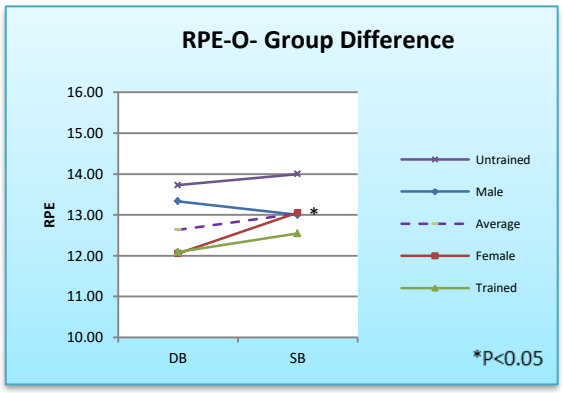
*RPE-O = overall rating of perceived exertion; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.05; SB = Spontaneous Breathing; DB = Deep Breathing;*

There was a main effect for training status (P = 0.006) and effect size was medium, the untrained group having a higher RPE-O compared to the trained group. Table 3-47 summarises the results.

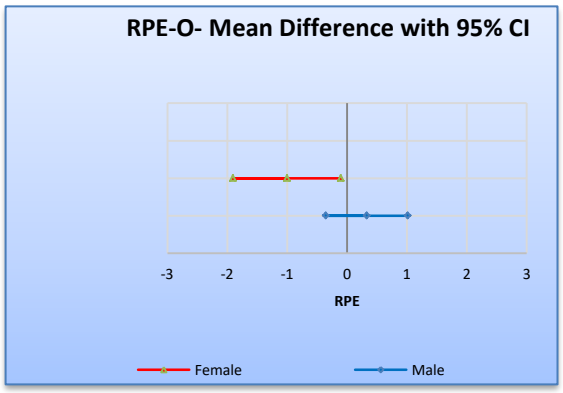
Table 3-47 RPE-O – Summary results for main effect for training

	Main Effect			Difference				
	P	Partial η2	ES	Trained	Untrained	Mean Diff	95% CI	
Training	**0.006***	0.234	M	12	14	-2	-3	-1

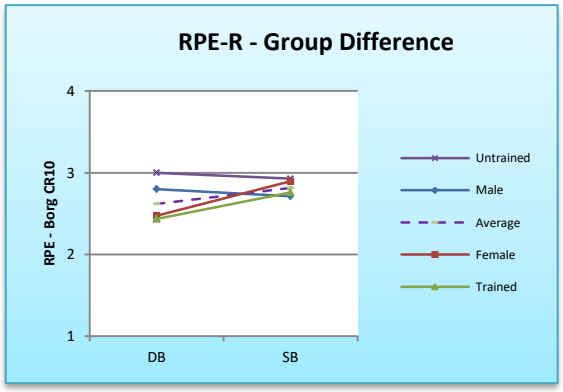
*RPE-O = overall rating of perceived exertion; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.01; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; M = Medium;*



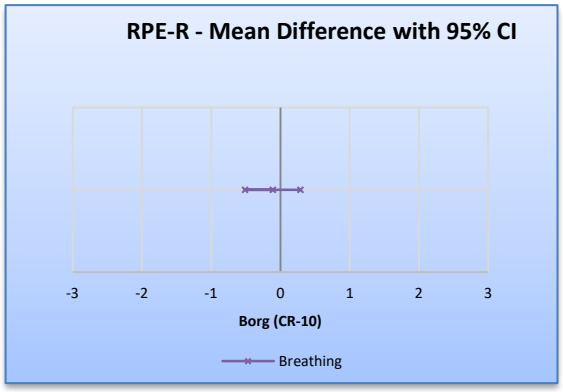
(A) RPE-O DB vs. SB



(B) RPE-O Mean difference with 95% CI



(C) RPE-R DB vs. SB



(D) RPE-R Mean difference with 95% CI

Figure 3-10 Differences in overall and respiratory perceived exertion
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects

and per group (trained, untrained, males and females). There is a significant decrease in overall rating of perceived exertion (RPE-O) for females only (A&B) and no significant difference in respiratory rating of perceived exertion (RPE-R).

Figure 3-10(A) presents mean data for each group in both DB and SB trials. Figure 3-10(B) presents the mean difference for breathing pattern.

3.4.6.2 RPE-R

There was no significant three-way interaction between breathing, gender and training status (P = 0.518).There was no significant two-way interaction between breathing and training (P = 0.303) or between breathing and gender (P = 0.253). Table 3-48 summarises the interaction results.

Table 3-48 RPE-R – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.518	0.014	T
Breathing *Training	0.303	0.035	S
Breathing*Gender	0.253	0.043	S

RPE-R = respiratory rating of perceived exertion;ES = Effect Size; T = Trivial; S = Small;

There was no main effect for breathing (P = 0.595), gender (P = 0.812) or training status (P = 0.405) and effect sizes were trivial or small. Table 3-49 summarises the results.

Table 3-49 RPE-R – Summary results for main effects

	Main Effect			Difference			
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**
Breathing	0.595	0.010	T	3	3	0	-1 0
				Female	**Male**	**Mean Diff**	**95% CI**
Gender	0.812	0.002	T	3	3	0	-1 1
				Trained	**Untrained**	**Mean Diff**	**95% CI**
Training	0.405	0.023	S	3	3	0	-1 1

RPE-R = respiratory rating of perceived exertion; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; S = Small;

Figure 3-9=10(C) presents mean data for each group in both DB and SB trials. Figure 3-10(D) presents the mean difference for breathing pattern.

3.4.6.2.1 HR

There was no significant three-way interaction between breathing, gender and training status (P = 0.774).There was no significant two-way interaction between breathing and

training (P = 0.925) or between breathing and gender (P = 0.072). Table 3-50 summarises the interaction results.

Table 3-50 HR – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.774	0.003	T
Breathing *Training	0.925	0.000	T
Breathing*Gender	0.072	0.104	S

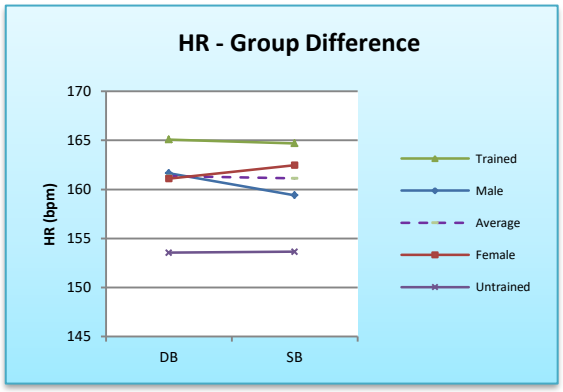
HR = heart rate; ES = Effect Size; T = Trivial; S = Small;

There was no main effect for breathing (P = 0.674) or gender (P = 0.460) and effect sizes were trivial. There was a main effects for training status (P = 0.007) with a medium effect size, HR significantly higher in the trained group. Table 3-51 summarises the results.

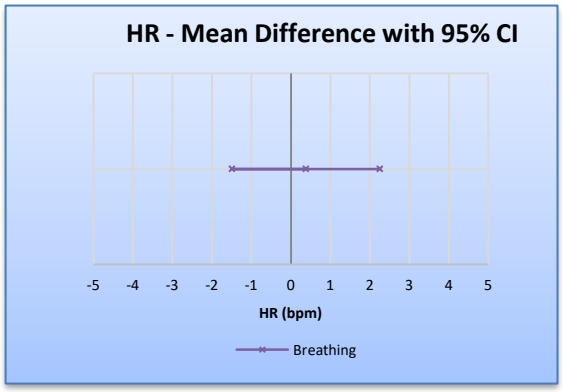
Table 3-51 HR (bpm) – Summary results for main effects

	Main Effect			Difference			
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**
Breathing	0.674	0.006	T	159	159	0	-1 2
				Female	**Male**	**Mean Diff**	**95% CI**
Gender	0.460	0.018	T	157	160	-3	-11 5
				Trained	**Untrained**	**Mean Diff**	**95% CI**
Training	**0.007***	0.221	M	165	153	12	4 20

*HR = heart rate; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *(P<0.05); SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; T = Trivial; M = Medium;*



(A) HR– DB vs. SB

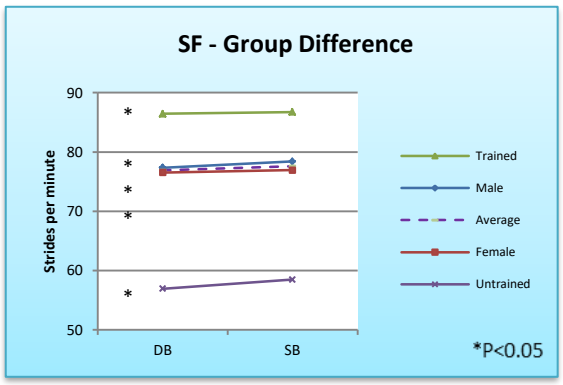


(B) HR – Mean difference with 95% CI

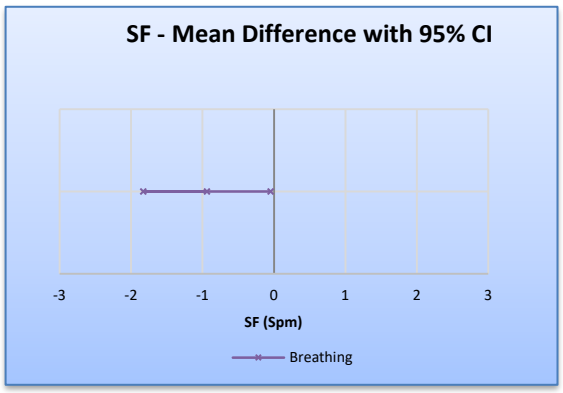
Figure 3-11 Heart rate difference
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels on the right show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). There is no significant difference in heart rate (HR) between trials (A&B).

Figure 3-11(A) presents mean data for each group in both DB and SB trials. Figure 3-11(B) presents the mean difference for breathing pattern.

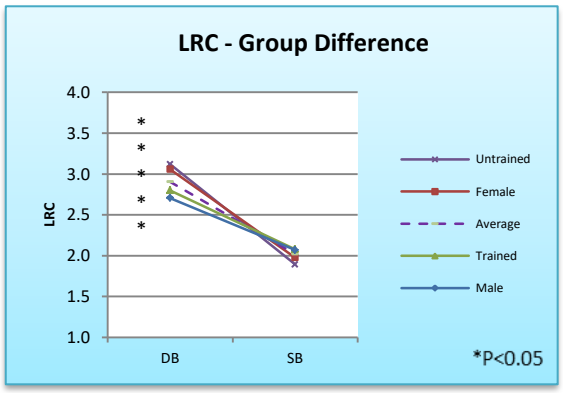
3.4.7 **Locomotor measures**



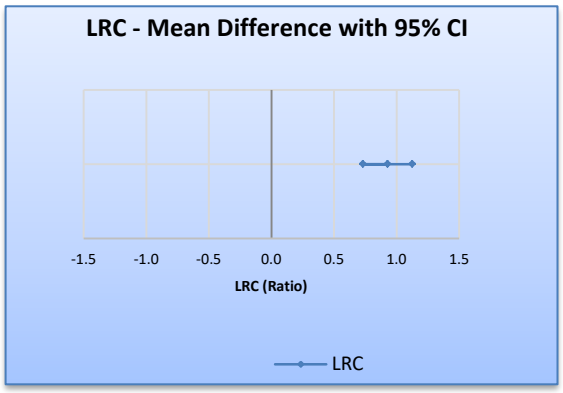
(A) SF – DB vs. SB



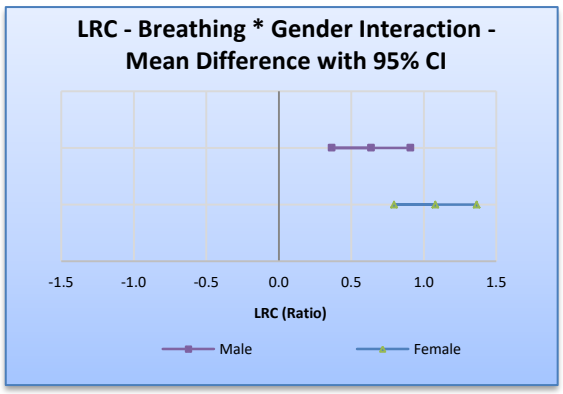
(B) SF – Mean difference with 95% CI



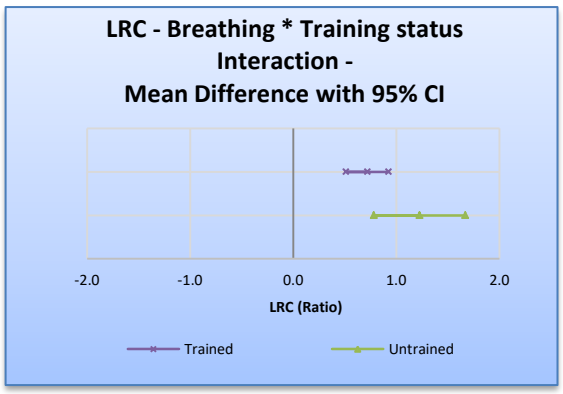
(C) LRC – DB vs. SB



(D) LRC – Mean difference with 95% CI



(E) LRC - Breathing*Gender Interaction



(F) LRC - Breathing*training Interaction

Figure 3-12 Locomotor paramters
Panels A and C show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) trials of Study 1 while panels B and D show the mean difference with the 95% confidence interval (CI) for all subjects and per group (trained, untrained, males and females). Panel E and F show the mean difference with the 95% confidence interval (CI) for the interaction effect between breathing pattern and Gender and training status respectively. There is a significant decrease in stride frequency (SF) (A&B) and a significant increase in locomotor respiratory coupling (LRC) (C&D) for all groups with DB. There is a significant difference in LRC only with gender, a greater increase in females (E) and untrained (F) subjects.

3.4.7.1 SF

There was no significant three-way interaction between breathing, gender and training status (P = 0.487).There was no significant two-way interaction between breathing and training (P = 0.178) or between breathing and gender (P = 0.539). Table 3-52 summarises the interaction results.

Table 3-52 SF – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.487	0.016	T
Breathing *Training	0.178	0.060	S
Breathing*Gender	0.539	0.013	T

SF = Stride Frequency; ES = Effect Size; T = Trivial; S = Small;

There was a main effects for breathing (P = 0.041) with a medium effect size, SF significantly lower under DB conditions. There was no main effect for gender (P = 0.277) and effect size was small. There was a main effects for training status (P = 0.000) with a large effect size trained subjects having a much greater SF. Table 3-53 summarises the results.

Table 3-53 SF (Strides per minute) – Summary results for main effects

	Main Effect			Difference			
	P	**Partial η2**	**ES**	**SB**	**DB**	**Mean Diff**	**95% CI**
Breathing	**0.041***	0.132	M	72	71	-1	-2 0
				Female	**Male**	**Mean Diff**	**95% CI**
Gender	0.277	0.039	S	73	71	-2	-6 2
				Trained	**Untrained**	**Mean Diff**	**95% CI**
Training	**0.000****	0.902	L	87	57	29	26 33

*SF = Stride Frequency; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.05; **P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; ES = Effect Size; S = Small; M = Medium; L= Large;*

Figure 3-12(A) presents mean data for each group in both DB and SB trials. Figure 3-12(B) presents the mean difference for breathing pattern.

3.4.7.2 LRC

No significant three-way interaction between breathing, gender and training status were observed (P = 0.446). There was a significant two-way interaction between breathing and training (P = 0.034). Table 3-54 summarises the interaction results.

Table 3-54 LRC – Three-way and two-way interaction results

	P	Partial η2	ES
Breathing*Gender*Training	0.446	0.020	T
Breathing *Training	**0.034***	0.142	M
Breathing*Gender	**0.025***	0.156	M

*LRC = Locomotor respiratory Coupling; *P<0.05; ES = Effect Size; T = Trivial; S = Small; M = Medium; L= Large;*

This interaction was further analysed (see Table 3-55) revealing no significant difference between males and female in the both the SB trial (P = 0.233) and the DB trial (P = 0.241). There was however a significant increase in the LRC ratio in both the untrained group (P = 0.000) and the trained group (P = 0.1000).

*Table 3-55 LRC - - Breathing*Training Interaction results*

	Untrained vs. Trained					DB vs. SB				
		P	Mean DIff	95% CI			P	**Mean Diff**	95% CI	
Breathing *Training	SB	0.233	0.2	-0.1	0.5	UT	**0.000***	1.2	0.8	1.7
	DB	0.241	-0.3	-0.9	0.2	T	**0.000***	0.7	0.5	0.9

*LRC = Locomotor Respiratory Coupling; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing; UT = Untrained; T = Trained;*

There was a significant two-way interaction between breathing and gender (P = 0.025). This interaction was further analysed (see Table 3-56) revealing no significant difference between males and female in the both the SB trial (P = 0.550) and the DB trial (P = 0.170). There was however a significant increase in the LRC ratio in both the female group (P = 0.000) and the male group (P = 0.1000).

*Table 3-56 LRC - Breathing*Gender Interaction results*

		P	**Mean Diff**	**95% CI**			**P**	Mean Diff	**95% CI**	
Breathing *Gender	SB	0.550	0.1	-0.2	0.4	Female	**0.000***	1.1	0.8	1.4
	DB	0.170	-0.4	-0.9	0.2	Male	**0.000***	0.6	0.4	0.9

*LRC = Locomotor Respiratory Coupling; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; SB = Spontaneous Breathing; DB = Deep Breathing;*

Figure 3-12(C) presents mean data for each group in both DB and SB trials. Figure 3-12(D) presents the mean difference for breathing pattern. Figure 3-12(E) presents the mean difference in male and female groups. Figure 3-12(F) presents the mean difference in trained and untrained groups.

3.5 Discussion

Previous research has investigated deep breathing (DB) primarily in the context of meditation and the affects it induces (Cysarz and Büssing, 2005; Vyas and Dikshit, 2002; Harinath et al., 2004). Research identifies the individuality of breathing pattern and the exercise hyperpnoiec response, and the diverse physiological and psychological inputs that influence it. It is therefore suspected that the ability to breathe more deeply during exercise, the primary way in how it is achieved (abdominal vs. thoracic), and therefore the potential to improve may also be highly individual.

Performance of dual tasks such as a motor task and a cognitive task (e.g. consciously deep breathing) can results in a decrement in the performance of either or both (Schott and Klotzbier, 2018, Grassmann et al., 2016). The effects are referred to as dual task effects (DTE) and are the results of competition for our limited attentional resources. Grassmann et al. (2016) examined the alterations in breathing associated with cognitive load, concluding that cognitive load caused overbreathing, resulting in decreased end-tidal CO_2 ($P_{ET}CO_2$) and increased VO_2 and VCO_2. It is therefore possible that the interaction between the consciously deep breathing may have a negative impact or at least represent a confounding factor in our results.

For deep breathing (DB) to be effective is must be achievable with reduced or minimal extra cost. Failure to synchronise the respiratory musculature can increase the cost of breathing and therefore negatively affect performance (Aliverti, 2008a, Hopkinson et al., 2010). Efficient breathing, especially efficient DB, requires the abdominal musculature to relax and not to oppose the diaphragm, as to do so will increase the cost of breathing and can also result in a sub-optimal thoracic breathing pattern. It is therefore crucial to identify how DB is accomplished, primarily via either abdominal or thoracic expansion. It is a major limitation of our study that we can only describe DB in terms of increased V_T and cannot make this differentiation We must therefore interpret our findings with caution as this is a possible confounding factor and may have impacts on the effectiveness of DB.

Benchetrit (2000) identified the highly individual nature of breathing pattern while Masoaka and Homma (2001, 2004, 2004, 2008) have highlighted the role of both cognitive and emotional state and trait anxiety as factors that influence breathing pattern. These factors may influence who may benefit from DB or be able to effectively achieve such a pattern.

Dempsey et al., (2006) have shown that reductions in respiratory muscle work improve endurance exercise performance. It remains to be seen if DB reduces respiratory work or in fact increases respiratory work by increased activation of the diaphragm which has been shown to compete with locomotor musculature for blood supply.

In this study, DB resulted in a significant change in respiratory pattern, with a decrease in respiratory rate (RR) and an increase in tidal volume (V_T) but differed between trained and untrained subjects. There was a significant increase in V_T for all groups with DB. Our results highlight interesting differences in the untrained group who had higher RR but similar V_T to trained subjects under spontaneous breathing (SB) conditions but increased V_T to a greater extent with DB. There was no difference between males and females when V_T was normalised (V_T/FVC). T_i, T_e and T_{tot} with DB were also significantly higher in the untrained group under both conditions and the increase with DB was also significantly greater in the untrained group. No significant change in duty cycle (T_i/T_{tot}) was observed, reflecting a proportionate increase in both T_i and T_E, noted in previous work by Faull et al. (2018) but contrary to the advantageous greater proportional increase in T_e observed in elite cyclists who have a decreased duty cycle (Lucia et al., 2001). The smaller change in breathing pattern in trained subjects is possibly due to enhanced interoception and ventilatory profile change that can occur with training that allows athletes to more closely match V_E to exercise demands (Faull et al., 2018). However, the interpretation of the differences in the trained group is confounded by the difference in locomotion between the two groups. The higher impact and vertical forces may impose greater restrictions on V_T increases, perhaps via the visceral piston (Bramble and Carrier, 1983; Bramble and Jenkins, 1993). Factors such as intensity and gradient (flat/uphill/downhill) may play and important role in the ability to deep breathe.

Locomotor efficiency was our primary outcome measure assessed by calculating both the unit oxygen cost, O_C (ml/kg/km) and the unit energy cost, E_C (kcal/kg/km). DB had a significant impact on $O_{C,}$ improving efficiency and to the greatest extent in the untrained group. There was significant difference between trained and untrained in both SB and DB conditions, the untrained group have significantly poorer efficiency as might be expected, however, due to the different types of locomotion, uphill walking in the untrained subjects versus 1 % gradient running subjects, a direct comparison is not possible. Notably, females were significantly more efficient than males, both in the trained group and the untrained group. Our interest was in the effect of DB and there was no effect of gender on the reductions in O_C. E_C was also higher in the untrained who were the only group to show a

significant improvement with DB. From the differences in these two measures it appears than while O_C is reduced, the increase in RER with DB, suggesting a possible shift in the substrate utilised, influenced E_C in trained subjects such that no significant energy saving was made. In the untrained subjects it is difficult to interpret whether this is advantageous or not and requires further study into the interaction between reduced O_C and E_C and the impact on total energy expenditure in an exercise bout. If the main objective for some individuals is to maximise energy expenditure this would have a negative impact unless the increased efficiency was accompanied by counteracting effects. These might include the ability to tolerate exercise in this domain that would not otherwise be achievable or the ability to exercise longer in this domain that would result in a higher overall energy expenditure.

There was also evidence of change in locomotor respiratory coupling (LCR) as evidenced by a significant decrease in stride frequency (SF) and an increase in stride rate to respiratory rate ratio (SR:RR). The significant change in SF clearly supports the previous work by Rabler and Kohl (2000) who demonstrated a bi-directional link within the coupling, suggesting a mutual attraction between the two processes. Alteration of the respiratory pattern resulted in a change in SF contrary to the unidirectional, subordinate theories behind LRC which suggest that respiratory pattern is entrained by the locomotor pattern (Bramble and Carrier, 1983). The magnitude of change in SF is not proportional to the change in RR and would suggest that while a mutual attraction may exist, locomotion is the dominant force in the relationship. McDermott et al. (2003) have shown that increasing mechanical and metabolic work affects both processes and we suggest that this may impose an overriding and limiting effect on the magnitude of this reverse coupling. Further to this, the magnitude of this effect is most likely constrained by the intensity and gradient of the exercise undertaken as well as mechanical limitations such as individual leg length constraining the ability to increase stride length to decrease SF.

It has also been demonstrated that various LRC ratios exist in humans and an integer ratio reflects a tight coupling between locomotion and respiration. These include 1:1, 2:1, 3:2, 3:1, 4:1 and 5:2 (Bramble and Carrier, 1983), with the 2:1 ratio being predominant (Bernasconi and Kohl, 1993). The transition between LRC ratios occurs seamlessly and unnoticed during steady state exercise (Bramble and Carrier, 1983) and with increasing intensity (McDermott et al. 2003). Mean values from this study for SF:RR during SB concur with the dominance of the 2:1 ratio. The SF:RR ratio for DB was approximately 3:1, with many subjects achieving an integer ratio suggestive of a *re-coupling* of the two

processes. Further research using more detailed and stringent analysis of the LRC is needed to elucidate if such a re-coupling exists. Individual analysis is also required to ascertain if the subjects with stronger coupling during SB achieve this re-coupling, or if indeed any correlation exists.

There was a significant decrease in absolute VO_2 of 0.138 ± 0.249L/min ($p<0.05$) and coincided with a significant reduction in V_E of 5.87 ± 6.49L/min ($p<0.001$). Benchetrit (2000) identifies considerable individuality and diversity in breathing patterns at rest but despite various combinations of RR, V_T and T_i:T_e ratios, V_E is maintained. Bernasconi and Kohl (1993) have also shown that an increase in coordination in LRC via paced breathing did not affect V_E despite a decrease in VO_2, which they suggested may be due to a decreased metabolic cost. This evidence that improvements can be induced by improved coordination supports the idea that the change in LRC ratio we observed with DB may produce further reductions in metabolic cost and may explain the significant reduction in VO_2 observed. Indeed, Bernasconi and Kohl (1993) proposed that the decreased sympathetic tone may underlie the reduction in metabolic cost and DB has been shown to have a dramatic modulating effect on the autonomic nervous system, affecting heart rate via RSA (Cysarz and Büssing, 2005; Giardino et al. 2003; Hayano et al., 1996) and suppressing the sympathetic nervous system (Bernardi et al., 2002) in particular in the exercising muscle modulating muscle sympathetic nerve activity (MSNA) (Seals et al., 1990).

The significant reduction in V_E in this study demonstrates an improvement in economy and strongly advocates the use of a deep breathing pattern and provides evidence of some optimisation within these systems. Ventilation, V_E, is primarily controlled by metabolic rate (Haouzi, 2006) and at intensities below LT, V_E is dynamically coupled with VCO_2 to maintain arterial PCO_2, while at intensities above LT, V_E is linked to the maintenance of arterial pH in the face of metabolic acidosis (Ward, 2007). As we discuss below, the reduction in V_E was not matched by a matching reduction in VCO_2, therefore the DB pattern appears to have broken this coupling. Despite a non-significant decrease in VCO_2, V_E/VCO_2 decreased significantly with DB due the disproportionate decrease in V_E which is related to the significant decrease in V_A also observed. The implications for this are evident in CO_2 parameters with significant increases in $P_{ET}CO_2$ reflecting an increase in arterial PCO_2 and disturbance to the acid-base balance. While not measured as part of this study, alterations to blood pH could affect a rightward shift in the O_2 disassociation curve, possibly enhancing O_2 unloading. The greater decrease in VO_2 relative to VCO_2 resulted in

a non-significant increase in the respiratory exchange ratio (RER), reflecting a change in substrate use and increase in carbohydrate metabolism. While there was no significant increase in blood lactate concentration (BLa^-) there was a non-significant increase with DB corroborating a greater reliance on glycolytic metabolism supported by the RER findings. Despite clear evidence of the powerful modulatory effect of DB on heart rate via RSA (Cysarz and Büssing, 2005), heart rate did not change significantly between trials. The intensity of exercise and metabolic stimuli most likely prevent a reduction in heart rate (HR) due to the metabolic demand for blood flow. Therefore, the same cardiac output coupled with a change in ventilation may suggest an alteration to ventilation/perfusion (V_A/Q) which may be a possible mechanism underlying the observed improvement in economy. This is supported by the work of Giardino et al. (2003) and Hayano et al. (1996) which demonstrated improvements in gas exchange and V_A/Q matching with deep slow breathing.

DB resulted in a significant reduction in overall RPE (RPE-O) for the female group but not in respiratory RPE (RPE-R). DB did not result in any significant changes in perceived exertion (RPE-O) or dyspnoea (RPE-R) for males. Significant research into gender differences identifying dysynapsis and increased susceptibility to EFL in females (Harms and Rosenkranz, 2008a) may be a possible reason why females may have benefited more from DB. Tong et al. (2004) have shown that dyspnoea may be an exercise limiting factor and Bernardi et al. (2002) have proposed that slow breathing may delay the onset of dyspnoea, however no significant results to support this were found in this study. This may have been influenced by the intensity of exercise above LT and therefore the need to counteract the greater chemical stimulus to respiration, the lack of familiarity with the RPE-O and RPE-R scales and therefore the validity of values from both trials is unclear. Also the large inter-individual variability in our subjects supports the findings of Bernasconi and Kohl (1993) who found that with comparatively smaller manipulation of respiratory pattern via paced breathing, some subjects expressed annoyance with forced pattern, a feeling echoed by many of our subjects despite non-significant changes in perceived exertion. Such feelings are understandable due to the autonomic control of respiration and the various, complex and poorly understood physiological control mechanisms underpinning respiration.

3.6 Conclusion

It can be concluded from this study that the adoption of deep breathing pattern results in increased locomotor efficiency as measured by a significant reduction in VO_2, O_2 cost and energy cost.

DB also effects a change in the LRC relationship, however the proposed decoupling of locomotion from respiration is not definitive and the possibility of a re-coupling and a more optimum coupling ratio is suggested as a possible mechanism explaining the improvements observed. These improvements were demonstrated in both trained and untrained males and females with a wide age range.

Research identifies the individuality of breathing pattern and the exercise hyperpnoiec response, and the diverse physiological and psychological inputs that influence it. It is therefore suspected that the ability to breathe more deeply during exercise, the primary way in how it is achieved (abdominal vs. thoracic), and therefore the potential to improve may also be highly individual. It is clearly necessary to investigate this individual pattern in future research to identify links between pattern type and the scope to increase depth of breath and exercise performance.

3.7 Limitations and future work

Due to technical limitations in our laboratory we were unable to measure certain parameters. We were unable to ascertain or categorise subject breathing patterns as abdominal or thoracic either under SB or DB conditions. This might be particularly useful in identifying individual differences. We were unable to measure blood gases, either arterial or capillary, and instead relied on the indirect measure of P_aCO_2 from $P_{ET}CO_2$. In light of the change in $P_{ET}CO_2$ with deep breathing arterial blood gases would have allowed confirmation of this as it is possible the altered breathing pattern and change measured at the mouth may not accurately reflect arterial partial pressure changes. While clearly identified in the literature as a limiting factor, expiratory flow limitation was not measured.

Both an advantage and disadvantage of this study is the heterogenous nature of the population. Inclusion of both a trained and untrained group allows for investigation of DB in two different populations. The within-subject design and the statistical analysis enabled this. However, the use of two different exercise protocols (running vs. walking) imposes different mechanical constraints and therefore between-group comparisons must be interpreted with caution. Variations in anthropometric variables between groups were also

a limitation as it is possible that factors such as excessive abdominal fat could restrict abdominal incursion of the diaphragm and therefore affect the breathing pattern.

Another consideration is the static ordering of trials with the DB trial following the SB trial however the order was chosen to prevent subjects' spontaneous pattern from being influenced by DB. We did not assess psychological parameters which may have influenced SB pattern and the ability to deep breath, such as trait anxiety or respiratory anxiety. Also, because subjects must consciously increase the depth of breath it constitutes a dual-task performance which has been shown to affect physiological parameters and is therefore a possible confounding factor. The RPE measures used may not have been sensitive enough to measure subtle changes in perceived exertion.

The selection of a method for selecting the same relative exercise intensity is problematic In retrospect, the adoption of the 1mmol lactate threshold, while achieving the desired effect of placing subjects above LT in the heavy intensity domain, was arbitrary and likely placed subjects at different relative intensities. It's known that the lactate concentrations at which LT and MLSS occur, the lower and upper bound for the heavy intensity domain, vary considerably. This poses the problem that a fixed concentration of 1mmol/L could place different subjects in different areas of this zone and possible even above MLSS and into the severe domain. Ideally both LT and MLSS would need to be identified and intensity set at a fixed percentage of this range to attempt to make it more accurate relative intensity. This is however problematic due to the difficulty and time consuming nature of MLSS identification. An alternative would be to identify VT1 and VT2 as the upper and lower intensity limits, however, these too are not always easily identified.

Future work to measure these parameters would deepen our understanding considerably to probe the individual basis for breathing and those susceptible to limitation and possibly more amenable to improvement from DB. The acute change in breathing pattern was necessitated to assess the effect of pattern alone as it is established that the respiratory muscles when trained can influence performance and also the neuro-respiratory centres can undergo both modulatory and plastic responses that may benefit performance. However such an acute change could trigger individual respiratory anxiety levels to increase, interfering with perceived exertion and/or ability to deep breathe. A chronic intervention which trained subjects to deep breathe in isolation or in conjunction with respiratory muscle training needs to be explored. The ability to DB and to maintain the pattern may be limited by respiratory muscle endurance and respiratory control circuits. Increased

endurance may facilitate DB and chronic training may allow for modulatory and plastic responses to occur which might allow DB to fully benefit subjects.

4. Study 2

‘To measure the effect of Deep Breathing on running performance in male endurance athletes’

4.1 Introduction

A principal aim of research in the area of sports performance is to determine the effect of a specific intervention on sports performance, however, the assessment of this is often problematic due to issues with reliability and validity of tests used (Hopkins and Hewson, 2001). Actual performance is rarely, if ever, assessed. Instead simulated endurance performance measures (Currell and Jeukendrup, 2008) or physiological performance parameters are assessed (Jacobs et al., 2011) despite the imprecise relationship to actual performance (Hopkins and Hewson, 2001). Indirect physiological measures include maximum oxygen uptake (VO_2max), lactate threshold (LT), exercise efficiency, peak running speed (Vpeak), critical speed (CS) (Buchheit et al., 2008, Galbraith et al., 2014), time trials (TT’s), time to exhaustion (TTE) (Jacobs et al., 2011, Machado et al., 2013b, Currell and Jeukendrup, 2008). Hopkins and Hewson (2001) recommend CV’s in predictive tests for running performance (CV < 2.5% for half and full marathon; CV < 1.5% for shorter distance). Time trials (TT’s) have better predictive validity and reliability and possibly sensitivity over time to exhaustion (TTE) tests, the CV for running less than 5% (60min run CV = 2.7%) (Currell and Jeukendrup, 2008), however these CV’s exceed that recommended by Hopkins and Hewson so an alternative method was chosen to evaluate endurance running performance in our study.

Peak running speed during an incremental test (Vpeak) or velocity at VO_2peak (vVO_2peak) is the best predictor of running performance (McLaughlin et al., 2010). Vpeak has been found to be highly correlated with both 5K and 10K TT running performance but is affected by stage duration, 3 minutes the recommended duration (Machado et al., 2013b). Machado et al. (2013a) compared three commonly used V_{peak} protocols the 1 minute ($V_{peak\_1\_min}$), 2 minute ($V_{peak\_2\_min}$) and 3 minute stages ($V_{peak\_3\_min}$). The stage duration has

an effect on peak lactate (BLa^-_{peak}), peak heart rate (HR_{peak}), V_{peak} and TT performance prediction. $V_{peak\_3\_min}$ producing significantly lower V_{peak} and BLa^-_{peak} compared to the other two protocols. The lower vVO_2peak for the 3 minute protocol is also support by the work of Midgley (2007c). The $V_{peak\_3\_min}$ protocol using $V_{peak\text{-}P}$ has the highest predictive scores and the lowest standard error of the estimates (SEE) for 5K (r^2 = 0.92; SEE = 0.8 min) and 10K (r^2 = 0.83; SEE = 2.5 min) performance and the recommended standard for 5K and 10K running performance prediction by Machado (2013b). Mclaughlin et al. (2010) has shown $V_{peak\_3\_min}$ to be the best predictor of 16km performance (r^2=0.94) explaining 94% of the variance in performance and superior to velocity just below the onset of plasma blood lactate accumulation, V_{OBLA} (r^2=0.83) and running economy (RE) (r^2=0.66). These recommendations are further supported by the more recent work of Peserico et al. (2014) confirming a 3 minute protocol used in conjunction with $V_{peak\text{-}P}$ to be the most reliable method used (1.5% ≤ CV ≥ 1.8%; SEM = 0.3; ICC = 0.9; Highly Reliable). They also noted the effect of increment, 0.5km/h more reliable than 1 km/h which was more reliable than 2km/h. The high coefficients of determination (r^2) and use of SEM CI's and ICC's supports the predictive capacity of this metric a go to meet the requirements for test validation set out by Impellizzeri and Marcora (2009). With this in mind the vVO_2peak protocol chosen for Study 2 and Study 3 used 3 minute stages with 1km/h increment (Midgley et al., 2007c) with and initial speed of 10km/h in endurance trained male athletes (Thevenet et al., 2008) and $V_{peak\text{-}P}$ as our vVO_2peak calculation method.

Research has sought to train and optimise various physiological systems to improve cardiovascular, metabolic and neuromuscular function to elicit improvements in performance (Joyner and Coyle, 2007, Midgley et al., 2007a). The respiratory system been only been recently added as a possible avenue of investigation and it's role is far more complex and pervasive than previously thought (McKenzie, 2012). The traditional consensus that the respiratory system did not limit performance has changed amidst growing evidence that it may limit exercise performance, especially in elite athletes, but more importantly, that it may be trained to improve performance (Dempsey et al., 2008a, Dempsey et al., 2008b, Dempsey et al., 2006, Romer and Dempsey, 2006, Tong et al., 2008, Tong et al., 2004, Guenette and Sheel, 2007b, Amann, 2011b, Gigliotti et al., 2006, McKenzie, 2012). Also, new developments in our understanding of fatigue mechanisms and the role peripheral metabolite accumulation, which the respiratory system may influence also highlights the need to take a deeper look at this often overlooked physiological system (Amann, 2011a).

The role of the respiratory system in many of the physiological and psychological factors contributing to the development of fatigue and ultimately to the limitation of exercise performance has been largely ignored. In light of a growing body of research challenging this view, emerging evidence suggests the respiratory system may fail to meet the demands imposed during exercise and therefore play a role in the development of fatigue, both locally and systematically, limiting exercise performance (Dempsey et al., 2008a, Dempsey et al., 2008b, McKenzie, 2012, Romer and Polkey, 2008, Amann, 2011b, Harms et al., 1997), especially in athletes (Guenette and Sheel, 2007a, Romer and Polkey, 2008) of which female athletes may be at even greater risk (Dominelli et al., 2011, Guenette et al., 2009, Guenette et al., 2007, Hopkins et al., 1998, Harms and Rosenkranz, 2008b). Ventilation patterns may have a considerable influence on ventilatory efficiency, the effectiveness of gas exchange, the development of respiratory limitation, the mechanics and therefore the metabolic cost of breathing, and also the mechanics of locomotion (Aliverti, 2008b, Koulouris and Hardavella, 2011, Dominelli et al., 2011). The control of respiration is still debated and not fully understood (Haouzi, 2012). This changing research landscape recognises the respiratory system as a contributing factor to fatigue, posing a limiting factor to exercise performance. It is proposed that an altered breathing pattern, specifically a *deep breathing*' pattern (DB) may improve athletic performance via moderation or amelioration of respiratory limiting factors.

The autonomic ventilatory pattern adopted during exercise may fail to meet the imposed functional demands placed upon the respiratory system leading to respiratory limitation of exercise. This may be due to impaired ventilation perfusion matching (VA/Q), impaired gas exchange, expiratory flow limitation (EFL) and/or exercise induced arterial hypoxemia (EIAH) (Wagner, 1992, McClaran et al., 1999, Dempsey et al., 2008b, Dempsey et al., 2008a). Evidence of respiratory system plasticity has shown that respiratory adaptations via respiratory muscle training (RMT) can enhance exercise performance in running (Tong et al., 2008), cycling (Gething, 2004, Johnson et al., 2007) and rowing performance (Volianitis et al., 2000). Currently there is a lack of research in the area of ventilator pattern manipulation and how this may effect respiratory limitation, respiratory efficiency, acid-base balance and how these may influence the development of fatigue and/or exercise performance.

The ventilatory pattern adopted is consequential to the combined and proportional influences of afferent inputs on autonomic control centres. There is considerable heterogeneity in respiratory pattern both at rest and during exercise demonstrating that

ventilatory requirements may be satisfied in varying ways and indeed some elite athletes exhibit unique ventilatory patterns during exercise (Benchetrit, 2000, Lucia et al., 2001). It is important to remember that while respiration is under autonomic control it can be consciously overridden allowing ventilatory pattern to be altered.

Ventilation pattern determines the mechanics and therefore the metabolic cost of breathing and also influences ventilatory efficiency. Its effects have implications both on the effectiveness in maintaining O_2, CO_2, and pH homeostasis and also the incurred cost in attempting to achieve this. An inefficient, sub-optimal ventilator pattern may result in an increased cost of breathing and the development of respiratory muscle fatigue which has been shown to result in competition for O_2 with locomotor muscles, negatively affecting exercise performance (Dempsey et al., 2006, Romer and Dempsey, 2006). In addition to these specific respiratory effects ventilation pattern may also affect the mechanics of locomotion (Baskurt, 2012, Bernasconi and Kohl, 1993, Rabler and Kohl, 2000) and therefore mechanical efficiency of exercise.

It has been proposed that an individual critical limit of peripheral metabolic disturbances exists which cannot be voluntarily surpassed (Amann, 2011a). During intense exercise when metabolic disruption is detected and relayed to the central nervous system (CNS) via metobosensitive afferent neural pathways inhibiting central motor drive (CMD), this threshold is reached leading to fatigue and ultimately reduced exercise intensity and/or exercise termination (Amann, 2011a). These afferent pathways also provide feedback which regulate ventilatory and cardiovascular responses to exercise (Amann, 2011a). Hydrogen ions (H^+) are one such metabolite which disrupt acid-base balance, and intramuscular levels of H^+ are related to metabolic CO_2 accumulation. Therefore the elimination of CO_2 plays a key role in the regulation and maintenance of 'acid-base' balance (Robergs et al., 2005). A ventilatory pattern that may be more effective and efficient in CO_2 elimination may decrease this afferent stimulus which may be responsible for driving an inefficient pattern. This may reduce the metabolic cost and/or delay acid-base disturbance, delay fatigue onset and improve exercise performance.

Deep breathing has been shown to affect the autonomic nervous system (ANS) causing sympathovagal modulation, affecting heart rate (HR) via heart rate variability (HRV), a phenomena called respiratory sinus arrhythmia (RSA), blood pressure, arterial oxgen saturation (S_aO_2), muscle sympathetic nerve activity (MSNA) in skeletal muscle and the peripheral microcirculation (Krasnikov et al., 2013, Yasuma and Hayano, 2004, Seals et al., 1990). It is suggested that deep breathing may improve gas exchange, ventilatory

efficiency, reduce the cost of breathing and/or improving mechanical efficiency. This has the potential to decrease VO_2, delay acid-base disturbance and ultimately improve exercise performance.

The conscious overriding of autonomic respiratory control altering ventilatory pattern may positively affect exercise performance if it can reduce the effects of these exercise limiting factors without incurring other deleterious side effects such as exacerbating disruptions to homeostatic balances of blood gases and pH that occur in exercise and possibly exacerbating fatigue and reducing exercise tolerance.

4.2 Aims and Objectives

- **Hypothesis**

  'Deep breathing improves vVO_2peak'

- ***Aims***

  - *To evaluate if deep breathing increases vVO_2peak*
  - *To explore what factors may underlie this improvement*

- ***Objectives***

  - *Measure and calculate cost of locomotion in the heavy intensity domain, VO_2, Oc and Ec, under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
  - *Measure other gas exchange parameters under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
  - *Measure overall and respiratory RPE under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*
  - *Measure stride frequency and calculate locomotor respiratory coupling under two breathing conditions, spontaneous and deep, while walking/running on a treadmill*

4.3 Methods

4.3.1 Subjects

Subjects were recruited by emailing athletics and triathlon clubs advertising for research volunteers. Healthy male endurance athletes between the 18 and 45 years of age were included if they were engaged in regular training (>5days per week) that included high intensity training, were injury free for the previous month and had normal respiratory function assessed via spirometry (forced expiratory volume in 1s (FEV_1 > 90% predicted, ratio between FEV_1 and forced vital capacity (FEV1/FVC) >70%). Subjects were excluded if they had any respiratory disease or musculoskeletal injury that could interfere with exercise testing. The study was approved by the Ethics Committee in Dublin City University.

4.3.2 Study Design

Figure 4-1 gives an overview of the structure of the study outlining the test sequence. The study used a within-subject, random crossover design, participants randomly allocated to either complete the deep breathing (DB) or spontaneous breathing (SB) vVO_2peak tests first.

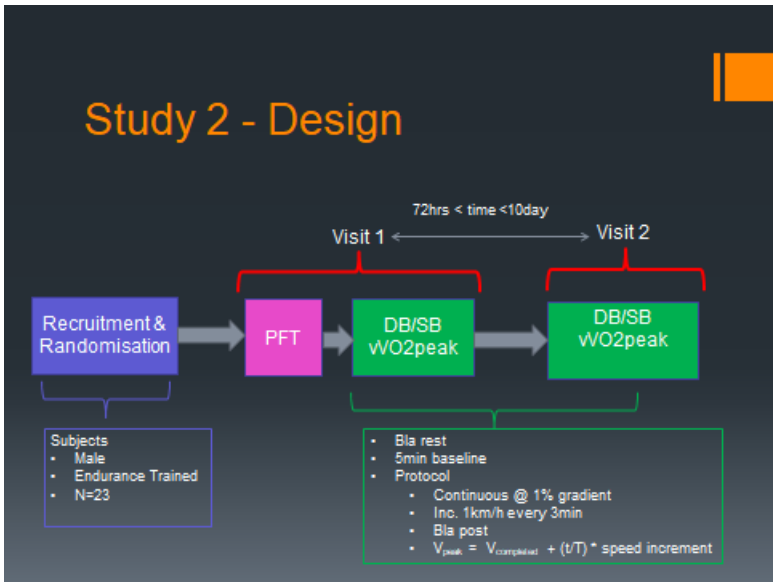


Figure 4-1 Study 2 – Design Overview

All the subjects visited DCU Human Performance Laboratory in the School of Health and Human Performance for testing on three separate occasions, separated by at least 72 hours and no more than 10 days between the two vVO_2peak tests. Following recruitment, on the

initial visit subjects completed a medical health screening form and informed consent before a pulmonary function was tested to screen for respiratory disease. Following this they performed the first vVO_2peak test. On the subsequent visit subjects completed the same vVO_2peak protocol with the alternate breathing pattern. Subjects were instructed to follow a similar diet and training regimen before all tests. This meant being well hydrated and abstaining from food and caffeine for 4 hours prior to testing, and performing no hard training in the 48 hours prior to testing. Every attempt was made to perform tests at a similar time and on the similar training day to control for diurnal changes, training fatigue and metabolic changes. Subjects had height, weight and resting heart rate measured prior to each test.

4.3.3 **Pulmonary Function Testing**

Spirometry was carried out with an automated pulmonary function testing system (Viasys Vmax Encore 299; SensorMedics, Yorba Linda, CA) via indirect calorimetry using open-circuit spirometry. Tests were carried out in the standing posture following recommended procedures. Pulmonary function measurements were expressed as absolute values and percentages of predicted values.

4.3.4 **Cardiopulmonary Exercise Testing**

Laboratory environment conditions were controlled at 18 degrees centigrade. Exercise testing was carried out on a COSMED T170 motorised treadmill. Pulmonary data collected breath-by-breath throughout all exercise tests with the ViasysVmax Encore 299 metabolic cart. The system was calibration in accordance with manufacturer guidelines prior to each test. Heart rate data was recorded with the Polar V800 heart rate monitor (Polar Electro, Inc., Kempele, Finland) using a 1 second sample rate and later downloaded for analysis. Perceived exertion was assessed on two scales, the standard Borg Rating of Perceived Exertion (RPE) scale for overall exertion which we shall refer to as RPE-O and the Borg CR-10 dyspnea scale. RPE was recorded at the end of each stage and on test termination, if during a stage. Maximal incremental exercise tests were terminated by exhaustion. Subjects were verbally encouraged through the final stages to give maximum effort.

4.3.5 **vVO_2peak protocol**

The protocol adopted was a three minute incremental vVO_2peak protocol with incline set to 1% (Jones and Doust, 1996). The protocol was programmed into the COSMED T170 and starting at 10km/h, speed alone was automatically increased every three minutes by 1km/h. The velocity at VO_2peak (vVO_2peak) was calculated using the equation 3Vpeak-P

= Vpeak-C + (t/T) * speed increment Equation 1 (Machado et al., 2013b)

V_{peak} = $V_{completed}$ + (t/T) * speed increment; Equation 3

In which V_{peak} is the maximal running speed, $V_{completed}$ is the speed of the last completed stage, *t* is the number of secons completed in the final stage and *T* is the number of seconds per stage (i.e. 180sec). The V_{peak} was used to set the intensity of the HIIE bouts.

Prior to the exercise test subjects sat still for 5min minutes after which resting heart rate and baseline lactate was sampled. Subjects began with 5 minutes standing still on the treadmill attached to the metabolic system to obtain baseline cardiopulmonary measurements. For the DB trial, subjects were given the final 2 minutes to practice the DB technique. There was no warm-up and subjects began by stepping onto the treadmill at the calculated intensity. The exercised at this intensity continuously until test termination.

4.3.5.1 Deep Breathing Instructions

The deep breathing (DB) pattern was self-paced by the subjects. Instructions were verbally conveyed to the subjects, in which they were instructed to breathe as deeply and slowly as the felt comfortable doing. During the test tidal volume (V_T) was monitored to ascertain if they maintained a DB pattern based on the V_T from the SB trial. Periodically during the test the instructions were repeated if the V_T was observed to be decreasing significantly to SB levels.

4.3.6 Stride Frequency (SF) measurement

SF was measured using the Polar Stride Sensor in conjunction with the Polar V800 heart rate monitor and recorded before the end of each stage manually to corroborated data.

4.3.7 Locomotor Respiratory Coupling (LRC) calculation

LRC was calculated by dividing SF taken for the last minute (19-20min) by the respiratory rate (RR). The manual counting of SF imposes limitation in the accuracy of assessment and does not allow for phase coupling to be assessed however it is a method that allows global assessment of the coordination (McDermott et al., 2003) and has been used previously (Bramble and Carrier, 1983).

4.3.8 Locomotor Efficiency

Efficiency of locomotion was assessed by calculating the O_2 unit cost (O_c) expressed as ml/kg/km and energy unit cost (E_c) expressed as kcal/kg/km (Fletcher et al., 2009). E_C was calculated using the updated formula of Jeukendrup & Wallis (2005) for moderate to high

intensity exercise (seeEC = (0.550 * VCO2 – 4.471 *VO2* (# min/km)) / Body Mass (kg) Equation 24).

E_C = (0.550 * VCO2 – 4.471 *VO2* (# min/km)) / Body Mass (kg) Equation 4

As this metric assume steady state it was not used at peak exercise for comparison. It was calculated on the first 6 stages with the understanding that steady state may not be achieved by all subjects within the 3 minutes stage duration except in the initial stages.

4.3.9 **Data processing and analysis**

All manually recorded data was entered into a Micrsoft Excel speeadsheet. HR data was uploaded from the V800 watch to Polar Flow software and downloaded in excel format. Due to limitations with the Vmax software version all data from the system was only downloadable as text files. Data for each test was in 10 second samples and exported in four separate files in order to get all parameters for analysis and spirometry data was exported separately. These files were parsed using a Python script to remove text headers and combine all the data for all subjects into Excel format. The Excel files were then imported to Microsoft Access for analysis and formatting for SPSS. Microsoft Access SQL queries were written to further analyse the data. One minute rolling averages were calculated on all data fields and combined with manually recorded data and spirometry data. Data was then exported in Excel format for import into SPSS for statistical analysis.

4.3.10 **Statistical Analysis**

SPSS was used for statistical analysis. All normally distributed quantitative variables were analysed using two-way Repeated Measures Anova. RPE data did not meet parametric requirements and was analysed using a Related-Samples Wilcoxen Signed Rank Test. Results are represented as mean with standard deviation (mean ± SD), mean difference (Mean Diff.) with 95% confidence intervals (95% CI) and Cohen's d and effect size (based on Cohen's d) are also shown where appropriate. The level of significance was set at $p < 0.05$.

Planned contrasts using paired t tests were implemented comparing SB and DB at all stages for V_T and RR as these were the metrics to confirm DB occurred. Since there was a substantial decrease in N following stage 6 the uncertainly of the effect became apparent at this point, while it was also apparent that the presence of DB diminished as the trials increased in intensity from stage 7 onward (Figure 1-1 to 1-4). Therefore, all subsequent submaximal analysis was completed on stages 1-6 comparing DB and SB, both as a result

of the presence of DB, as well as the insufficient sample size to draw any conclusions from further stages. Analysis of peak data for primary outcomes was of course still undertaken.

A two-way RM ANOVA was used to test for differences across the first 6 stages that all subjects had completed (n=23). Stage data failed Mauchly's test of Sphericity so Huynh-Feldt correction was used and a Bonferroni adjustment was applied for multiple comparisons.

4.4 Results

4.4.1 -Descriptives

Table 4-1 Participant characteristics

N = 23	Mean ± SD
Age (years)	34.3 ± 8.9
Height (cm)	178 ± 7
Weight (kg)	72.9 ± 10.1
BMI (kg/m^2)	23 ± 2.2
FVC (L)	5.93 ± 1.198
FVC (% Reference)	117 ± 18
FEV1 (L/min)	4.837 ± 1.051
FEV1(% Reference)	119 ± 20
FEV1/FVC	82 ± 7
SB vVO$_2$Peak (km/h)	17.2 ± 1.5
SB vVO$_2$Peak (km/h)	17.2 ± 1.5
SB VO$_2$peak (ml/kg/min)	62.6 ±5.3
DB VO$_2$peak (ml/kg/min)	62.7 ± 7.3
SB VO$_2$peak (L/min)	4.512 ± 0.625
DB VO$_2$peak (L/min)	4.524 ± 0.645
DB HRpeak	182 ± 10 bpm
SB HRpeak	181 ± 9 bpm

Summary of key subject characteristics (N=23). BMI = Body Mass Index; FVC = Forced Vital Capacity; %Ref = % of reference value based on normative data; FEV1 = Forced Expiratory Volume in 1 second; SB = Spontaneous Breathing; DB = Deep Breathing; VO$_2$peak = peak oxygen uptake; vVO$_2$peak = velocity at peak oxygen uptake; HRpeak = peak Heart Rate; SD = Standard Deviation;

Table 4-1 presents summary physical and physiological characteristics of the participants (n=23) in the study. All participants were healthy, well-trained (VO$_2$peak = 62.7 ± 7.3 ml/kg/min, 4.524 ± 0.645 L/min; vVO$_2$peak = 17.2 ± 1.5km/h) male endurance athletes

(runners and triathletes) regularly engaged in running training (>5 days per week). All participants had normal respiratory function at rest (FVC (%Ref) = 117 ± 18%; FEV1/FVC = 82 ± 7).

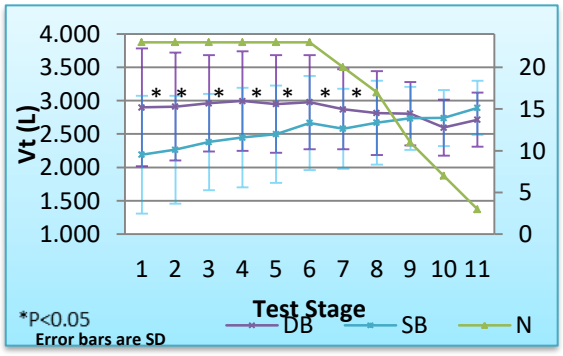
4.4.2 Breathing Pattern Analysis

Analysis was conducted on each stage to investigate if a significantly different breathing pattern had been achieved with DB. Primary indices V_T, RR and V_E, and secondary indices T_{tot} , T_i, T_e and T_i/T_{tot} were analysed across all stages. 23 subjects completed stages 1-6, but the number completing each subsequent stage (7-11) decreases as subjects completed the tests.

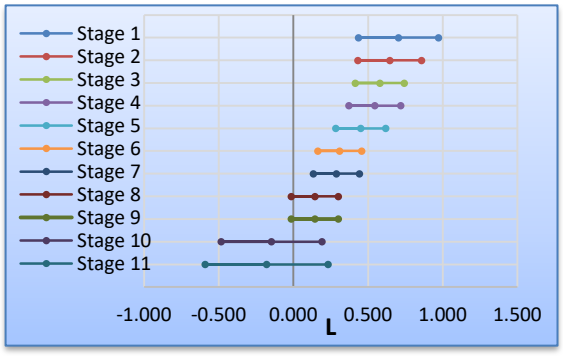
Planned contrasts using paired t tests were implemented comparing SB and DB at all stages for V_T and RR as these were the metrics to confirm DB occurred. Since there was a substantial decrease in N following stage 6 the uncertainly of the effect became apparent at this point, while it was also apparent that the presence of DB diminished as the trials increased in intensity from stage 7 onward (Figure 1-1 to 1-4). Therefore, all subsequent submaximal analysis was completed on stages 1-6 comparing DB and SB, both as a result of the presence of DB, as well as the insufficient sample size to draw any conclusions from further stages. Analysis of peak data for primary outcomes was of course still undertaken.

4.4.3 Primary Indices of Breathing Pattern (V_T and RR)

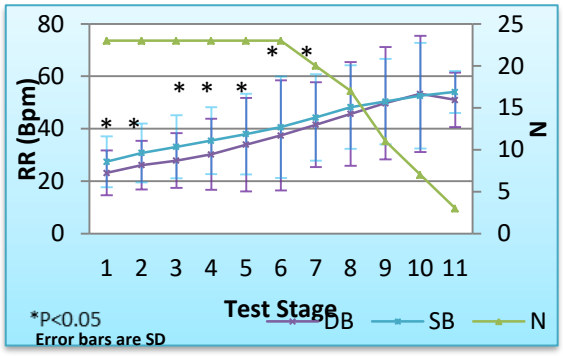
When examining both V_T and RR, the difference decreased as stage increased, reaching non-significance at stage 8 (stages 1-7 $P < 0.01$). Specific results of each stage can be observed in Table 4-2 below, and is presented visually in Figures 4-2(A&B) V_T, and Figure 4-2(C&B) for RR. As a result, analysis of the first six stages, as discussed above, is presented in the following results.



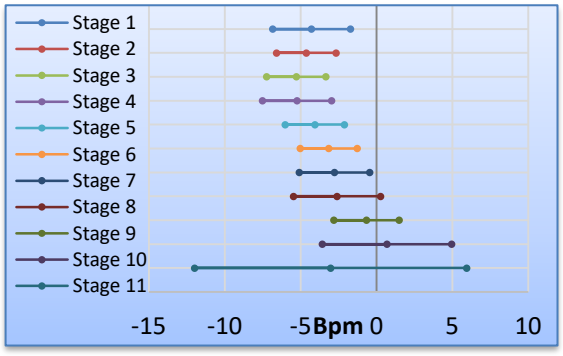
(A) V_T comparing DB vs. SB over 11 stages



(B) V_T – Mean ± 95% CI comparing DB vs. SB



(C) RR comparing DB vs. SB over 11 stages



(D) RR – Mean ± 95% CI comparing DB vs. SB

Figure 4-2 Stage by stage changes in breathing pattern
Panels on the left show the mean response observed for the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 while panels on the right show the mean difference with the 95% confidence interval (CI)). There is a significant increase in tidal volume (V_T) for stages 1-7 with DB and a significant decrease in respiratory rate (RR) for stage 1-6. The magnitude of difference decreases with each advancing stage. (*P<0.05)

Table 4-2 Stage by stage results (SB vs. DB) for V_T and RR to confirm the presence of DB.

	V_T				RR			
Stage	P	Cohen's d	ES	N	P	Cohen's d	ES	N
1	.000*	0.96	Large	23	.002**	0.67	Medium	23
2	.000*	0.92	Large	23	.000*	0.71	Medium	23
3	.000*	0.88	Large	23	.000*	0.77	Medium	23
4	.000*	0.80	Medium	23	.000*	0.71	Medium	23
5	.000*	0.67	Medium	23	.000*	0.57	Medium	23
6	.000*	0.46	Small	23	.002**	0.44	Small	23
7	.001**	0.53	Medium	20	.022***	0.35	Small	20
8	.070	0.24	Small	17	.074	0.29	Small	17
9	.433	0.15	Trivial	11	.525	0.06	Trivial	11
10	.334	0.29	Small	7	.696	0.07	Trivial	7
11	.205	0.44	Small	3	.286	0.33	Small	3

*V_T = tidal volume; RR = Respiratory Rate; *P<0.001; **P<0.01; ***P<0.05; ES = Effect Size.*

4.4.3.1 V_T

We examined the first six stages of V_T using a 2 Way RM ANOVA and identified a significant interaction between Breathing and Stage (P = 0.003; Partial η2 = 0.215; ES = Medium). We examined this interaction using simple main effects (Figure 4-3 A & B) and identified significantly greater V_T when DB compared to SB at all stages (All stages 1-6 P <0.05). When examining the DB trial over the 6 stage duration, there was no change in V_T. In the SB trial, there was a significant increase in V_T between stages 2-3, and 5-6, but not between any other stages. Stage by stage data is presented in Table 4-3.

Table 4-3 V_T (L) Data from all six initial stages comparing DB vs. SB

Stage	DB	SB	p	Mean Diff	95% CI	
1	2.899 ± 0.882	2.193 ± 0.557	**0.000***	0.706	0.438	0.973
2	2.912 ± 0.81	2.265 ± 0.573	**0.000***	0.647	0.434	0.860
3	2.961 ± 0.722	2.381 ± 0.594	**0.000***	0.580	0.416	0.744
4	2.994 ± 0.745	2.447 ± 0.619	**0.000***	0.547	0.373	0.721
5	2.95 ± 0.73	2.498 ± 0.618	**0.000***	0.452	0.284	0.620
6	2.978 ± 0.706	2.665 ± 0.64	**0.000***	0.312	0.164	0.460

*V_T = tidal volume; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; *P<0.001; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD).*

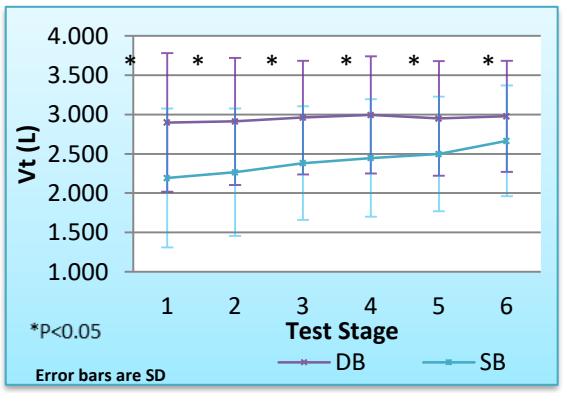
4.4.3.2 RR

When examining RR, there was a main effect for breathing pattern on RR with a large effect size, with DB significantly lower than SB (Mean diff: 4.42 95% CI [2.86 to 5.97]; P = 0.000; Partial η2 = 0.613; ES = Large) and no interaction between breathing pattern and stage (P = 0.349; Partial η2 = 0.048; ES = Small). Descriptives for each stage are presented in Table 4-4 and results are presented in Figure 4-3 C and D.

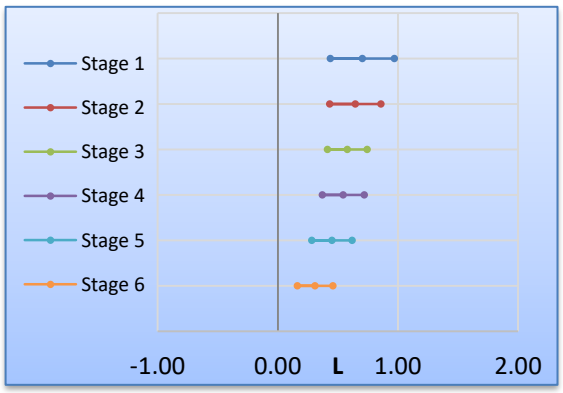
Table 4-4 RR Data from all six initial stages comparing DB vs. SB

Stage	DB	SB
1	23 ± 8 Bpm	27 ± 5 Bpm
2	26 ± 7 Bpm	31 ± 6 Bpm
3	28 ± 7 Bpm	33 ± 7 Bpm
4	30 ± 7 Bpm	35 ± 8 Bpm
5	34 ± 7 Bpm	38 ± 7 Bpm
6	37 ± 7 Bpm	41 ± 7 Bpm

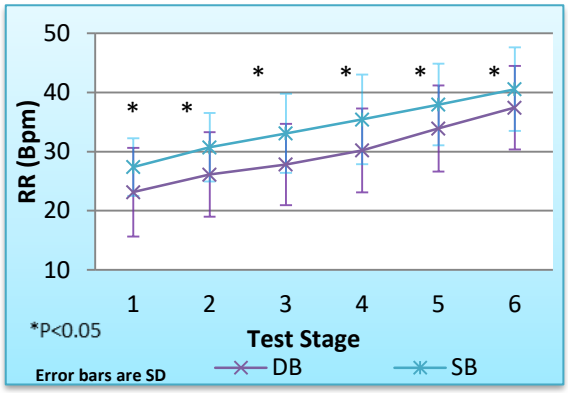
*RR = Respiratory Rate (Bpm); *P<0.001; DB = Deep Breathing; SB = Spontaneous Breathing; Bpm = Breaths per minute; Results are mean ± Standard Deviation (SD);*



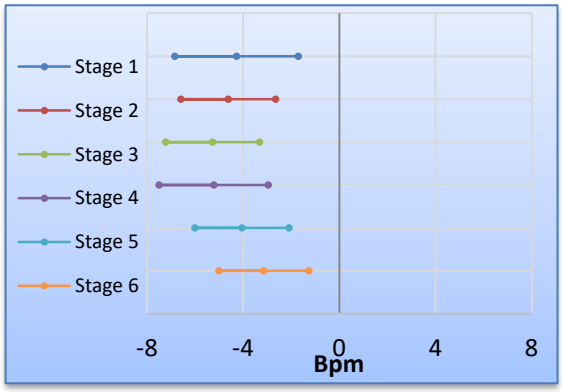
(A) V_T comparing DB vs. SB over 6 stages



(B) V_T – Mean ± 95% CI comparing DB vs. SB



(C) RR comparing DB vs. SB over 6 stages



(D) RR – Mean ± 95% CI comparing DB vs. SB

Figure 4-3 Breathing pattern response for stages 1-6
Panels on the left show the mean response observed for the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 while panels on the right show the mean difference with the 95% confidence interval (CI)). There is a significant increase in tidal volume (V_T) and a significant decrease in respiratory rate (RR) with DB. The magnitude of difference decreases with each advancing stage. (*P<0.05)

4.4.4 Primary Breathing Indices (V_T and RR) measured at VO_2peak

Results of analysis of RR and V_T at VO_2peak are presented in Table 4-5. There was no significant difference between DB and SB trials at VO_2peak in V_T, or RR. The effect sizes for both variables were trivial.

Table 4-5 Primary Breathing Indices Results

	P	SB	DB	Mean Diff	95% CI		Cohen's d	ES
V_T	0.311	2.740 ± 0.556	2.823 ± 0.639	0.083	-0.083	0.248	0.14	T
RR	0.443	52 ± 9	51 ± 10	-1	-4	2	0.11	T

*V_T = tidal volume; RR = Respiratory Rate (Bpm); Mean Diff = mean difference between DB and SB; CI = Confidence Interval;*P<0.001; Mean Difference with 95% CI of the difference; DB = Deep Breathing; SB = Spontaneous Breathing; Bpm = Breaths per minute; Results are mean ± Standard Deviation (SD); ES = Effect Size;*

Figure 4-4 presents the mean difference in primary indices with 95% CI.

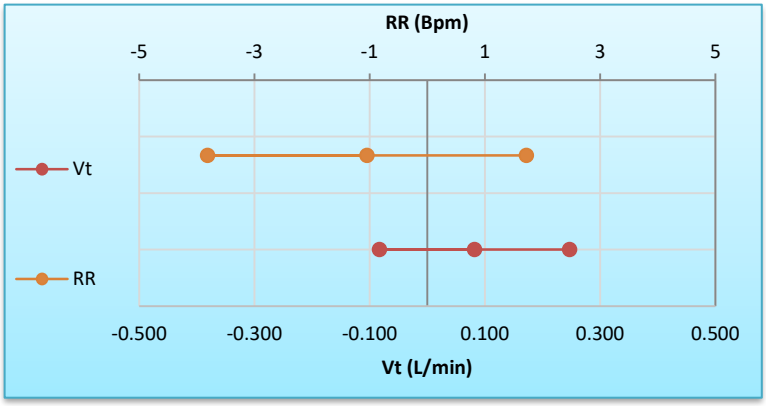


Figure 4-4 Primary Indices of Breathing Pattern (V_T and RR) at VO_2peak comparing SB vs. DB
The figure shows the mean difference with the 95% confidence interval (CI) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak . There is no significant difference in tidal volume (V_T) or respiratory rate (RR).

4.4.4.1 Individual response

Figure 4-5 presents the difference in the V_T/FVC between the DB and the SB sessions. There is considerable individual variation both in direction and magnitude of change. The difference ranged from an increase of ~22% to a decrease of ~12% with DB.

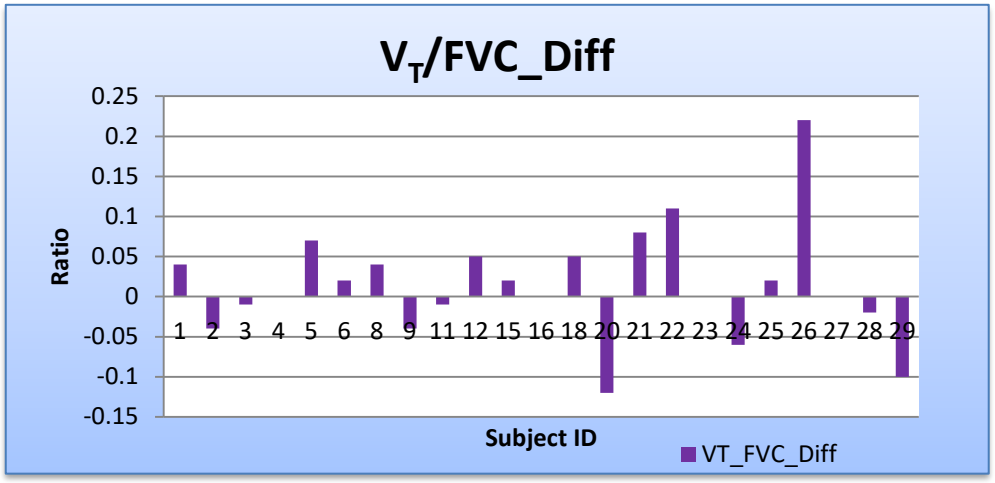
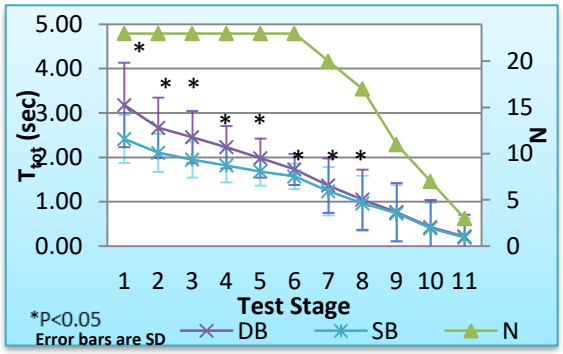


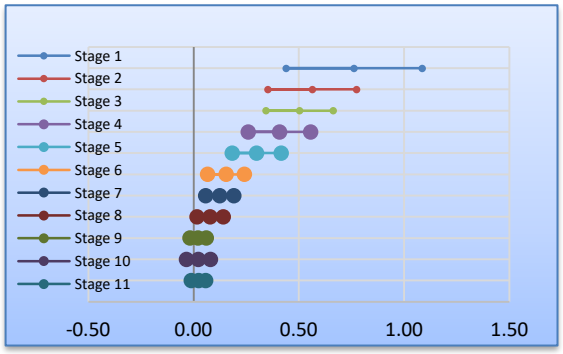
Figure 4-5 Individual V_T response
The figure shows the percentage change in tidal volume (V_T) when normalized for FVC (V_T/FVC) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. The response is quite variable with individual subjects showing, no difference, an increase or decreases in tidal volume (V_T). The magnitude of the difference is also quite varied.

4.4.5 Secondary Indices of Breathing Pattern (T_{tot}, T_i, T_e, T_i/T_{tot})

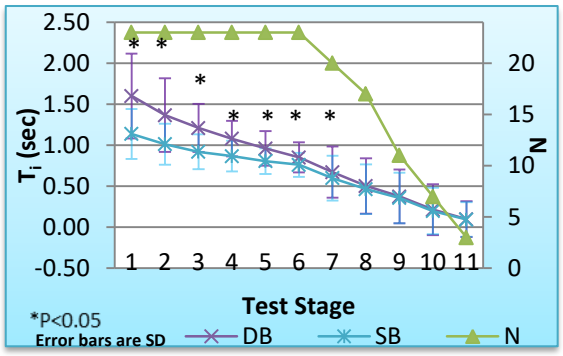
Secondary indices of breathing pattern were compared to assess if there was a difference in the duration of each aspect of the breath cycle. Figure 4-6(A-H) presents mean values for DB versus SB across all stages in the left panels and the mean difference with 95% CI in the right hand panels. Both sets of figures show the difference between DB and SB diminishing with increasing speed, but also with a substantial drop in N.



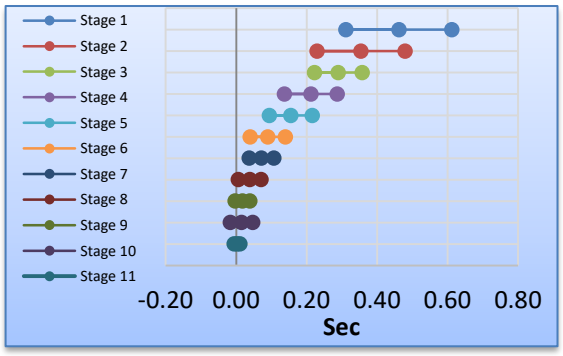
(A) T_{tot} comparing DB vs. SB over 11 stages



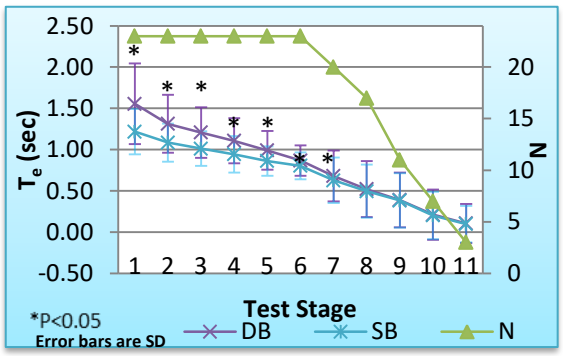
(B) T_{tot} – Mean ± 95% CI comparing DB vs. SB



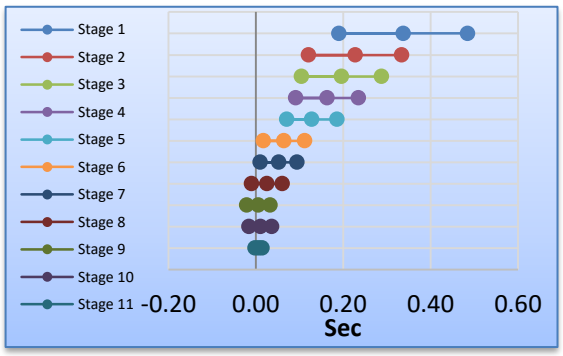
(C) T_i comparing DB vs. SB over 11 stages



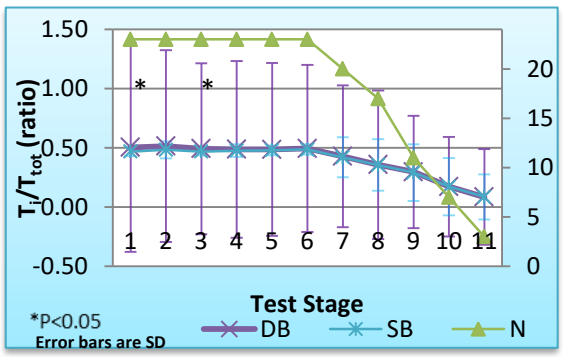
(D) T_i – Mean ± 95% CI comparing DB vs. SB



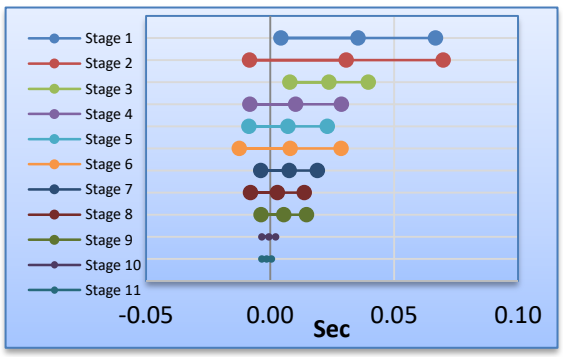
(E) T_e comparing DB vs. SB over 11 stages



(F) T_e – Mean ± 95% CI comparing DB vs. SB



(G) T_i/T_{tot} comparing DB vs. SB over 11 stages



(H) T_i/T_{tot} – Mean ± 95% CI comparing DB vs. SB

Figure 4-6 Temporal indices of breathing pattern
Panels on the left show the mean response observed for the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 while panels on the right show the mean difference with the 95% confidence interval (CI)). Panel A and B present the mean difference showing a significant increase in total breath time (T_{tot}), C and D inspiratory time (T_i), E and F expiratory time (T_e) while G and H show no significant increase in duty cycle (T_i/T_{tot}) except for stages 1 and 3. The magnitude of difference for T_{tot}, T_i and T_e diminishes with advancing stages until non-significant. (*P<0.05)

There was a significant increase in T_{tot} and T_i for stages 1-8 only and T_e for stages 1 – 7, when participants were asked to DB. Despite an increase in T_i, T_e and T_{tot} the duty cycle (T_i/T_{tot}) was maintained in all stages, except stages 1 and 3.

4.4.6 Secondary Breathing Indices measured at VO_2Peak

There was no significant difference for any of the secondary indices at peak exercise, as observed in Table 4-6, and Figure 4-7.

Table 4-6 Secondary Breathing Indices Results

	P	SB	DB	Mean Diff	95% CI		Cohen's d	ES
T_i	0.698	0.58 ± 0.11	0.6 ± 0.14	0.02	-0.01	0.05	0.14	T
T_e	0.242	0.62 ± 0.14	0.64 ± 0.17	0.02	-0.02	0.07	0.14	T
T_{tot}	0.240	1.21 ± 0.24	1.25 ± 0.3	0.04	-0.03	0.11	0.15	T
T_i/T_{tot}	0.950	0.48 ± 0.03	0.48 ± 0.04	0.00	-0.01	0.01	0.05	T
MEFR	0.242	4.576 ± 1.012	4.524 ± 0.776	-0.052	-0.327	0.224	0.06	T
Peak Flow	0.321	6.66 ± 1.433	6.648 ± 1.138	-0.012	-0.413	0.388	0.01	T

T_{tot} = total breath time (sec); T_i,= inspiratory time (sec); T_e = expiratory time; T_i/T_{tot} = duty cycle; MEFR = maximum expiratory flow rate (L/sec); peak flow (L/sec); DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); ES = Effect Size; T= Trivial;

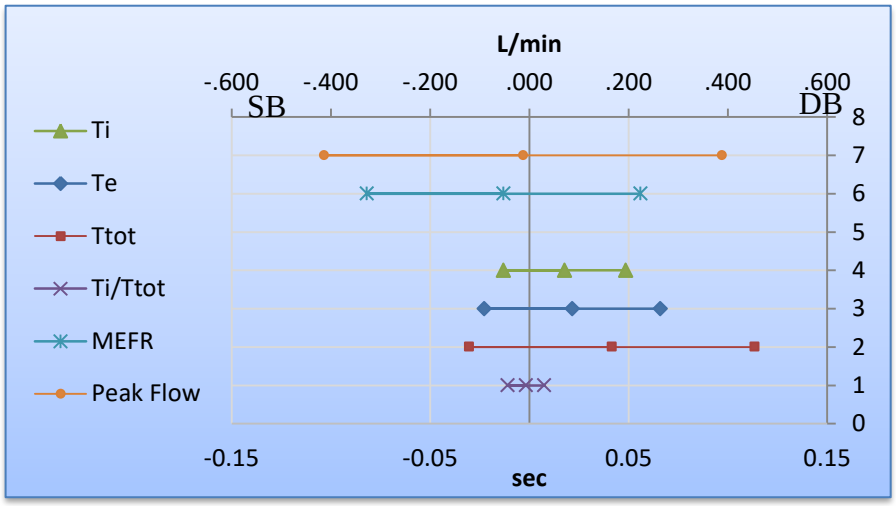


Figure 4-7 Secondary Indices comparing SB vs. DB (mean ± 95% CI)
The figure shows the mean difference with the 95% confidence interval (CI) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. There is no significant change in total breath time (T_{tot}), inspiratory time (T_i), expiratory time (T_e), duty cycle (T_i/T_{tot}), maximum expiratory flow rate (MEFR) or peak flow.

4.4.7 Primary outcomes

When examining the primary performance related outcomes, deep breathing had no significant effect on performance when compared to SB assessed by vVO_2peak and VO_2peak (vVO_2peak P=0.366; VO_2peak P=0.91) (Table 4-7and Figure 4-8).

Table 4-7 vVO_2peak & VO_2peak Results

	P	SB	DB	Mean diff	95% CI		Cohen's d	ES
vVO_2peak	0.366	17.2 ± 1.5	17.2 ± 1.5	0.1	-0.1	0.3	0.05	T
VO_2peak	0.910	4.524 ± 0.645	4.512 ± 0.6	-0.012	-0.230	0.206	0.02	T

*vVO_2peak = velocity at VO_2peak; VO_2peak = peak oxygen uptake; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); *P<0.001; ES = Effect Size, T = Trivial.*

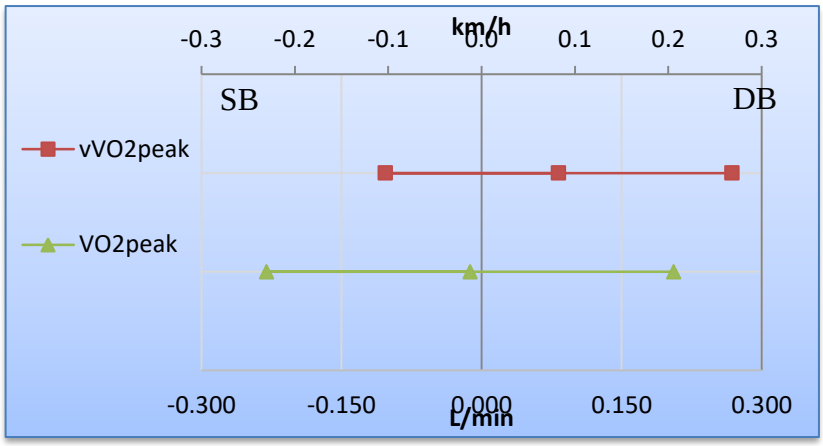


Figure 4-8 Performance measures (vVO_2peak and VO_2peak) comparing SB vs. DB (mean ± 95% CI)
The figure shows the mean difference with the 95% confidence interval (CI) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. There is no significant change in velocity at VO_2peak (vVO_2peak) or VO_2peak.

4.4.7.1 Individual response

Figure 4-9 presents the difference in the number of work intervals (# Reps) completed between the DB and the SB HIIE sessions. There is considerable individual variation both in direction and magnitude of change. There is considerable individual variation both in direction and magnitude of change. The difference in # Reps ranged from an increase of 5 intervals to a decrease of 9 intervals with DB.

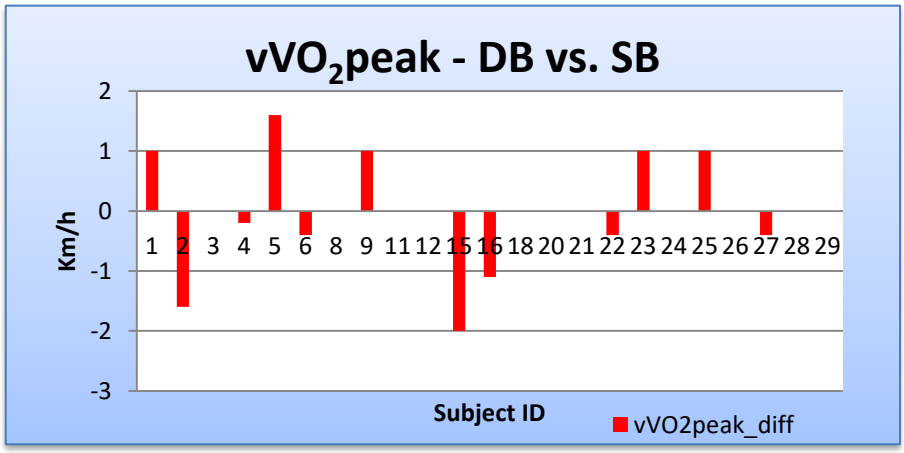


Figure 4-9 Individual response in vVO_2peak
The figure shows the difference in vVO_2peak between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. The response is quite variable with individual subjects showing, no difference, an increase or decreases in tidal volume (V_T). The magnitude of the difference is also quite varied.

4.4.8 Secondary Outcomes

Secondary analysis examined indices of cardiovascular function (HR), perceived exertion (RPE-O and RPE-R), ventilation (V_E, V_A, VCO_2, V_E/VCO_2, $P_{ET}CO_2$, and RER) and locomotion (SF and LRC), and are presented in the following sections.

4.4.8.1 HR

There was no significant difference between DB and SB trials for HR, 182 ± 10 bpm for DB versus 181 ± 9 bpm for SB (P = 0.683).; Mean diff = 0.5 bpm; [-1.8 to 2.7] 95% CI; Cohen's d = 0.05 [*trivial*]).

4.4.8.2 RPE

RPE does not meet requirements for parametry and was analysed using a Related-Samples Wilcoxon Signed Rank Test. There was no significant difference between trial in either RPE-O (P=0.664; Trivial) or RPE-R (P=1.000; Trivial).

4.4.8.3 Ventilation

There was no significant difference between DB and SB at VO_2peak in secondary measures of ventilation and effect size was trivial for V_E, V_A, VCO_2, V_E/VCO_2 and $P_{ET}CO_2$ and Small for RER. Table 4-8 and Figure 4-10 presents these results for each variable.

Table 4-8 Ventilation Parameters comparing DB vs. SB at VO2peak.

	P	SB ± SD	DB ± SD	Mean Diff	95% CI		Cohen's d	ES
V_E	0.829	131.7 ± 29.8	130.9 ± 24.6	-0.878	19.312	4.027	0.03	T
V_A	0.433	116.2 ± 24.4	113.7 ± 18.9	-2.509	15.052	3.139	0.11	T
VCO_2	0.879	4.747 ± 0.697	4.766 ± 0.587	0.019	-0.242	0.280	0.03	T
V_E/VCO_2	0.723	29 ± 4	29 ± 3	-0.1	-0.9	0.6	0.04	T
$P_{ET}CO_2$	0.439	37.9 ± 4.9	38.3 ± 4.7	0.4	-0.6	1.4	0.08	T
RER	0.267	1.05 ± 0.04	1.07 ± 0.07	0.01	-0.01	0.04	0.25	S

V_E = minute ventilation (L/min); V_A = alveolar ventilation (L/min); VCO_2 = volume of expired CO_2 (L/min); V_E/VCO_2 = ventilatory equivalent for VCO_2; $P_{ET}CO_2$ = partial pressure of end tidal CO2 (mmHg); RER = Respiratory Exchange Ratio; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); T = Trivial; S = Small; ES = Effect Size;

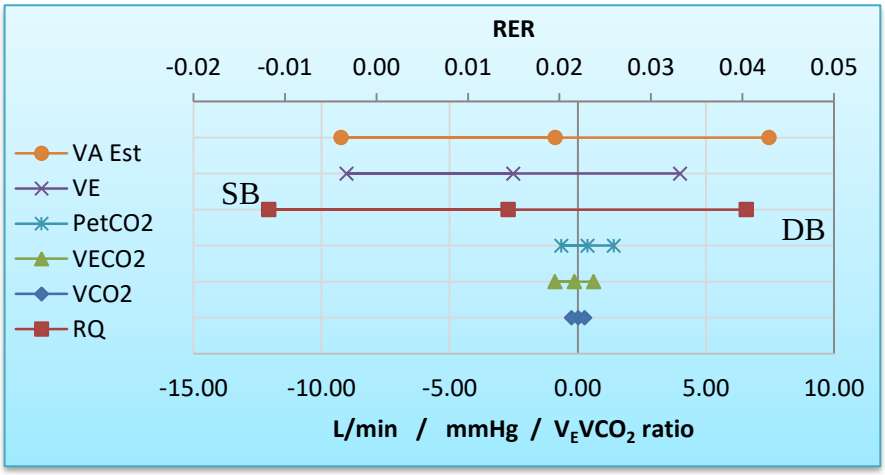


Figure 4-10 Ventilation changes comparing SB vs. DB (mean ± 95% CI)
The figure shows the mean difference with the 95% confidence interval (CI) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. There is no significant change in alveolar ventilation (V_A), minute ventilation (V_E), end-tidal CO_2 ($P_{ET}CO_2$), ventilatory efficiency ($VECO_2$), CO_2 production (VCO_2) or respiratory quotient (RQ).

4.4.8.4 Locomotor Variables

Analysis of locomotor variables shows a small but significant decrease in SF for DB when compared to SB. There was no significant difference in LRC between trials. Results are presented in Table 4-9 and Figure 4-11.

Table 4-9 Locomotor parameters (SF and LRC) comparing DB vs. SB at VO2peak

	P	SB	DB	Mean Diff	95% CI		Cohen's d	ES
SF	**0.015***	**89 ± 4**	**88 ± 5**	**-1**	**-2**	**0**	**0.30**	**S**
LRC	0.782	1.8 ± 0.4	1.8 ± 0.4	0.0	-0.1	0.1	0.04	T

*SF = Stride Frequency; LRC = Locomotor Respiratory Coupling; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD);*P<0.05; T = Trivial; S = Small; ES = Effect Size;*

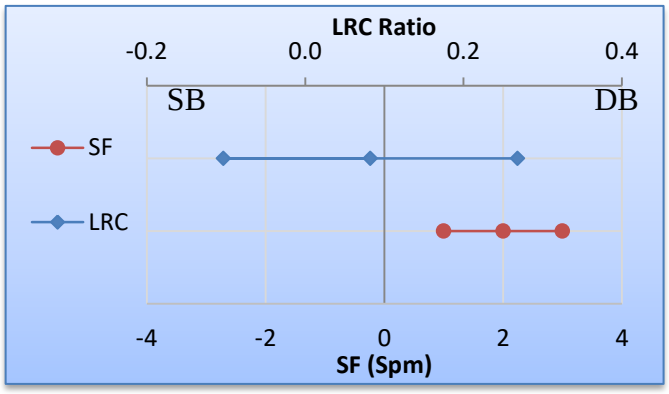


Figure 4-11 Locomotor changes comparing SB vs. DB (mean ± 95% CI)
The figure shows the mean difference with the 95% confidence interval (CI) between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. There is a significant decrease in stride frequency (SF) but no significant change in locomotor respiratory coupling (LRC) with DB.

4.4.9 Analysis of outcome variables over the 6 initial stages

4.4.9.1 Ventilatory Parameters

No two-way interaction was identified when examining V_E, V_A, VO_2, RER, V_E/VCO_2 and $P_{ET}CO_2$. There was a two-way interaction between breathing and stage for VCO_2 and $P_{ET}CO_2$, which are explored individually below. There was no main effect of breathing for any variable. Results are presented in Table 4-10.

Table 4-10 Primary Outcome results for the 2-way RM ANOVA

			P	partial η^2	ES	DB	SB	P	95% CI	
V_E (STPD)	Breathing		0.261	0.057	S	76.9	74.0	0.261	-2.3	8.1
	Breathing Stage	*	0.893	0.013	T					
V_A	Breathing		0.978	0.000	T	65.3	65.4	0.978	-4.2	4.1
	Breathing Stage	*	0.939	0.008	T					
VO_2	Breathing		0.450	0.026	S	3.340	3.269	0.450	-0.119	0.260
	Breathing Stage	*	0.755	0.022	S					
VCO_2	Breathing		0.321	0.045	S					
	Breathing Stage	*	**0.048***	**0.104**	**S**					
RER	Breathing		0.115	0.109	S	0.96	0.94	0.16	-0.01	0.42
	Breathing Stage	*	0.278	0.056	S					
V_E/VCO_2	Breathing		0.602	0.013	T	26	26	0	-1	1
	Breathing Stage	*	0.198	0.070	S					
$P_{ET}CO_2$	Breathing		0.294	0.050	S					
	Breathing Stage	*	**0.032***	**0.123**	**S**					

*V_E = minute ventilation (L/min); V_A = alveolar ventilation (L/min)); VO_2 = volume of O_2 uptake (L/min); VCO_2 = volume of expired CO_2 (L/min); RER = Respiratory Exchange Ratio; V_E/VCO_2 = ventilatory equivalent for VCO_2; $P_{ET}CO_2$ = partial pressure of end tidal CO2 (mmHg); Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); ES = Effect Size; T= Trivial; S = Small; *P<0.05;*

4.4.9.1.1 VCO_2

The 2 Way RM ANOVA identified a significant but small interaction between Breathing and Stage (P = 0.048, partial η^2= 0104; Small). We examined this interaction using simple

main effects (Table 4-11, Figure 4-12(A&B) and identified no difference in VCO_2 when DB at all stages (All stages 1-6 P >0.05). When examining the DB trial over the 6 stages there was a significant increase in VCO_2 between all stages (P<0.05). The same was true for the SB trial (all P<0.05). No clear effects of this interaction are identified.

Table 4-11 VCO_2 (L/min) Data from all six initial stages comparing DB vs. SB

Stage	DB	SB	p	Mean Diff	95% CI	
1	2.148 ± 0.433	2.175 ± 0.374	0.75	-0.027	-0.200	0.147
2	2.677 ± 0.482	2.599 ± 0.419	0.39	0.077	-0.107	0.262
3	3.04 ± 0.478	2.941 ± 0.444	0.28	0.099	-0.087	0.285
4	3.29 ± 0.551	3.21 ± 0.519	0.37	0.080	-0.103	0.263
5	3.622 ± 0.630	3.46 ± 0.704	0.20	0.163	-0.094	0.420
6	4.029 ± 0.685	3.872 ± 0.696	0.16	0.156	-0.069	0.382

VCO_2 = volume of expired CO_2 (L/min); Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD);

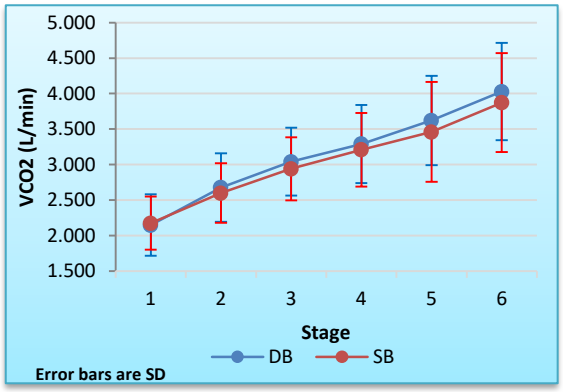
4.4.9.1.2 $P_{ET}CO_2$

The 2 Way RM ANOVA identified a significant interaction between Breathing and Stage for $P_{ET}CO_2$ (P = 0.032; partial η^2 = 0.123). We examined this interaction using simple main effects (Table 4-12, Figure 4-12(C&D) and identified a significant difference between SB and DB at stage 5 (P=0.035), but no difference in $P_{ET}CO_2$ at all other stages (P >0.05).

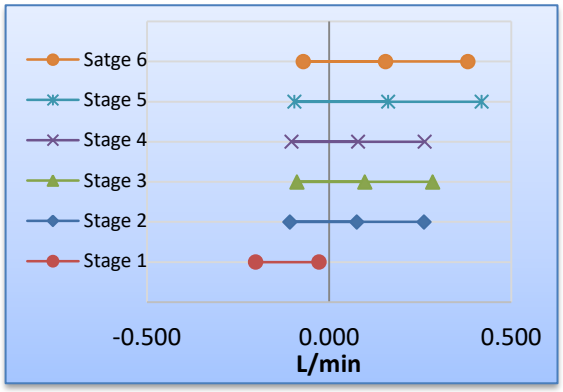
Table 4-12 $P_{ET}CO_2$ (mmHg) Data from all six initial stages comparing DB vs. SB

Stage	DB	SB	p	Mean Diff	95% CI	
1	41.7 ± 4.8	42.1 ± 3.4	.606	-0.4	-2.0	1.2
2	43.5 ± 4.7	43.1 ± 3.6	.523	0.5	-1.0	2.0
3	44.0 ± 5.2	43.3 ± 4.0	.318	0.8	-0.8	2.4
4	43.9 ± 4.9	43.3 ± 4.0	.160	1.0	-0.4	2.4
5	**43.2 ± 5.0**	**42.9 ± 4.2**	**.035**	**1.5**	**0.1**	**2.8**
6	41.8 ± 4.9	41.8 ± 4.6	.224	0.7	-0.4	1.7

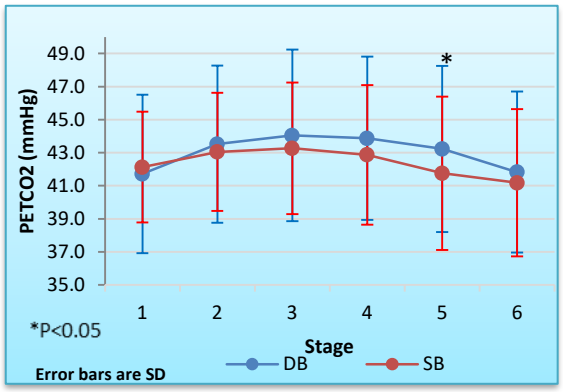
*$P_{ET}CO_2$ = partial pressure of end tidal CO2 (mmHg); Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); *P<0.05;*



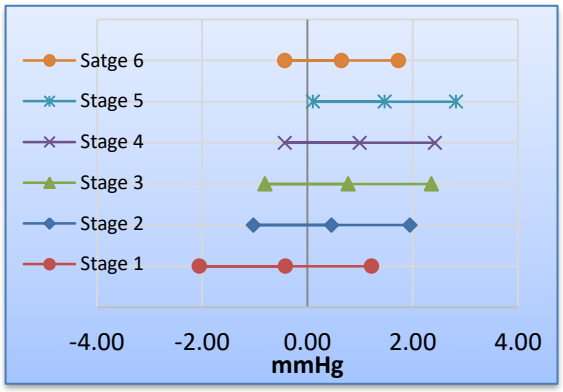
(A) VCO_2 comparing DB vs. SB over 6 stages



(B) VCO_2 – Mean ± 95% CI comparing DB vs. SB



(C) $P_{ET}CO_2$ comparing DB vs. SB over 6 stages



(D) P_{ET}CO2 Mean ± 95% CI comparing DB vs. SB

Figure 4-12 Changes in CO_2 parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 while panels on the right show the mean difference with the 95% confidence interval (CI). There is no significant difference in expired CO_2 (VCO_2) (A&B).There is a significant increase in end-tidal CO_2 ($P_{ET}CO_2$) for stage 5 only (C&D).

When examining the DB trial over the 6 stages, there was a significant increase in $P_{ET}CO_2$ between stages 1 and 2 (P=0.008), but at no other stage (all P<0.05). In the SB trial, a significant difference was observed between trials 4 and 4 (P=0.001), but not between any other stages (all P<0.05).

4.4.9.1.3 Individual responses in ventilation

Figure 4-13 presents the magnitude of change in both V_A and V_E with DB for individual subjects. There is considerable variability with some subjects increasing ventilation and others decreasing ventilation with DB. The magnitude of change is also individual.

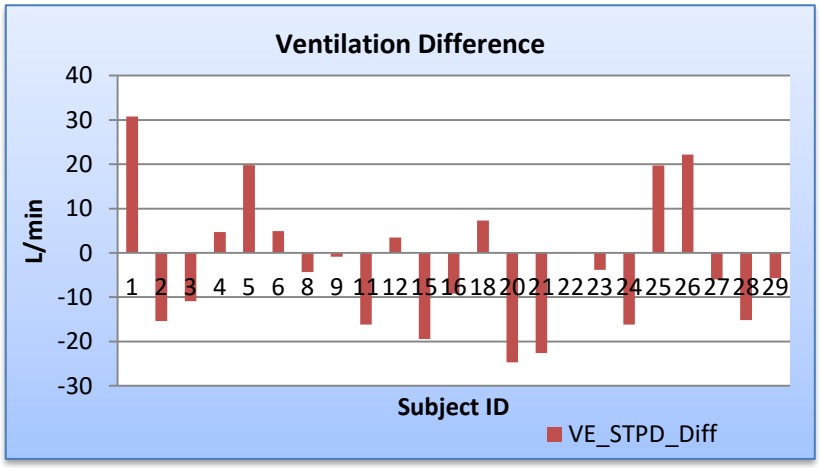


Figure 4-13 Individual response in ventilation parameters
The figure shows the difference in vVO_2peak between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 at VO_2peak. The response is quite variable with individual subjects showing, no difference, an increase or decreases in either minute ventilation (V_E) or alveolar ventilation (V_A). The magnitude of the difference is also quite varied.

4.4.9.2 Non ventilatory parameters

With respect to the non-ventilatory parameters over the 6 stages, there was no main effect or interactions for HR, RPE-O or RPE-R. However, an interaction between breathing and stage for LRC was observed (P=0.011; partial η2 = 0.173; ES = Medium), and there was a main effect for SF. Table 4-13 presents a summary of the results and the interaction and main effect are explored below.

Table 4-13 Non-Ventilatory parameter results for the 2-Way RM ANOVA

		P	partial η^2	ES
HR	Breathing	0.929	0.000	Trivial
	Breathing * Stage	0.623	0.021	Small
RPE-O	Breathing	0.085	0.129	Small
	Breathing * Stage	0.239	0.061	Small
RPE-R	Breathing	0.452	0.026	Small
	Breathing * Stage	0.370	0.044	Small
SF	Breathing	**0.004****	0.342	Large
	Breathing * Stage	0.225	0.073	Small
LRC	Breathing	**0.000*****	0.515	Large
	Breathing * Stage	**0.011***	0.173	Medium

*HR = Heart Rate (BPM); RPE-O = overall rating of perceived exertion (Borg); RPE-R = respiratory rating of perceived exertion (Borg CR-10); SF = Stride Frequency (strides per minute); LRC = Locomotor Respiratory Coupling; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); ES = Effect Size; *P<0.05;*P<0.05; **P<0.01; ***P<0.001;*

4.4.9.3 Locomotor parameters

4.4.9.3.1 SF

There was a main effect of breathing pattern on SF, where DB had a lower SF than SB (P = 0.004; partial η^2= 0.34; ES = Large). Comparisons and 95% CI of the difference are presented in Table 4-14, Table 4-15 and Figure 4-14(A&B).

Table 4-14 Primary Outcome differences for each stage

		P	partial η^2	ES	DB	SB	P	95% CI	
SF	Breathing	**0.004***	0.342	L	83	84	0.004	-2	-1

*SF = Stride Frequency (strides per minute); CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); ES = Effect Size; *P<0.01;*

Table 4-15 SF Data from all six initial stages comparing DB vs. SB

	SF (Spm)	
Stage	DB	SB
1	79 ± 6	82 ± 4
2	81 ± 4	83 ± 6
3	83 ± 4	84 ± 5
4	83 ± 4	85 ± 5
5	84 ± 4	86 ± 4

SF = Stride Frequency (strides per minute); DB = Deep Breathing; SB = Spontaneous Breathing; Spm = Strides per minute; Results are mean ± Standard Deviation (SD);

4.4.9.3.2 LRC

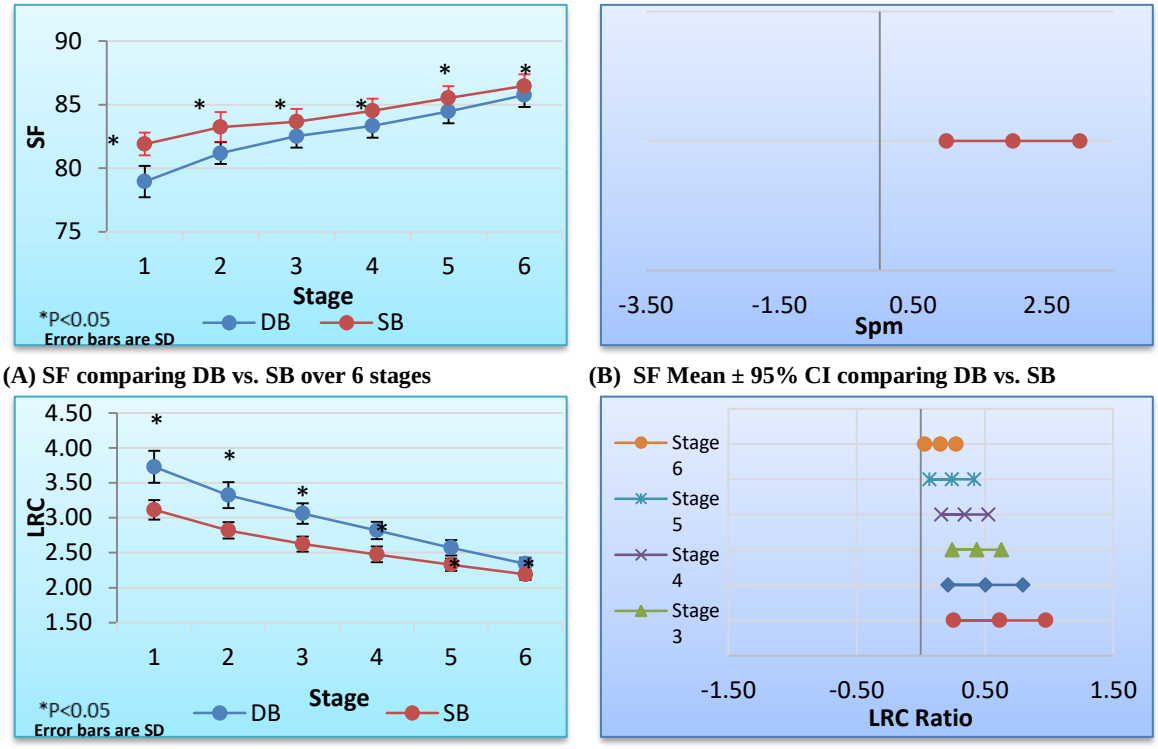
The 2 Way RM ANOVA identified a significant interaction between Breathing and Stage for LRC (P ≤ 0.001; partial η^2= 0.173: ES = Medium).

Table 4-16 LRC Data from all six initial stages comparing DB vs. SB

Stage	DB	SB	P	Mean Diff	95% CI	
1	3.7 ± 1.1	3.1 ± 0.7	**0.002***	0.6	0.3	1.0
2	3.3 ± 0.8	2.8 ± 0.5	**0.002***	0.5	0.2	0.8
3	3.1 ± 0.7	2.6 ± 0.5	**0.000***	0.4	0.2	0.6
4	2.8 ± 0.6	2.5 ± 0.5	**0.001***	0.3	0.2	0.5
5	2.6 ± 0.5	2.3 ± 0.4	**0.009***	0.2	0.1	0.4
6	2.3 ± 0.4	2.2 ± 0.4	**0.017****	0.2	0.0	0.3

*LRC = Locomotor Respiratory Coupling; Mean Diff = mean difference between DB and SB; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); *P<0.01; ** P<0.05;*

We examined this interaction using simple main effects (Table 4-16, Figure 4-14(C&D)) and identified a significant difference between SB and DB at all stages (P<0.05). When examining the DB trial over the 6 stages, there was a significant decrease in LRC between stages 4-5 (P = 0.014), and 5-6 (P = 0.003), but at no other stage (all P<0.05). In the SB trial, a significant decrease was observed between all stages, except 3-4 (P = 0.122) and 4-5 (P=0.617).



(A) SF comparing DB vs. SB over 6 stages

(B) SF Mean ± 95% CI comparing DB vs. SB

(C) LRC comparing DB vs. SB over 6 stages

(D) LRC Mean ± 95% CI comparing DB vs. SB

Figure 4-14 Locomotor parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2 while panels on the right show the mean difference with the 95% confidence interval (CI). There is a significant decrease in stride frequency (SF) (A&B) with DB. There was a significant increase in locomotor respiratory coupling (LRC) with DB and an interaction between breathing and stage (C&D). (*P<0.05)

4.4.10 Efficiency of movement (O_C and E_C)

With respect to parameters of efficiency over the 6 stages, there was no main effect or interactions for O_C or E_C. Table 4-17 presents a summary of the results for the 2-Way RM ANOVA.

Table 4-17 Efficiency results for the 2-Way RM ANOVA (O_C and E_C)

			P	partial η^2	ES	DB	SB	P	95% CI	
O_C	Breathing		0.388	0.034	S	221.6	216.3	0.388	5.3	6.0
	Breathing * Stage	*	0.832	0.016	T					
E_C	Breathing		0.401	0.032	S	0.88	0.86	0.401	-0.03	0.07
	Breathing * Stage	*	0.767	0.020	T					

O_C = O_2 Cost; E_C = Energy Cost; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD);

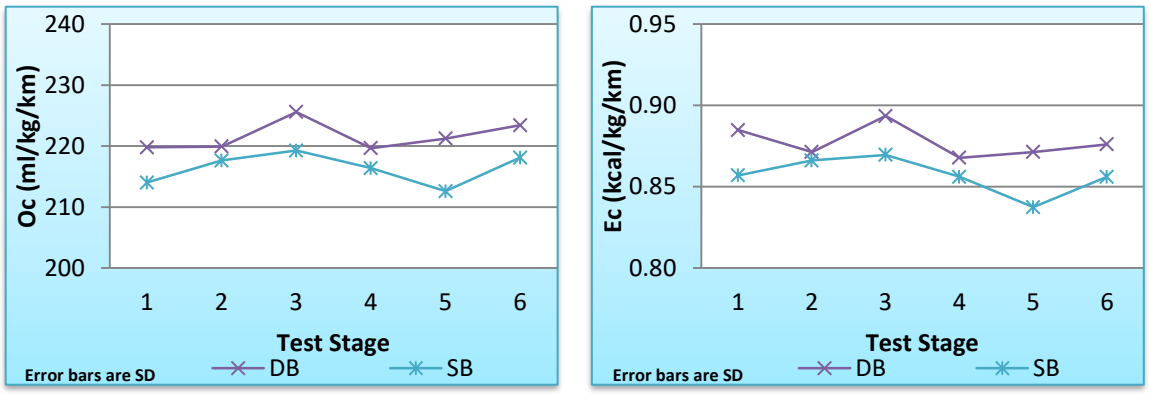
Table 4-18 presents the results for both O_C and E_C for stages 1 to 6.

Table 4-18 Oc & Ec data from all six initial stages comparing DB vs. SB

	O_C (ml/kg/km)		E_C (kcal/kg/km)	
Stage	DB	SB	DB	SB
1	219.8 ± 36	219.9 ± 32.7	0.88 ± 0.15	0.87 ± 0.13
2	225.6 ± 30.1	219.7 ± 26.9	0.89 ± 0.12	0.87 ± 0.1
3	221.2 ± 27.1	223.4 ± 19.6	0.87 ± 0.11	0.88 ± 0.07
4	214.0 ± 27.5	217.6 ± 27.4	0.86 ± 0.11	0.87 ± 0.11
5	219.3 ± 19	216.4 ± 19.5	0.87 ± 0.08	0.86 ± 0.08
6	212.6 ± 27.2	218.1 ± 21.4	0.84 ± 0.11	0.86 ± 0.08

O_C = O_2 Cost; E_C = Energy Cost; CI = Confidence Interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD);

Stage by stage comparisons have been presented below in Figure 4-15.



(A) O_C comparing DB vs. SB over 6 stages **(B) Ec comparing DB vs. SB over 6 stages**

Figure 4-15 Efficiency of movement parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) vVO_2peak trials of Study 2. There is no significant difference in the oxygen cost (O_C) (A) or in the energy cost (E_C) (B).

4.5 Discussion

Determining the effect of a specific intervention on sports performance is often problematic due to issues with reliability and validity of tests used (W. G. Hopkins & Hewson, 2001).While vVO_2peak provides one of the most valid and reliable indirect methods of evaluating endurance running performance (Machado et al., 2013b, McLaughlin et al., 2010, Midgley et al., 2007c) the results from our study show the inabilty to change breathing pattern at higher intensities and limit our ability to assess DB on performance. Endurance performance often takes place in the heavy intensity domain or the lower end of the severe domain for prolonged periods where pattern change is possible without neural control overpowering volitional control as a result of heavy metabolic and affective afferent input (Amann, 2011a, Babb et al., 2010, Bussotti et al., 2008). During intense exercise metobosensitive afferent neural pathways inhibiting central motor drive (CMD), lead to fatigue and ultimately reduced exercise intensity and/or exercise termination (Markus Amann, 2011). While a definitive picture of respiratory limitation is lacking, various possible mechanisms have been suggested including gas exchange inefficiency, metaboreflex mediated blood flow limitation and expiratory flow limitation (Wagner, 1992). One possible avenue for DB to improve performance would be alterations in gas exchange that might mitigate these limiting effects.

Initial analysis focused on establishing whether DB was achievable throughout the incremental protocol. Increases in exercise intensity results in ever greater afferent feedback from mechanical and metabolic sensors driving exercise hyperpnea. In the face of this powerful drive to breathe if deep breathing cannot be maintained, we are not able to compare the effects of DB to SB. At peak exercise there was no significant difference with DB in any indices of breathing pattern or gas exchange parameters. Furthermore, analysis revealed that DB was only achieved in stages 1 to 6 with maximum subject numbers for analysis. SB resulted in a typical exercise hyperpnoeic response, however the tachypnoeic response resulting in a decrease in V_T at maximal exercise was not observed.

As no difference was observed in breathing pattern in stages above Stage 7 or when analysis was conducted on peak data, DB was not achieved at peak exercise. There was no difference in vVO_2peak or VO_2peak between SB and DB indicating that DB didn't improve running performance but neither did it have any negative effect. The one notable effect was a small but significant decrease in SF with DB supporting the bidirectional nature of the locomotor respiratory coupling (LRC) (Rabler and Kohl, 2000). There was however no change in LRC, suggesting a recoupling and supported by our previous work

(see Study 1). As we will discuss next, significant differences were observed during submaximal stages, however, these didn't influence performance at peak.

We then looked at sub-maximal stages which traverse the moderate, heavy and severe intensity domains in which endurance performance takes place for changes in efficiency and gas exchange that we speculate might influence performance. In Stages 1 to 6, a significant increase in V_T was observed between Stages 2-3 and 5-6 with DB while in contrast with DB V_T remained constant and was significantly higher than SB for the first six stages, the difference decreasing with successive stages (~0.7 to 0.3 L). RR was significantly lower with DB (~4-5 Bpm) for Stages 1-6 and increased with each stage for both conditions. Despite this there was no significant difference in V_E (though higher in DB) or V_A between conditions. This was unexpected as it was anticipated that the deep breathing pattern, with less dead space ventilation, would improve V_A. Other differences were observed in temporal indices as expected with an increase in V_T and a decrease in RR, T_I, T_E and T_{TOT} were significantly increased with DB, the difference decreasing with increasing intensity to non-significance by Stage 8. Duty cycle (T_I/T_{TOT}) was significantly higher in Stages 1 and 3 only, as a result of a greater increase in T_I versus T_E, contrary to the advantageous relative increase in T_E observed by Lucia et. al (2001) in elite cyclists. Duty cycle is usually tightly maintained by powerful central drive and a relative increase in T_E would reduce flow rates. It remains unclear if this opposite change has any negative effect when it occurs in conjunction with DB when the T_E is increased.

Despite differences in breathing pattern, significant differences were only seen in VCO_2 and $P_{ET}CO_2$ in the first six stages. There was a significant interaction between breathing pattern and stage. While there was no significant difference between DB and SB, the mean difference increased with each stage, VCO_2 increasing to a greater degree with DB. There was also a significant interaction between breathing and stage for $P_{ET}CO_2$, reversing from slightly lower with DB in Stage 1 to becoming increasingly greater in each subsequent stage and reaching significance in Stage 5 only before decreasing in Stage 6.

Alterations in respiration may also have indirect effects on exercise performance by producing changes in locomotion which may affect mechanical efficiency of movement. Autonomic control is responsible for locomotor-respiratory coupling (LRC), a relationship between breathing rates and step frequency and describes the synchronisation of the two cyclical processes of locomotion and respiration (Bramble and Carrier, 1983; Lafortuna et al., 1996; Siegmund et al., 1999; Rabler and Kohl, 2000). Therefore we also looked for changes in locomotor efficiency, SF and LRC. There was a significant difference in SF and

LRC across all stages. SF was significantly lower with DB for Stages 1-6 but there was no interaction between breathing and stage. Interestingly, there was an interaction between breathing pattern and stage for LRC, the LRC ratio was greater with DB, however decreased with each stage. There was only a significant difference between Stages 4-5 and 5-6 with DB but difference between all six stages with SB. This provides evidence that DB may alter the change in LRC that occurs with increasing intensity with the coupling preserved at lower intensities, however, whether this is advantageous or not is not discernible from our study. No significant difference was found in locomotor efficiency across all six stages. However, oxygen cost and energy cost tended to slightly higher with DB contrary to our previous findings of improved efficiency with DB during heavy intensity exercise. Again, we speculated an interaction between altered gas exchange kinetics and stage length that may be the source of this discrepancy. Further work looking at the changes in kinetics is warranted to fully elucidate the effects of DB.

Despite evidence that athletes have improved interoception and a more accurate anticipatory response to perceived breathlessness than non-athletes, Faull (2016) suggested that some athletes may be more susceptible to breathing anxiety, either due to lower respiratory muscle endurance or higher ventilatory sensitivity, and at increased risk for performance limitation and would possibly benefit from psychophysiological interventions. We assessed both RPE-O and RPE-R to assess overall and respiratory effort and found no positive or negative effect of DB. We did not measure anxiety, either trait or perceived, which could have acted as a confounding factor. It is well established that dyspnoea and anticipatory or perceived breathlessness have a negative effect on performance and the acute change to respiratory pattern could interact with individual sensitivity.

4.6 Conclusion

In male endurance athletes DB doesn't improve vVO_2peak but neither does it impair it, however, there is considerable heterogeneity in the individual response. Research identifies the individuality of breathing pattern and the exercise hyperpnoiec response, and the diverse physiological and psychological inputs that influence it. It is therefore suspected that the ability to breathe more deeply during exercise, the primary way in how it is achieved (abdominal vs. thoracic), and therefore the potential to improve may also be highly individual. It is clearly necessary to investigate this individual pattern in future research to identify links between pattern type and the scope to increase depth of breath and exercise performance.

It was observed in this study that intensity plays a significant role in the ability to breathe deeply, at higher intensities in a three-minute incremental running test DB was not achieved and as such our tests failed to compare SB and DB except at the lower submaximal intensities. DB results in no significant improvement in running performance in well-trained endurance runners, contrary to our hypothesis. Neither did we find any difference in submaximal or maximal gas exchange parameters that might suggest improved gas exchange or ventilatory efficiency. No changes were found in ratings of perceived exertion. While we found changes in SF and LRC at both submaximal and at peak (SF only) these changes didn't impact performance. While vVO_2peak is one of the best predictors of endurance running performance, the respiratory demands of incremental exercise differ from the constant work load of endurance running performance and so therefore may not be the most suitable test to evaluate DB. Other constant work load test such as time trials and time to exhaustion tests, despite their questionable reliability, might be more suitable, mimicking the respiratory demands of performance more accurately.

4.7 Limitations and future work

Due to technical limitations in our laboratory we were unable to measure certain parameters. We were unable to ascertain or categorise subject breathing patterns as abdominal or thoracic either under SB or DB conditions. This might be particularly useful in identifying individual differences.

We were unable to measure blood gases, either arterial or capillary, and instead relied on the indirect measure of P_aCO_2 from $P_{ET}CO_2$. In light of the change in $P_{ET}CO_2$ with deep breathing, arterial blood gases would have allowed confirmation of this as it is possible the altered breathing pattern and change measured at the mouth may not accurately reflect arterial partial pressure changes. While clearly identified in the literature as a limiting factor, expiratory flow limitation was not measured. While vVO_2peak is a validated and reliable measure of endurance running performance, the incremental nature of the test and imposed exercise hyperpnea, to levels above performance intensity, rendered DB impossible and is therefore a severe limitation in the study.

We did not assess the effect of DB at peak or approaching peak intensity as it was not possible for subjects to increase depth at these intensities.

Finally, we did not assess psychological parameters which may have influenced SB pattern and the ability to deep breath, such as trait anxiety or respiratory anxiety. Also because subjects must consciously increase the depth of breath it constitutes a dual-task

performance which has be shown to affect physiological parameters and is therefore a possible confounding factor. The RPE measures used may not have been sensitive enough to measure subtle changes in perceived exertion.

It was not possible to categorise subject breathing patterns as abdominal or thoracic either under SB or DB conditions. This would be particularly useful in identifying individual breathing patterns and the effect of this aspect of breathing pattern on the ability to breathe deeply and performance measures.

We were unable to measure blood gases, either arterial or capillary, and instead relied on the indirect measure of P_aCO_2 from $P_{ET}CO_2$. In light of the change in $P_{ET}CO_2$ with deep breathing arterial blood gases would have allowed confirmation of this as it is possible the altered breathing pattern and change measured at the mouth may not accurately reflect arterial partial pressure changes. While clearly identified in the literature as a limiting factor, expiratory flow limitation was not measured. Another consideration is the static ordering of trials with the DSB trial following the SB trial however the order was chosen to prevent subjects spontaneous pattern from being influenced by DB. We did not assess psychological parameters which may have influenced SB pattern and the ability to deep breath, such as trait anxiety or respiratory anxiety. The RPE measures used may not have been sensitive enough to measure subtle changes in perceived exertion.

Future work to measure these parameters would deepen our understanding considerably to probe the individual basis for breathing and those susceptible to limitation and possibly more amenable to improvement from DB. The acute change in breathing pattern was necessitated to assess the effect of pattern alone as it is established that the respiratory muscles when trained can influence performance and also the neuro-respiratory centres can undergo both modulatory and plastic responses that may benefit performance. However such an acute change could trigger individual respiratory anxiety levels to increase, interfering with perceived exertion and/or ability to deep breathe. A chronic intervention which trained subjects to deep breathe in isolation or in conjunction with respiratory muscle training needs to be explored. The ability to DB and to maintain the pattern may be limited by respiratory muscle endurance and respiratory control circuits. Increased endurance may facilitate DB and chronic training may allow for modulatory and plastic responses to occur which might allow DB to fully benefit subjects.

5. Study 3

To measure the effect of Deep Breathing on high intensity interval exercise performance in male endurance athletes'

5.1 Introduction

High intensity interval exercise has existed is various forms for over 100 years and is considered one of the most effective training methods to promote greater physiological adaptations (Tschakert and Hofmann, 2013, Buchheit and Laursen, 2013b, Billat, 2001a, Billat, 2001b, Buchheit and Laursen, 2013a). While athletes regularly engage in heavy and severe intensity exercise, it is less common in the non-athletic population. High intensity interval exercise (HIIE) has moved from the almost exclusive realm of the trained athlete to the domain of the recreational athlete, physically active adolescents and adults, and clinical populations, although with some safety concerns (Costigan et al., 2015, Gosselin et al., 2012, De Nardi et al., 2018). A possible barrier to exercise adherence and in particularly HIIE is perceived exertion and the resultant affective feelings of motivation, mood state, arousal and exercise enjoyment, which may be mitigated by choosing intervals not exceeding 60 seconds with a 1:1 work-to-rest ratio may minimize negative feelings and promote better continued adherence while maintaining a high cardio-metabolic stimulus (Kilpatrick et al., 2015, Seiler and Sjursen, 2004).

HIIE protocols are diverse, with variations in intensity and duration of both the work and recovery phases with at least nine variable that can be manipulated (Buchheit and Laursen, 2013b). The intensity is above maximum lactate steady state MLLSS and critical speed (CS) and below the maximum exercise intensity, the maximum sprint speed (MSS) which characterises the severe and extreme intensity domains (Jones et al., 2011). HIIE generally elicits a RPE ≥ 6 on the Borg CR-10 scale and ≥ 15 on the standard Borg scale. HIEE has been shown to be a powerful stimulus for improving endurance performance using a different signalling pathway to high volume, lower intensity training to signal oxidative fibres, promoting various physiological adaptations including, muscle remodelling, mitochondrial biogenesis, increased fat oxidative capacity and increased GLUT4, MCT 1and 4, and glycogen content (Laursen, 2010, Gibala, 2009, Kohn et al., 2011, Perry et al., 2008).

HIIE can be categorised into very short (3 to 7 second) repeated sprint training (RST) in the 120-170% vVO_2max intensity range, short all-out effort(~30sec) sprint interval training (SIT) in the >160%vVO_2max to MSS range, short (<60sec) intervals (HIT short) in the 100-120% vVO_2max range, and long (>60sec) intervals (HIT long) in the 90-100% vVO_2max range (> MLSS/CS) (Buchheit and Laursen, 2013b).Depending on the method adopted the physiological stimulus challenges cardiopulmonary, metabolic, neuromuscular, autonomic nervous system (ANS) and musculoskeletal systems to different extents and therefore elicits different physiological adaptions. Importantly, the differing physiological stresses of such protocols can result in different stresses on the ANS which play a vital role in both adaptation and recovery (Seiler et al., 2007). There is no consensus on the dose-response to HIIE and not enough evidence to link specific protocols with specific adaptation, however, some global recommendations can be made. Metabolic stress will vary, placing higher or lower emphasis on oxidative and glycolytic fibres and energy pathways and HIEE protocols can be programmed based on specific loading of ATP/PCr, glycolytic and oxidative pathways (Buchheit and Laursen, 2013b, Tschakert and Hofmann, 2013).

Cardiopulmonary stress can be assessed by quantifying the time spent at or near VO_2peak and it has been suggested that time accumulated at high intensities (>T90%) are necessary to attain maximal or near-maximal cardiac output and optimally signal cardiac and oxidative muscle fiber adaptation (Buchheit and Laursen, 2013b). HIIE protocols are commonly used to elicit VO_2max and suggested to be an optimal training stimulus for improving VO_2max and have been assessed by calculating the accumulated time above 90% VO_2max (T90%), 95%VO_2max (T95%) and 100%VO_2max (T100%) (Midgley et al., 2007c, Turnes et al., 2016, Buchheit and Laursen, 2013b). Also recovery intensity will affect overall T90% with recovery intensities of 50% vVO_2max show to elicit greater T90% and greater total VO_2 than either 67% or 84% (Thevenet et al., 2008). The quantification is based on the valid measurement of VO_2max and Kuiper et al. has shown no significant difference between protocols ranging from 1 to 6 minutes in stage length but a significant difference can occur with different time-averaging calculation of VO_2max. Indeed, the large inter-breath variability with breath-by-breath gas exchange analysis can results in an inverse relationship between VO_2max and rolling average duration, higher estimations of VO_2max with smaller rolling averages (Hill et al., 2003). VO_2max has been reported to have day-to-day variation as high as 5.6% and therefore the less stringent T95% recommended for intermittent running (Midgley et al., 2007c). These metrics has however been shown to have poor reproducibility with high coefficients of variation (CV),

T90% (CV = 24.5%) and T95% (CV= 34.5%) (Midgley et al., 2007b). In the absence of a more reliable and valid measure these metrics were used to tentatively assess HIIE performance in Study 3. They were extended to include time above 80% VO_2max (T80%) and 85%VO_2max (T85%) and used in conjunction with number of completed repetitions to assess overall HIIE performance. While we could find no CV's for lower metrics such as time above 80% (T80%) and time above 85% (T80%) it was decided to include these metrics post hoc as many subjects failed to record any time above T90% and T95%.

One protocol used by endurance runners is 60sec intervals with a 1:1 work rest ratio, completing approximately 24 work intervals (Seiler and Sjursen, 2004, Kilpatrick et al., 2015). This specific session when self-paced resulted in a lower work VO_2peak, higher VO_2 in recovery but a similar average over the entire session when to 2, 4 and 6 minute protocols. When the interval session is broken into sets the T@VO_2peak is reduced. Intervals not exceeding 60 seconds may minimize negative feelings and promote better continued adherence and exercise enjoyment (Kilpatrick et al., 2015, Seiler and Sjursen, 2004).

Research has sought to train and optimise various physiological systems to improve cardiovascular, metabolic and neuromuscular function to elicit improvements in performance (Joyner and Coyle, 2007, Midgley et al., 2007a). The respiratory system been only been recently added as a possible avenue of investigation and it's role is far more complex and pervasive than previously thought (McKenzie, 2012). The traditional consensus that the respiratory system did not limit performance has changed amidst growing evidence that it may limit exercise performance, especially in elite athletes, but more importantly, that it may be trained to improve performance (Dempsey et al., 2008a, Dempsey et al., 2008b, Dempsey et al., 2006, Romer and Dempsey, 2006, Tong et al., 2008, Tong et al., 2004, Guenette and Sheel, 2007b, Amann, 2011b, Gigliotti et al., 2006, McKenzie, 2012). Also, new developments in our understanding of fatigue mechanisms and the role peripheral metabolite accumulation, which the respiratory system may influence also highlights the need to take a deeper look at this often overlooked physiological system (Amann, 2011a).

The role of the respiratory system in many of the physiological and psychological factors contributing to the development of fatigue and ultimately to the limitation of exercise performance has been largely ignored. In light of a growing body of research challenging this view, emerging evidence suggests the respiratory system may fail to meet the demands imposed during exercise and therefore play a role in the development of fatigue, both

locally and systematically, limiting exercise performance (Dempsey et al., 2008a, Dempsey et al., 2008b, McKenzie, 2012, Romer and Polkey, 2008, Amann, 2011b, Harms et al., 1997), especially in athletes (Guenette and Sheel, 2007a, Romer and Polkey, 2008) of which female athletes may be at even greater risk (Dominelli et al., 2011, Guenette et al., 2009, Guenette et al., 2007, Hopkins et al., 1998, Harms and Rosenkranz, 2008b). Ventilation patterns may have a considerable influence on ventilatory efficiency, the effectiveness of gas exchange, the development of respiratory limitation, the mechanics and therefore the metabolic cost of breathing, and also the mechanics of locomotion (Aliverti, 2008b, Koulouris and Hardavella, 2011, Dominelli et al., 2011). The control of respiration is still debated and not fully understood (Haouzi, 2012). This changing research landscape recognises the respiratory system as a contributing factor to fatigue, posing a limiting factor to exercise performance. It is proposed that an altered breathing pattern, specifically a deep breathing' pattern (DB) may improve athletic performance via moderation or amelioration of respiratory limiting factors.

The autonomic ventilatory pattern adopted during exercise may fail to meet the imposed functional demands placed upon the respiratory system leading to respiratory limitation of exercise. This may be due to impaired ventilation perfusion matching (VA/Q), impaired gas exchange, expiratory flow limitation (EFL) and/or exercise induced arterial hypoxemia (EIAH) (Wagner, 1992, McClaran et al., 1999, Dempsey et al., 2008b, Dempsey et al., 2008a). Evidence of respiratory system plasticity has shown that respiratory adaptations via respiratory muscle training (RMT) can enhance exercise performance in running (Tong et al., 2008), cycling (Gething, 2004, Johnson et al., 2007) and rowing performance (Volianitis et al., 2000). Currently there is a lack of research in the area of ventilator pattern manipulation and how this may effect respiratory limitation, respiratory efficiency, acid-base balance and how these may influence the development of fatigue and/or exercise performance.

The ventilatory pattern adopted is consequential to the combined and proportional influences of afferent inputs on autonomic control centres. There is considerable heterogeneity in respiratory pattern both at rest and during exercise demonstrating that ventilatory requirements may be satisfied in varying ways and indeed some elite athletes exhibit unique ventilatory patterns during exercise (Benchetrit, 2000, Lucia et al., 2001). It is important to remember that while respiration is under autonomic control it can be consciously overridden allowing ventilatory pattern to be altered.

Ventilation pattern determines the mechanics and therefore the metabolic cost of breathing and also influences ventilatory efficiency. Its effects have implications both on the effectiveness in maintaining O_2, CO_2, and pH homeostasis and also the incurred cost in attempting to achieve this. An inefficient, sub-optimal ventilator pattern may result in an increased cost of breathing and the development of respiratory muscle fatigue which has been shown to result in competition for O_2 with locomotor muscles, negatively affecting exercise performance (Dempsey et al., 2006, Romer and Dempsey, 2006). In addition to these specific respiratory effects ventilation pattern may also affect the mechanics of locomotion (Baskurt, 2012, Bernasconi and Kohl, 1993, Rabler and Kohl, 2000) and therefore mechanical efficiency of exercise.

It has been proposed that an individual critical limit of peripheral metabolic disturbances exists which cannot be voluntarily surpassed (Amann, 2011a). During intense exercise when metabolic disruption is detected and relayed to the central nervous system (CNS) via metobosensitive afferent neural pathways inhibiting central motor drive (CMD), this threshold is reached leading to fatigue and ultimately reduced exercise intensity and/or exercise termination (Amann, 2011a). These afferent pathways also provide feedback which regulate ventilatory and cardiovascular responses to exercise (Amann, 2011a). Hydrogen ions (H^+) are one such metabolite which disrupt acid-base balance, and intramuscular levels of H^+ are related to metabolic CO_2 accumulation. Therefore the elimination of CO_2 plays a key role in the regulation and maintenance of 'acid-base' balance (Robergs et al., 2005). A ventilatory pattern that may be more effective and efficient in CO_2 elimination may decrease this afferent stimulus which may be responsible for driving an inefficient pattern. This may reduce the metabolic cost and/or delay acid-base disturbance, delay fatigue onset and improve exercise performance.

Deep breathing has been shown to affect the autonomic nervous system (ANS) causing sympathovagal modulation, affecting heart rate (HR) via heart rate variability (HRV), a phenomena called respiratory sinus arrhythmia (RSA), blood pressure, arterial oxgen saturation (S_aO_2), muscle sympathetic nerve activity (MSNA) in skeletal muscle and the peripheral microcirculation (Krasnikov et al., 2013, Yasuma and Hayano, 2004, Seals et al., 1990). It is suggested that deep breathing may improve gas exchange, ventilatory efficiency, reduce the cost of breathing and/or improving mechanical efficiency. This has the potential to decrease VO_2, delay acid-base disturbance and ultimately improve exercise performance.

The conscious overriding of autonomic respiratory control altering ventilatory pattern may positively affect exercise performance if it can reduce the effects of these exercise limiting factors without incurring other deleterious side effects such as exacerbating disruptions to homeostatic balances of blood gases and pH that occur in exercise and possibly exacerbating fatigue and reducing exercise tolerance.

5.2 Aims and Objectives

- **Hypothesis**

  'Deep breathing during High Intensity Interval exercise can increase the number of repetitions completed'

- ***Aims***

  - *Evaluate the effect of deep breathing on high intensity interval exercise (HIIE) performance*
  - *To explore what factors may underlie this effect*

- **Objectives**

  - *Measure and compare the number of HIIE repetitions completed under deep and spontaneous breathing conditions*
  - *Measure and compare metabolic and gas exchange parameters during HIIE under deep and spontaneous breathing*
  - *Measure overall and respiratory RPE during HIIE under two breathing conditions, spontaneous and deep*
  - *Measure stride frequency and calculate locomotor respiratory coupling during HIIE under two breathing conditions, spontaneous and deep*

5.3 Methods

5.3.1 Subjects

Twenty three male subjects were recruited, nineteen completing all testing. Four subjects failed to complete testing, two subjects were injured outside of testing and two subjects could not complete testing within the allotted time. Subjects were aged 37.6 ± 4.6 years and were healthy, well-trained (VO_2peak = 62.6 ± 7.1 ml/kg/min; vVO_2peak = 17.7 ± 1.2km/h) male endurance athletes (runners and triathletes) regularly engaged in running

training (>5days per week). All participants had normal respiratory function at rest (FVC = 125 ± 16 % predicted. Subjects were recruited by emailing athletics and triathlon clubs advertising for research volunteers. Healthy male endurance athletes between the 18 and 45 years of age were included if they were engaged in regular training (>5days per week) that included high intensity training, were injury free for the previous month and had normal respiratory function assessed via spirometry (forced expiratory volume in 1s (FEV_1 > 90% predicted, ratio between FEV_1 and forced vital capacity (FEV1/FVC) >70%). Subjects were excluded if they had any respiratory disease or musculoskeletal injury that could interfere with exercise testing. The study was approved by the Ethics Committee in Dublin City University.

5.3.2 **Study Design**

Figure 5-1 gives an overview of the structure of the study outlining the test sequence. The study used a within-subject, random crossover design, participants randomly allocated to either complete the *deep breathing* (DB) or *spontaneous breathing* (SB) *high intensity interval exercise* (HIIE) bout first.

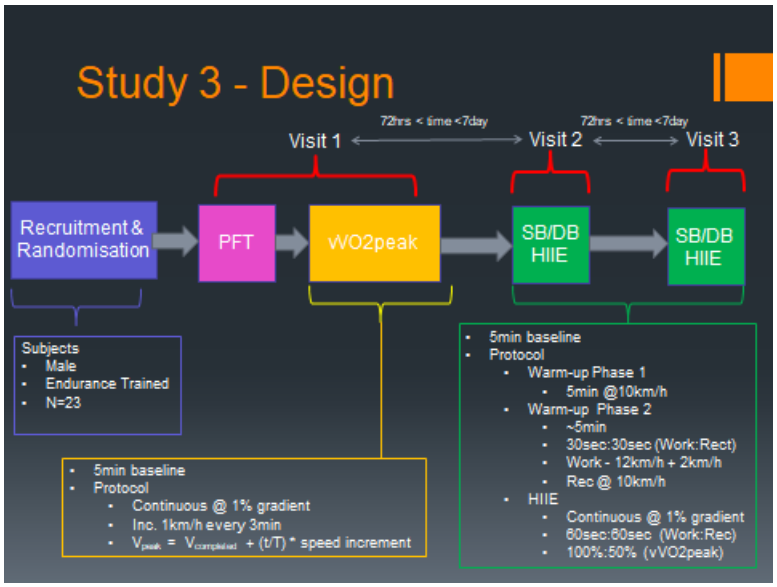


Figure 5-1 Study 3 – Design Overview

All the subjects visited DCU Human Performance Laboratory in the School of Health and Human Performance for testing on three separate occasions, separated by at least 72 hours and no more than two weeks between the two HIIE bouts. . Following recruitment, on the initial visit subjects completed a medical health screening form and informed consent before a pulmonary function was tested to screen for respiratory disease. This was

followed by a maximal incremental treadmill running test. On the two subsequent visits subjects completed the two HIIE tests to exhaustion. Subjects were instructed to follow a similar diet and training regimen before all tests. This meant being well hydrated and abstaining from food and caffeine for 4 hours prior to testing, and performing no hard training in the 48 hours prior to testing. Every attempt was made to perform tests at a similar time and on the similar training day to control for diurnal changes, training fatigue and metabolic changes. Subjects had height, weight and resting heart rate measured prior to each test.

5.3.3 **Pulmonary Function Testing**

Spirometry was carried out with an automated pulmonary function testing system (Viasys Vmax Encore 299; SensorMedics, Yorba Linda, CA) via indirect calorimetry using open-circuit spirometry. Tests were carried out in the standing posture following recommended procedures. Pulmonary function measurements were expressed as absolute values and percentages of predicted values.

5.3.4 **Cardiopulmonary exercise testing**

Laboratory environment conditions were controlled at 18 degrees centigrade. Exercise testing was carried out on a COSMED T170 motorised treadmill. Pulmonary data collected breath-by-breath throughout all exercise tests with the ViasysVmax Encore 299 metabolic cart. The system was calibration in accordance with manufacturer guidelines prior to each test. Heart rate data was recorded with the Polar V800 heart rate monitor (Polar Electro, Inc., Kempele, Finland) using a 1 second sample rate and later downloaded for analysis. Perceived exertion was assessed on two scales, the standard Borg Rating of Perceived Exertion (RPE) scale for overall exertion which we shall refer to as RPE-O and the Borg CR-10 dyspnea scale. RPE was recorded at the end of each stage and on test termination, if during a stage. Maximal incremental exercise testing and high intensity interval exercise tests were terminated by exhaustion. Subjects were verbally encouraged through the final stages to give maximum effort.

5.3.5 **vVO_2peak protocol**

The protocol adopted was a three minute incremental vVO_2peak protocol with incline set to 1% (Jones and Doust, 1996). The protocol was programmed into the COSMED T170 and starting at 10km/h, speed alone was automatically increased every three minutes by 1km/h. The velocity at VO_2peak (vVO_2peak) was calculated using the Vpeak-P = Vpeak-C + (t/T) * speed increment Equation 1 (Kuipers et al., 2003)

$$V_{peak} = V_{completed} + (t/T) * \text{speed increment;} \qquad \text{Equation 5}$$

In which V_{peak} is the maximal running speed, $V_{completed}$ is the speed of the last completed stage, t is the number of secons completed in the final stage and T is the number of seconds per stage (i.e. 180sec). The V_{peak} was used to set the intensity of the HIIE bouts.

5.3.6 High intensity interval exercise (HIIE)

The high intensity interval protocol consisted of warm-up phase followed by the main HIIE interval phase to exhaustion. Both phases were separately programmed into the treadmill and when the warm-up phase was completed the interval phase began within 30 seconds. Pulmonary data was assessed breath-by-breath throughout the exercise.

5.3.6.1 Warm-up phase

The warm-up consisted of 5 minutes constant speed running at 10 km/h followed by a set of 30 second work intervals interspersed by 30 seconds recovery at 10km/h. The work intervals began at 12 km/h and increased by 2 km/h on each subsequent interval. The number of work intervals was pre-determined based on V_{peak}, if a 2 km/h increment was equal to it this was the last interval, if not then the next increment above it.

5.3.6.2 HIIE phase

The intervals consisted of a 60 second recovery phase followed by a 60 second work phase. The work speed was set at 100% V_{peak} and the recovery speed was set at 50% V_{peak}. Subjects completed as many intervals as possible and were encouraged to give maximum effort. HR, RPE-O and RPE-R were recorded after every work interval. After work repetition 5 (Rep_10), 10 (Rep_10) and the final repetition (Last Rep) subjects were instructed to briefly step off the treadmill with feet either side of the belt and a capillary blood sample was taken from the left earlobe and analysed immediately with the *Lactate Pro 2* (Arkray KDK, Japan) handheld analyser to measure blood lactate BLa^-.

5.3.6.2.1 Blood Lactate Sampling

A Lactate Pro™ (Arkray KDK, Japan) handheld blood lactate analyser was used to measure blood lactate (BLa^-) from capillary blood sampled from the left earlobe, the device requiring 5µL of blood sampled via capillary action with a coded reagent strip, calculating BLa^- amperometricallly (Tanner et al., 2010). The device was calibrated following manufacturer guidelines with a manufacturer supplied calibration strip and check strip specific to each box of sampling strips prior to each test. Results took 60 s to analyse.

Following calibration, test strips were only removed from foil wrapping ~60 seconds prior to each sample and inserted into the analyser. The puncture site was cleaned with an alcohol pad prior to the initial puncture and prior to each sample, dried with sterile gauze, the first drop of blood obtained by applying pressure to the surrounding site was wiped away to remove any contaminants (alcohol or perspiration) and the second drop of blood touched to the tip of the test strip.

5.3.6.2.2 Deep Breathing Instructions

The deep breathing (DB) pattern was self-paced by the subjects. Instructions were verbally conveyed to the subjects, in which they were instructed to breathe as deeply and slowly as the felt comfortable doing. During the test tidal volume (V_T) was monitored to ascertain if they maintained a DB pattern based on the V_T from the SB trial. Periodically during the test the instructions were repeated if the V_T was observed to be decreasing significantly to SB levels.

5.3.6.2.3 Stride Frequency (SF) measurement

SF was measured using the Polar Stride Sensor in conjunction with the Polar V800 heart rate monitor and recorded before the end of each stage manually to corroborated data. SF Strides were measured by counting the number of times the right foot contacted the treadmill for 30 seconds and doubled.

5.3.6.2.4 Locomotor Respiratory Coupling (LRC) calculation

LRC was calculated by dividing SF taken for the last minute (19-20min) by the respiratory rate (RR). The manual counting of SF imposes limitation in the accuracy of assessment and does not allow for phase coupling to be assessed however it is a method that allows global assessment of the coordination (McDermott et al., 2003) and has been used previously (Bramble and Carrier, 1983).

5.3.7 Data processing and analysis

All manually recorded data was entered into a Micrsoft Excel speeadsheet. HR data was uploaded from the V800 watch to Polar Flow software and downloaded in excel format. Due to limitations with the Vmax software version all data from the system was only downloadable as text files. Data for each test was in 10 second samples and exported in four separate files in order to get all parameters for analysis and spirometry data was exported separately. These files were parsed using a Python script to remove text headers and combine all the data for all subjects into Excel format. The Excel files were then imported to Microsoft Access for analysis and formatting for SPSS. Microsoft Access SQL

queries were written to further analyse the data. One minute rolling averages were calculated on all data fields and combined with manually recorded data and spirometry data. Data was then exported in Excel format for import into SPSS for statistical analysis.

5.3.8 Statistical Analysis

SPSS was used for statistical analysis. All normally distributed quantitative variables were analysed using paired t-test. RPE data did not meet requirements for parametry and was analysed using a Related-Samples Wilcoxen Signed Rank Test. Results are represented as mean with standard deviation (mean ± SD), mean difference (Mean Diff.) with 95% confidence intervals (95% CI) and Cohen's d and effect size (based on Cohen's d) are also shown where appropriate. The level of significance was set at $p < 0.05$.

Analysis was conducted on each stage to investigate if a significantly different breathing pattern had been achieved with DB. Primary indices V_T, RR and VE, and secondary indices T_{tot}, T_i, T_e and T_i/T_{tot} were analysed at 3 time-points, after work repetitions 5 (Rep 5), 10 (Rep 10) and after the last work repetition (Last Rep). Data was also averaged across all work intervals (Work_Avg) and all recovery intervals (Rec_Avg).

5.4 Results

5.4.1 Subjects

Table 5-1 presents summary physical and physiological characteristics of the participants in the study. All 19 participants were healthy, well-trained (VO_2peak = 62.6 ± 7.1 ml/kg/min; vVO_2peak = 17.7 ± 1.2km/h) male endurance athletes (runners and triathletes) regularly engaged in running training (>5days per week). All participants had normal respiratory function at rest (FVC (%Ref) = 125 ± 16; FEV1/FVC = 79 ± 6).

Table 5-1 Participant characteristics

	Mean ± SD
Age (years)	37.6 ± 4.6
Height (cm)	178 ± 6
Weight (kg)	75.6 ± 7.5
BMI (kg/m^2)	24 ± 1.7
FVC (L)	6.204 ± 0.878
FVC (% Reference)	125 ± 16
FEV1 (L/min)	4.921 ± 0.891

FEV1(% Reference)	122 ± 21
FEV1/FVC	79 ± 6
vVO_2Peak (km/h)	17.7 ± 1.2
VO_2peak (ml/kg/min)	62.6 ± 7.1
VO_2peak (L/min)	4.724 ± 0.605

Summary of key subject characteristics (N=23). BMI = Body Mass Index; FVC = Forced Vital Capacity; %Ref = % of reference value based on normative data; FEV1 = Forced Expiratory Volume in 1 second; SB = Spontaneous Breathing; DB = Deep Breathing; VO2peak = peak oxygen uptake; vVO2peak = velocity at peak oxygen uptake; SD = Standard Deviation;

5.4.2 Breathing Pattern Analysis

5.4.2.1 Primary Indices

5.4.2.1.1 V_T

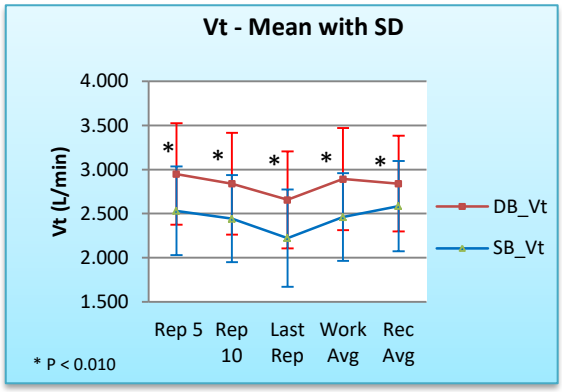
Table 5-2 V_T Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.000****	2.949 ± 0.576	2.532 ± 0.502	0.416	0.255	0.578	0.77	M
Rep 10	**0.000****	2.840 ± 0.577	2.443 ± 0.494	0.397	0.246	0.548	0.74	M
Last Rep	**0.001***	2.656 ± 0.551	2.223 ± 0.553	0.433	0.199	0.667	0.79	M
Work_Avg	**0.000****	2.892 ± 0.579	2.462 ± 0.495	0.430	0.286	0.575	0.80	M
Rec_Avg	**0.000****	2.84 ± 0.543	2.586 ± 0.511	0.254	0.143	0.365	0.48	S

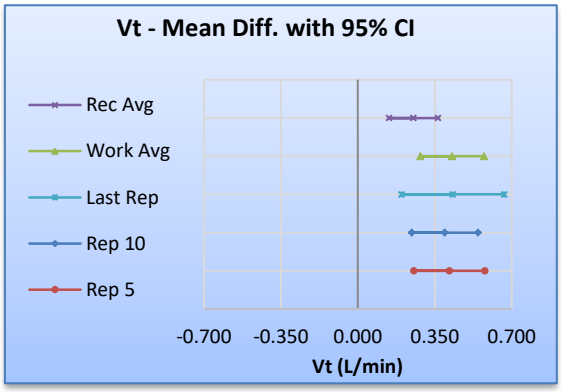
*Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); *P<0.01; **P<0.001; CI = Confidence Interval; ES = Effect Size; S = Small; M = Medium;*

When examining V_T there was a significant increase in V_T for Rep 5, Rep 10, Work_Avg and Rec_Avg. Effect size was medium for all parameters except Rec_Avg for which it was small. Table 5-2 summarises the results.

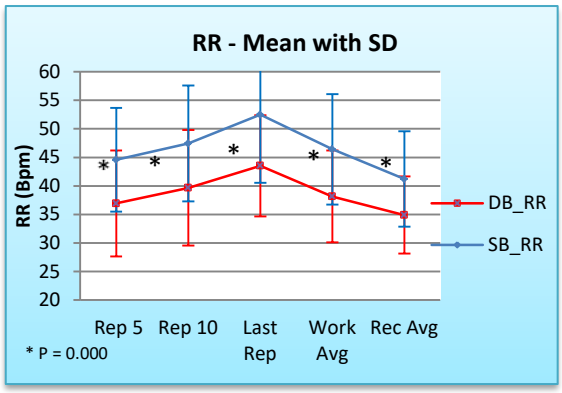
Figure 5-2(A) presents mean data for V_T with SD for DB and SB trials. V_T is significantly higher in the DB trial in all measures ($P < 0.01$). Figure 5-2(B) presents the mean difference with 95% CI.



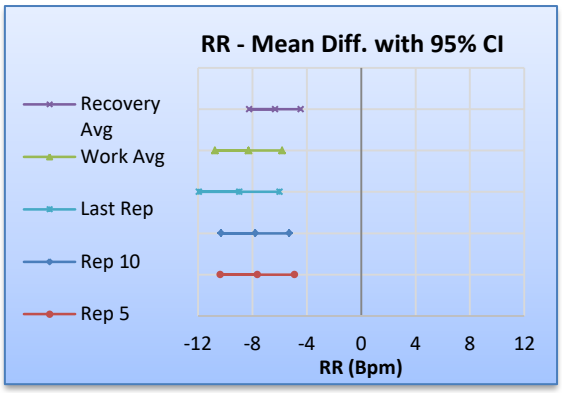
(A) V_T - DB vs. S



(B) V_T - DB vs.SB - Mean diff. (95% CI)



(C) RR – DB versus SB



(D) RR - DB vs.SB - Mean diff. (95% CI)

Figure 5-2 Breathing pattern differences
Panels on the left show the mean response observed for the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± standard deviation (Error Bars). There is a significant increase in tidal volume (V_T) and a significant decrease in respiratory rate (RR) with DB. (*P<0.001)

5.4.3 Secondary indices of Breathing Pattern

5.4.3.1 T_{tot}

There was a significant increase in T_{tot} with DB for Rep 5, Rep 10, Work_Avg and Rec_Avg. Effect size was large for all parameters except Rec_Avg for which it was medium. Table 5-3 summarises the results.

Table 5-3 T_{tot} Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.000***	1.79 ± 0.42	1.43 ± 0.32	0.36	0.24	0.49	0.97	L
Rep 10	**0.000***	1.65 ± 0.37	1.34 ± 0.32	0.31	0.21	0.40	0.89	L
Last Rep	**0.000***	1.48 ± 0.3	1.23 ± 0.31	0.25	0.16	0.33	0.82	L
Work_Avg	**0.000***	1.76 ± 0.45	1.37 ± 0.32	0.38	0.23	0.54	0.98	L
Rec_Avg	**0.001****	1.88 ± 0.5	1.57 ± 0.34	0.31	0.14	0.47	0.71	M

T_{tot} = total breath time; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB =

*Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; S = Small; M = Medium; L = Large; *P<0.001; **P<0.01;*

Figure 5-3(A) presents the results for DB and SB conditions. Figure 5-3(B) shows the mean difference between DB and SB conditions.

5.4.3.2 T_i

There was a significant increase in T_i with DB for Rep 5, Rep 10, Last Rep, Work_Avg and Rec_Avg. Effect size was large for Rep 5 and Rep 10 and medium for Last Rep, Work_Avg and Rec_Avg. Table 5-4 summarises the results.

Table 5-4 Ti Results

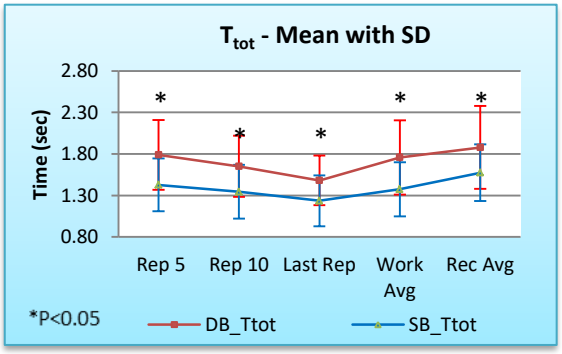
	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.000***	0.85 ± 0.21	0.68 ± 0.16	0.17	0.09	0.24	0.89	L
Rep 10	**0.000***	0.79 ± 0.2	0.64 ± 0.17	0.15	0.09	0.21	0.82	L
Last Rep	**0.000***	0.72 ± 0.16	0.59 ± 0.15	0.13	0.07	0.18	0.79	M
Work_Avg	**0.001****	0.81 ± 0.18	0.66 ± 0.18	0.14	0.07	0.21	0.79	M
Rec_Avg	**0.000***	0.85 ± 0.19	0.73 ± 0.18	0.12	0.07	0.17	0.66	M

*T_i = inspiratory time; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.001; **P<0.01; ES = Effect Size; M = Medium; L = Large;*

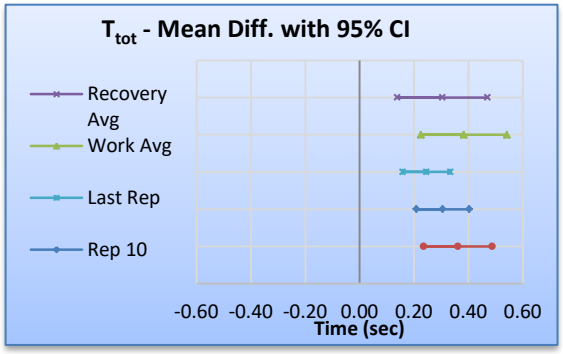
Figure 5-3(C) presents the results for DB and SB conditions. Figure 5-3(D) shows the mean difference between DB and SB conditions.

5.4.3.3 T_e

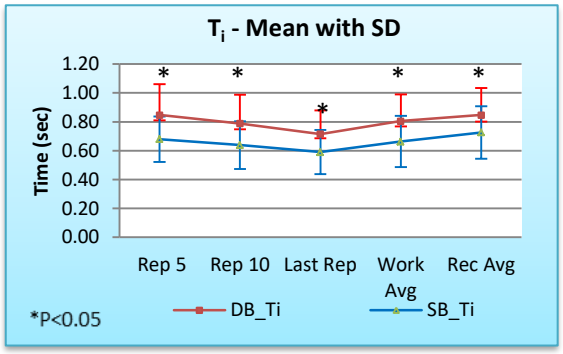
There was a significant increase in T_e with DB for Rep 5, Rep 10, Last Rep, Work_Avg and Rec_Avg. Effect size was large for Rep 5, Rep 10 and Work_Avg and medium for Last Rep and Rec_Avg. Table 5-5 summarises the results.



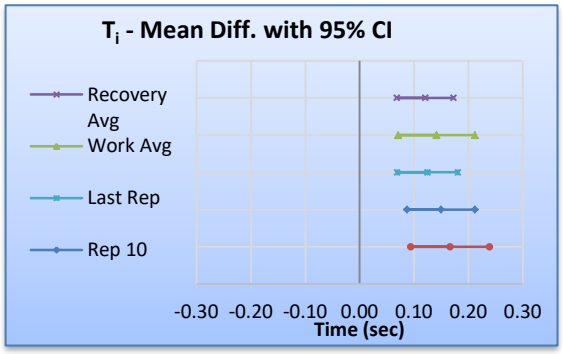
(A) T_{tot} - DB vs. SB



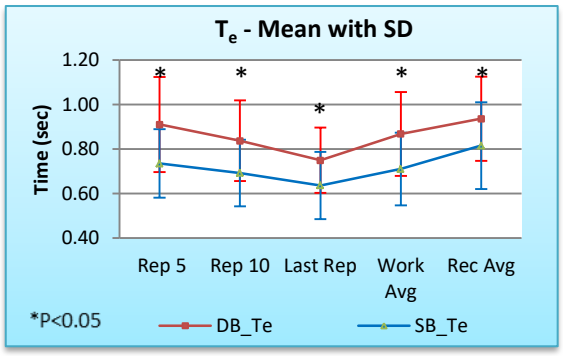
(B) T_{tot} - DB vs.SB - Mean diff. (95% CI)



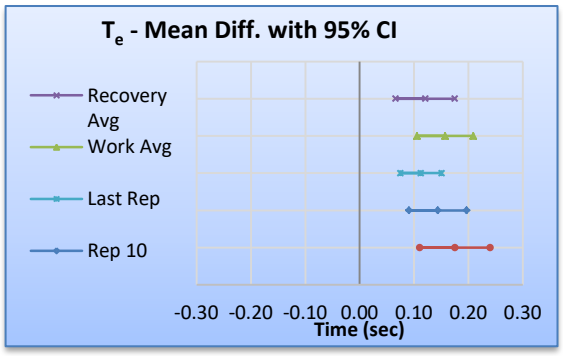
(C) T_i - DB vs. SB



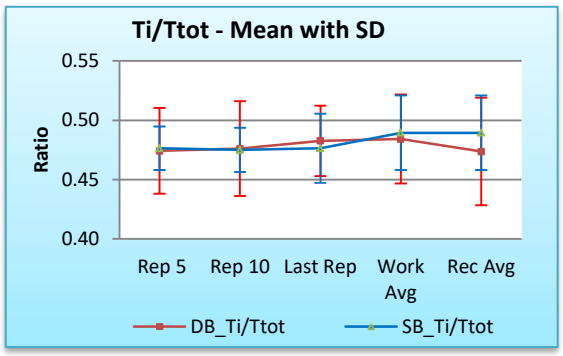
(D) T_i - DB vs.SB - Mean diff. (95% CI))



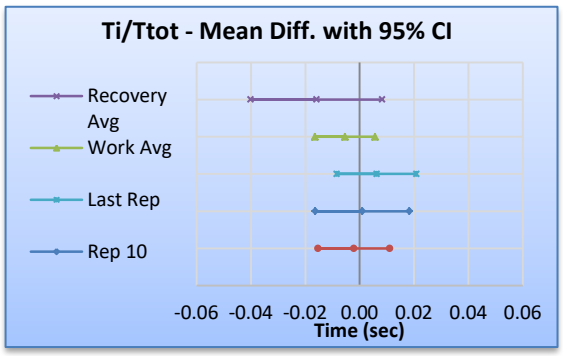
(E) T_e - DB vs. SB



(F) T_e - DB vs.SB - Mean diff. (95% CI)



(G) T_i/T_{tot} – DB vs. SB



(H) T_i/T_{tot} - DB vs.SB - Mean diff. (95% CI

Figure 5-3 Temporal Indices of breathing pattern
Panels on the left show the mean response observed for the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI)). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); Panel A and B present the mean difference showing a significant increase in total breath time (T_{tot}), C and D inspiratory time (T_i), E and F expiratory time (T_e) while G and H show no significant increase in duty cycle (T_i/T_{tot}) across all measures. (*P<0.05)

Table 5-5 T_e Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.000***	0.91 ± 0.21	0.74 ± 0.15	0.18	0.11	0.24	0.94	L
Rep 10	**0.000***	0.84 ± 0.18	0.69 ± 0.15	0.14	0.09	0.20	0.87	L
Last Rep	**0.000***	0.75 ± 0.15	0.64 ± 0.15	0.11	0.08	0.15	0.76	M
Work_Avg	**0.000***	0.87 ± 0.19	0.71 ± 0.16	0.16	0.11	0.21	0.90	L
Rec_Avg	**0.000***	0.94 ± 0.19	0.82 ± 0.2	0.12	0.11	0.24	0.63	M

*T_e = expiratory time; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.001; ES = Effect Size; S = Small; M = Medium; L = Large;*

Figure 5-3(E) presents the results for DB and SB conditions. Figure 5-3(F) shows the mean difference between DB and SB conditions.

5.4.3.4 T_i/T_{tot}

There was no significant difference in T_i/T_{tot} with DB and effect sizes were small or trivial. Table 5-6 summarises results.

Table 5-6 T_i/T_{tot} Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.742	0.47 ± 0.04	0.48 ± 0.02	0.00	-0.02	0.01	0.07	T
Rep 10	0.894	0.48 ± 0.04	0.48 ± 0.02	0.00	-0.02	0.02	0.04	T
Last Rep	0.377	0.48 ± 0.03	0.48 ± 0.03	0.01	-0.01	0.02	0.21	S
Work_Avg	0.331	0.48 ± 0.04	0.49 ± 0.03	-0.01	-0.02	0.01	0.15	T
Rec_Avg	0.187	0.47 ± 0.05	0.49 ± 0.03	-0.02	-0.04	0.01	0.40	S

T_i/T_{tot} = duty cycle (ratio of inspiratory time to total breath time); Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial; S = Small;

Figure 5-2(G) presents the results for DB and SB conditions. Figure 5-3(H) shows the mean difference between DB and SB conditions.

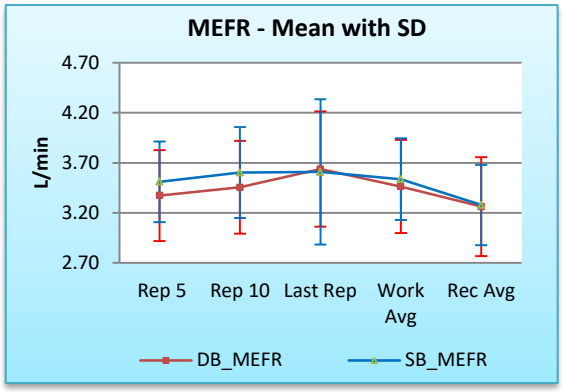
5.4.3.5 MEFR

There was no significant difference in MEFR with DB and effect sizes were small or trivial. Table 5-7 summarises the results.

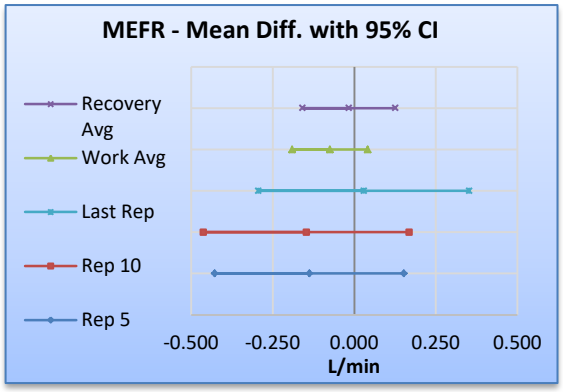
Table 5-7 MEFR Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.334	3.374 ± 0.454	3.511 ± 0.403	-0.137	-0.426	0.153	0.32	S
Rep 10	0.339	3.455 ± 0.464	3.602 ± 0.454	-0.146	-0.462	0.169	0.32	S
Last Rep	0.847	3.638 ± 0.576	3.608 ± 0.726	0.030	-0.293	0.353	0.05	T
Work_Avg	0.197	3.463 ± 0.466	3.537 ± 0.409	-0.074	-0.189	0.042	0.17	T
Rec_Avg	0.818	3.263 ± 0.494	3.279 ± 0.401	-0.016	-0.158	0.126	0.04	T

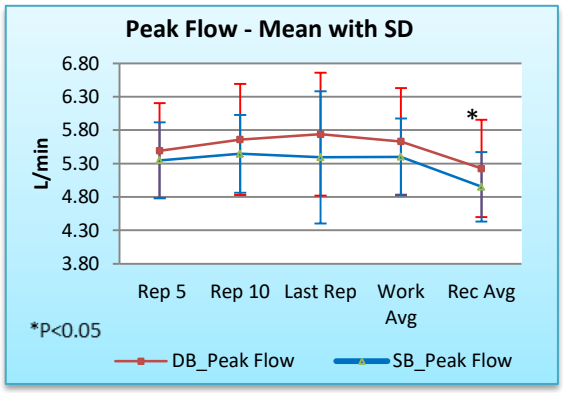
MEFR = maximum Expiratory Flow Rate; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial; S = Small;



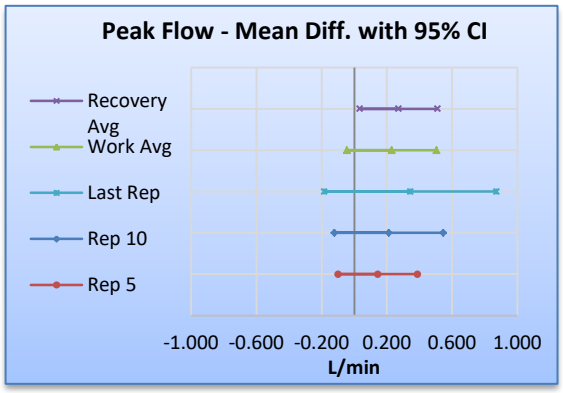
(A) MEFR – DB vs. SB



(B) MEFR - DB vs.SB - Mean diff. (95% CI)



(C) Peak Flow – DB s. SB



(D) Peak Flow - DB vs.SB - Mean diff. (95% CI)

Figure 5-4 Change in flow rate parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Panel A and B shows no significant difference in maximum expiratory flow rate (MEFR) and panel C and D show a significant increase in peak flow for recovery average only. (*P<0.05)

Figure 5-4(A) presents the results for DB and SB conditions. Figure 5-2(B) shows the mean difference between DB and SB conditions.

5.4.3.6 Peak Flow

There was significant increase in Peak Flow with DB for Rec_Avg only with a small effect size. There was a significant increase with DB for Rep 5, Rep 10, Last Rep and Work_Avg with small effect sizes. Table 5-8 summarises the results.

Table 5-8 Peak Flow Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.226	5.493 ± 0.710	5.347 ± 0.568	0.145	-0.098	0.389	0.23	S
Rep 10	0.195	5.660 ± 0.830	5.446 ± 0.582	0.214	-0.120	0.548	0.30	S
Last Rep	0.185	5.739 ± 0.919	5.394 ± 0.989	0.346	-0.182	0.873	0.36	S
Work_Avg	0.093	5.632 ± 0.799	5.400 ± 0.573	0.232	-0.043	0.506	0.33	S
Rec_Avg	**0.026***	5.226 ± 0.727	4.953 ± 0.519	0.274	0.036	0.511	0.43	S

*Peak Flow = highest flow rate; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.05; ES = Effect Size; S = Small;*

Figure 5-4 (C) presents the results for DB and SB conditions. Figure 5-4(D) presents the mean difference between DB and SB conditions.

5.4.4 Outcome Measures

The primary outcome measures were the number of repetitions completed, time accumulated above 80% (T80%), 85% (T85%), 90% (T90%) and 95% VO_2peak (T95%), overall rating or perceived exertion (RPE-O) and respiratory rating of perceived exertion (RPE-R). Secondary measures of overall performance were analysed after Work Rep 5, Work Rep 10 and, also averaged data from all Work intervals and averaged data from all Recovery intervals.

5.4.5 End of test Performance Measures

5.4.5.1 Number of Reps

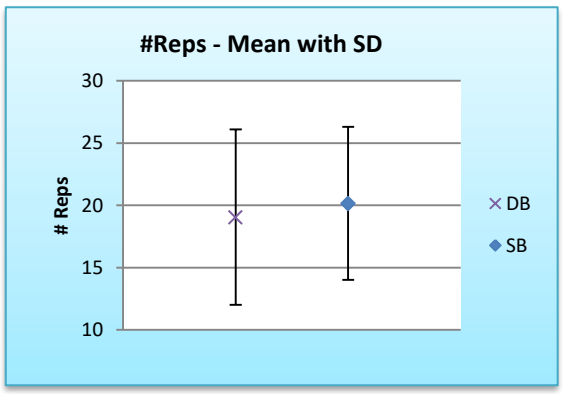
Deep breathing has no significant difference on the number of completed intervals or time accumulated above 95% (T95%), 90% (T90%), 85% (T85%),80% (T80%), VO_2peak. Effect sizes were trivial. Table 5-9 summarises the results.

Table 5-9- Performance measures – DB vs. SB

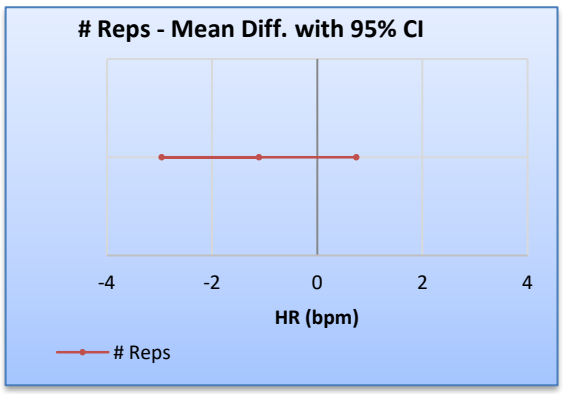
	N	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
# Reps	19	0.226	19 ± 7	20 ± 6	-1	-3	1	0.17	T
T95%	8	0.364	670 ± 563 sec	568 ± 643 sec	103	-147	352	0.17	T
T90%	13	0.454	663 ± 562 sec	601 ± 606 sec	62	-113	238	0.11	T
T85%	18	0.437	842 ± 563 sec	769 ± 590 sec	73	-120	266	0.13	T
T80%	18	0.866	1219 ± 562 sec	1201 ± 529 sec	18	-202	237	0.03	T

 # Reps = number of completed work intervals; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; #Reps = Number of Repetitions completed; T95% = Time above 95% VO_2peak; T90% = Time above 90% VO_2peak; T85% = Time above 85% VO_2peak; T80% = Time above 80% VO_2peak; ES = Effect Size; T = Trivial;

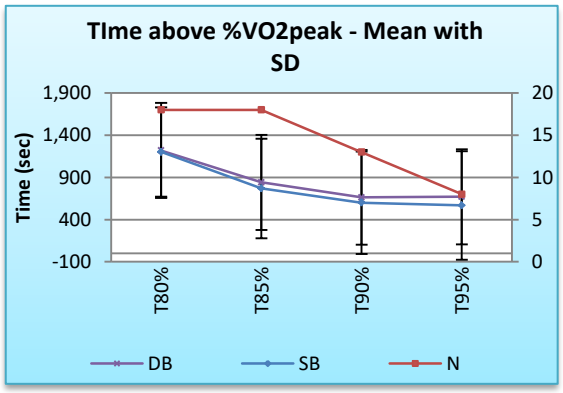
Figure 5-5(A) presents the results for DB and SB conditions for number of completed intervals and Figure 5-5(B) shows the mean difference between DB and SB conditions.



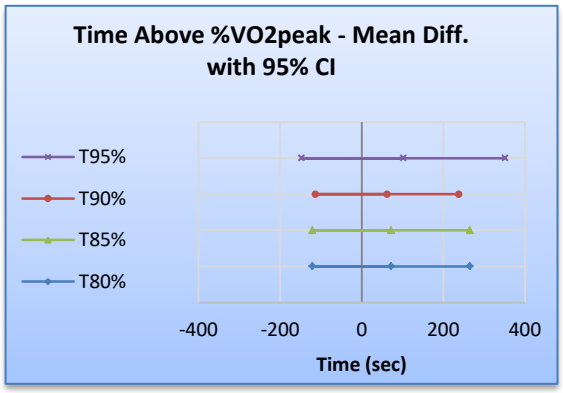
(A) # Repetitions – DB versus SB



(B) # Repetitions - DB vs.SB - Mean diff. (95% CI)



(C) Time above %VO2peak – DB vs. SB



(D) Time above %VO2peak - Mean diff. (95% CI)

Figure 5-5 End of test performance measures
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI). Panel A and B shows no significant difference in the number of repetition completed (# Reps) or the time accumulated above 80%, 85%, 90% and 100% of VO_2peak; T95% = Time above 95% VO_2peak; T90% = Time above 90% VO_2peak; T85% = Time above 85% VO_2peak; T80% = Time above 80% VO_2peak.

Figure 5-5(C) presents the results for DB and SB conditions for time above % VO_2peak and Figure 5-5(D) shows the mean difference between DB and SB conditions.

5.4.5.2 Individual Response

Figure 5-6 presents the difference in the number of work intervals (# Reps) completed between the DB and the SB HIIE sessions. There is considerable individual variation both in direction and magnitude of change. There is considerable individual variation both in direction and magnitude of change. The difference in # Reps ranged from an increase of 5 intervals to a decrease of 9 intervals with DB.

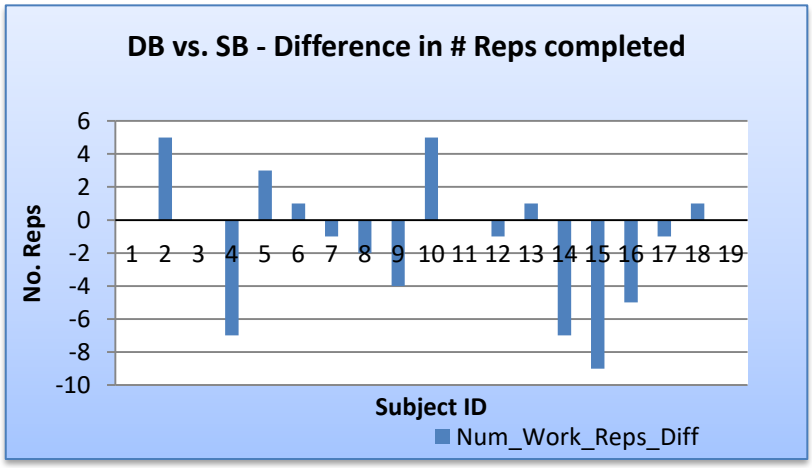


Figure 5-6 Difference in number of repetitions completed per subject
The figure shows the difference in number of work intervals completed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 for individual subjects. The response is quite variable with individual subjects showing, no difference. The magnitude of the difference is also quite varied.

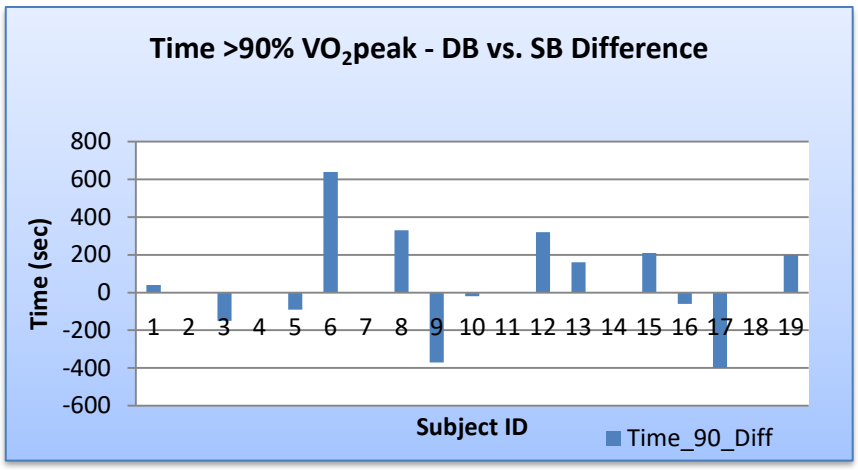
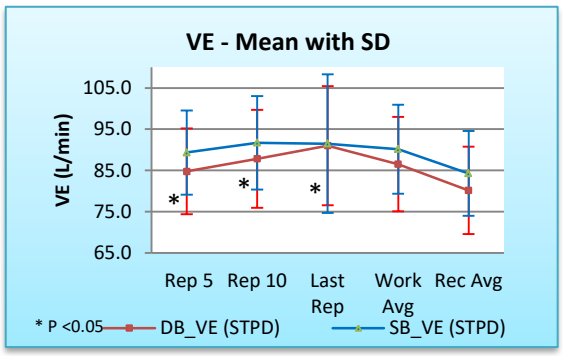


Figure 5-7 Difference in time accumulated above 90% VO_2peak
The figure shows the difference time accumulated above 90% VO_2peak between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 for individual subjects. The response is quite variable with individual subjects showing, no difference. The magnitude of the difference is also quite varied.

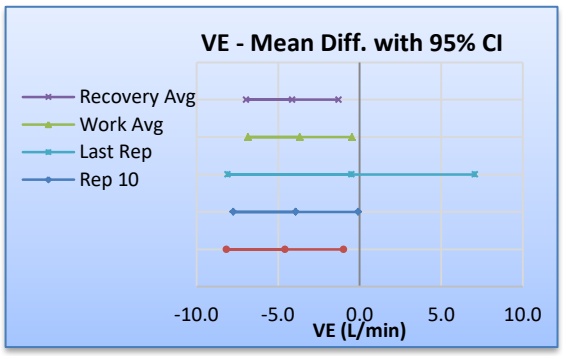
Figure 5-7 presents the difference in the amount of time accumulated above 90% of VO_2peak (T90%) between the DB and the SB HIIE sessions. There is considerable

individual variation both in direction and magnitude of change. The difference in T90% ranged from an increase of ~600 seconds to a decrease in ~400 seconds with DB.

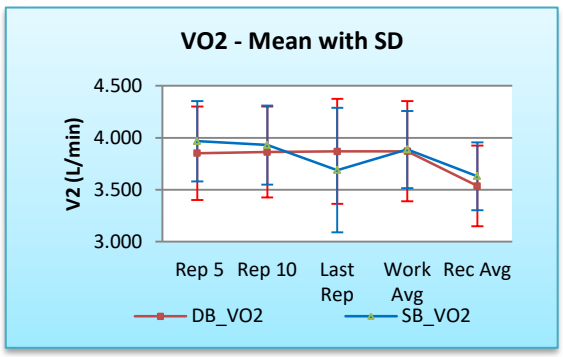
5.4.6 **Primary Ventilatory outcomes**



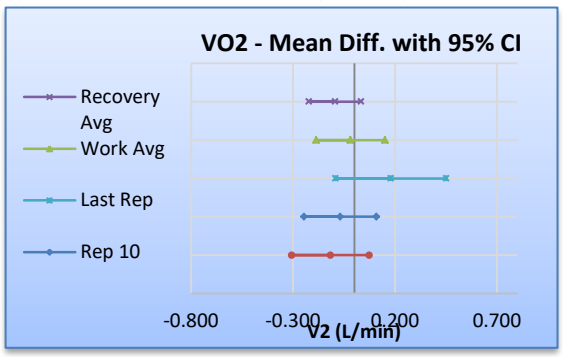
(A) VE – DB vs SB



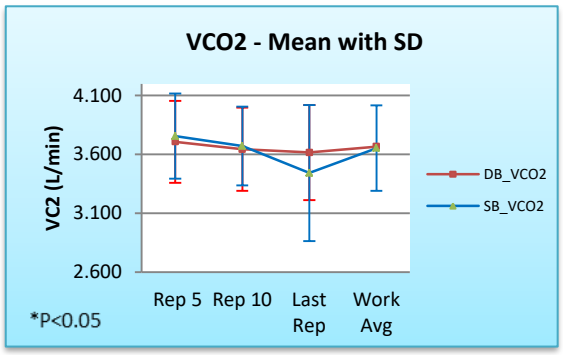
(B) VE - DB vs.SB - Mean diff. (95% CI)



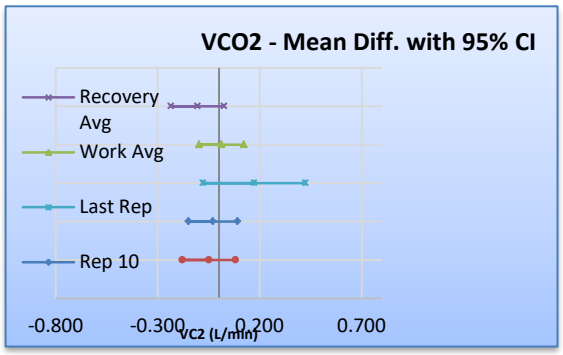
(C) VO_2 – DB vs. SB



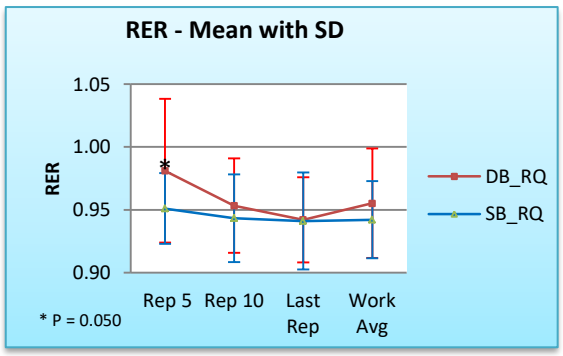
(D) VO_2 - DB vs.SB - Mean diff. (95% CI)



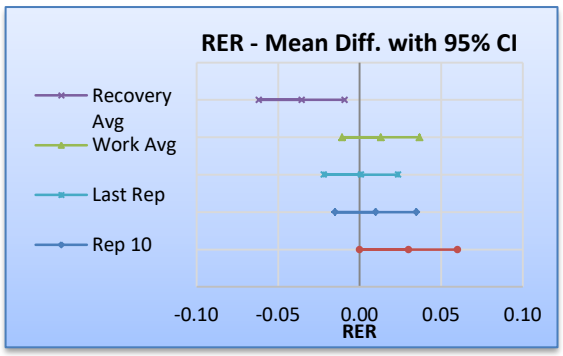
(E) VCO_2 - DB vs.



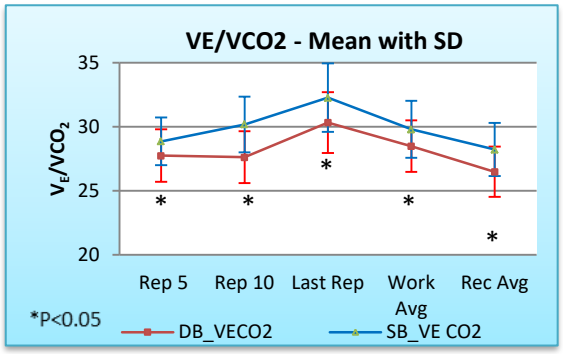
(F) VCO2 - DB vs.SB - Mean diff. (95% CI))



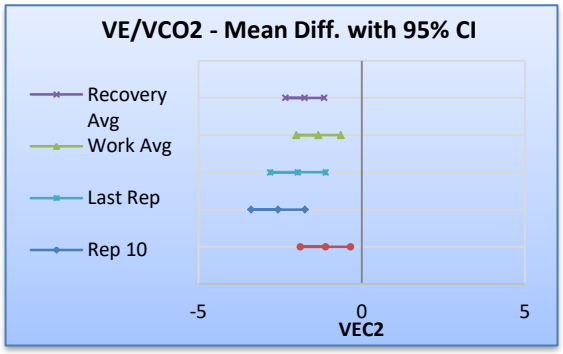
(G) RER - DB vs. SB



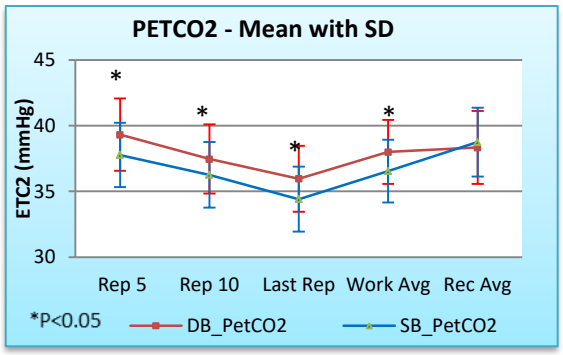
(H) RER - DB vs.SB - Mean diff. (95% CI)



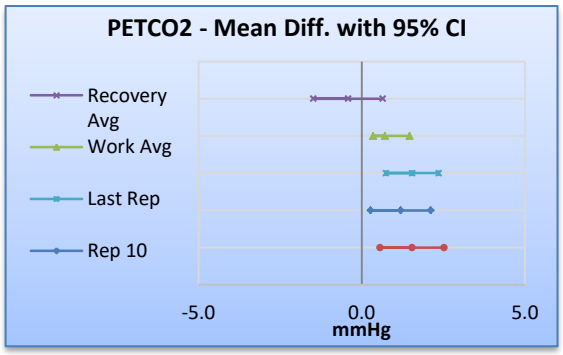
(I) VE/VCO_2 - DB vs.SB



(J) VE/VCO_2 - DB vs.SB - Mean diff. (95% CI)



(K) $P_{ET}CO_2$ - DB vs. SB (G) VE/VCO_2 - DB vs. SB



(L) $P_{ET}CO_2$ - DB vs.SB - Mean diff. (95% CI

Figure 5-8 Vetilatory parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); There is a significant decrease in minute ventilation (V_E) after Rep 5, Rep 10 and the Last Rep (A&B). There is no significant difference in O_2 consumption (VO_2) (C&D) or expired CO_2 (VCO_2) (E&F) but there is a significant increase in respiratory exchange rate (RER) with DB for Rec Avg only (G&H).There is a significant improvement ventilatory efficiency (V_E/VCO_2) for all measures (I&J), and a significant increase in end-tidal CO_2 ($P_{ET}CO_2$) except for Rec Avg (K&L). (*P<0.05)

5.4.6.1 V_E

There was a significant decrease in V_E with DB for Rep 5, Rep 10, Work_Avg and Rec_Avg but no significant difference for the Last Rep. Effect size was small or trivial for all parameters. Table 5-10 summarises the results.

Table 5-10 V_E Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.016****	84.8 ± 10.4	89.3 ± 10.2	-4.6	-8.2	-1.0	0.44	S
Rep 10	**0.046****	87.8 ± 11.9	91.7 ± 11.4	-3.9	-7.7	-0.1	0.34	S
Last Rep	0.893	91 ± 14.5	91.5 ± 16.8	-0.5	-8.1	7.1	0.03	T
Work_Avg	**0.027****	86.5 ± 11.4	90.2 ± 10.8	-3.6	-6.8	-0.5	0.33	S
Rec_Avg	**0.007***	80.2 ± 10.6	84.3 ± 10.3	-4.1	-7.0	-1.3	0.39	S

*V_E = minute ventilation; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.01; **P<0.05; ES = Effect Size; T = Trivial; S = Small;*

Figure 5-8(A) presents the results for DB and SB conditions. Figure 5-8(B) shows the mean difference between DB and SB conditions.

5.4.6.2 VO_2

There was no significant difference in VO_2 with DB and effect sizes were small or trivial. Table 5-11 summarises the results.

Table 5-11 VO_2 Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.217	3.967 ± 0.386	3.852 ± 0.45	-0.116	-0.306	0.074	0.28	S
Rep 10	0.435	3.864 ± 0.436	3.864 ± 0.436	-0.067	-0.244	0.110	0.16	T
Last Rep	0.180	3.931 ± 0.38	3.87 ± 0.506	0.180	-0.092	0.452	0.32	S
Work_Avg	0.837	3.87 ± 0.506	3.87 ± 0.482	-0.017	-0.186	0.152	0.04	T
Rec_Avg	0.145	3.689 ± 0.6	3.537 ± 0.387	-0.093	-0.306	0.074	0.26	S

VO_2 = volume of oxygen uptake (L/min); Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial; S = Small;

Figure 5-2(C) presents the results for DB and SB conditions. Figure 5-8(D) shows the mean difference between DB and SB conditions.

5.4.6.3 VCO_2

There was no significant difference in VCO_2 with DB and effect sizes were small or trivial. Table 5-12 summarises the results.

Table 5-12 VCO_2 Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.441	3.755 ± 0.361	3.706 ± 0.348	-0.049	-0.179	0.081	0.14	T
Rep 10	0.628	3.671 ± 0.334	3.643 ± 0.354	-0.028	-0.148	0.092	0.08	T
Last Rep	0.163	3.442 ± 0.578	3.616 ± 0.453	0.174	-0.078	0.425	0.33	S
Work_Avg	0.795	3.652 ± 0.363	3.666 ± 0.404	0.014	-0.096	0.123	0.04	T
Rec_Avg	0.111	3.623 ± 0.372	3.519 ± 0.377	-0.104	-0.234	0.026	0.28	S

VCO_2 = volume of expired carbon dioxide (L/min); Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial; S = Small;

Figure 5-8(E) presents the results for DB and SB conditions. Figure 5-8(F) shows the mean difference between DB and SB conditions.

5.4.6.4 RER

There was a significant increase in RER with DB for Rec_Avg, only with a large effect size. There was no significant difference with DB for Rep 5, Rep 10, Last Rep or Work_Avg. Effect sizes were small or trivial except for Rep_5 which was medium. Table 5-13 summarises the results.

Table 5-13 RER Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.050	0.98 ± 0.06	0.95 ± 0.03	0.03	0.00	0.06	0.66	M
Rep 10	0.410	0.95 ± 0.04	0.95 ± 0.04	0.01	-0.01	0.03	0.28	S
Last Rep	0.924	0.94 ± 0.03	0.94 ± 0.03	0.00	-0.02	0.02	0.03	T
Work_Avg	0.259	0.96 ± 0.04	0.94 ± 0.03	0.01	-0.01	0.04	0.35	S
Rec_Avg	**0.011***	0.97 ± 0.04	0.94 ± 0.04	-0.04	-0.06	-0.01	0.89	L

*RER =Respiratory Exchange ratio; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.05; ES = Effect Size; T = Trivial; S = Small; M = Medium; L = Large;*

Figure 5-8(G) presents the results for DB and SB conditions. Figure 5-8(H) shows the mean difference between DB and SB conditions.

5.4.6.5 V_E/VCO_2

There was a significant decrease in V_E/VCO_2 with DB for Rep 5, Rep 10, Last Rep, Work_Avg and Rec_Avg. Effect sizes were medium to very large. Table 5-14 summarises the results.

Table 5-14 V_E/VCO_2 Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.007***	**27.7 ± 2.1**	**28.8 ± 1.9**	**-1.1**	**-1.9**	**-0.3**	**0.56**	**M**
Rep 10	**0.000****	**27.6 ± 2**	**30.2 ± 2.2**	**-2.6**	**-3.4**	**-1.7**	**1.21**	**VL**
Last Rep	**0.000****	**30.3 ± 2.4**	**32.3 ± 2.7**	**-1.9**	**-2.8**	**-1.1**	**0.77**	**M**
Work_Avg	**0.001***	**28.5 ± 2**	**29.8 ± 2.2**	**-1.3**	**-2.0**	**-0.6**	**0.62**	**M**
Rec_Avg	**0.000****	**26.5 ± 2**	**28.2 ± 2.1**	**-1.7**	**-2.3**	**-1.1**	**0.86**	**L**

V_E/VCO_2 = ventilatory equivalent for CO_2; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery

*intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.01; **P<0.001; ES = Effect Size; M = Medium; L = Large; VL = Very Large;*

Figure 5-8 (I) presents the results for DB and SB conditions. Figure 5-8(J) shows the mean difference between DB and SB conditions.

5.4.6.6 $P_{ET}CO_2$

There was a significant increase in $P_{ET}CO_2$ with DB for Rep 5, Rep 10, Last Rep and Work_Avg with medium and small effect sizes but no significant difference for a Rec_Avg with a trivial effect size. Table 5-15 summarises the results.

Table 5-15 $P_{ET}CO_2$ Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.004***	39.3 ± 2.8	37.8 ± 2.4	1.5	0.6	2.5	0.59	M
Rep 10	**0.014****	37.5 ± 2.6	36.3 ± 2.5	1.2	0.3	2.1	0.47	S
Last Rep	**0.001***	36.0 ± 2.5	34.4 ± 2.5	1.6	0.7	2.4	0.62	M
Work_Avg	**0.001***	38.0 ± 2.4	36.5 ± 2.4	1.5	0.7	2.2	0.61	M
Rec_Avg	0.430	38.3 ± 2.8	38.8 ± 2.6	-0.4	-1.5	0.7	0.15	T

*$P_{ET}CO_2$ = partial pressure of end tidal CO_2; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.01; **P<0.05; ES = Effect Size; T = Trivial; S = Small; M = Medium;*

Figure 5-8(K) presents the results for DB and SB conditions. Figure 5-8(L) shows the mean difference between DB and SB conditions.

5.4.6.7 BLa^-

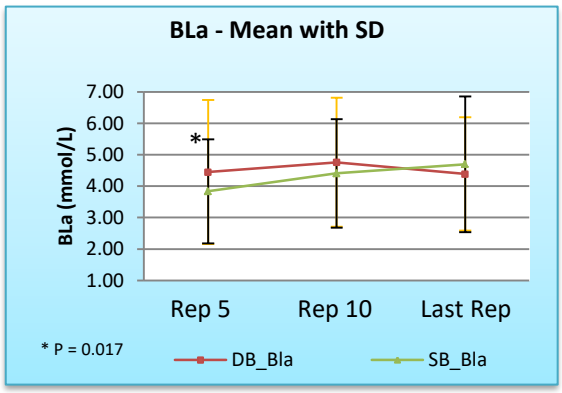
There was a significant increase in BLa^- with DB for Rep 5 only with a small effect size but no significant difference for Rep 10 and Last Rep with trivial effect sizes. Table 5-16 summarises the results.

Table 5-16 BLa^- (mmol/L) Results

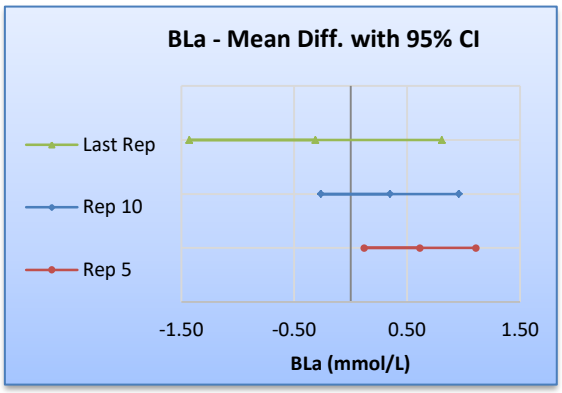
	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.017***	4.5 ± 2.3	3.8 ± 1.7	0.6	0.1	1.1	0.31	S
Rep 10	0.243	4.8 ± 2.1	4.8 ± 2.1	0.4	-0.3	1.0	0.18	T
Last Rep	0.557	4.4 ± 1.8	4.4 ± 1.7	-0.3	-1.4	0.8	0.15	T

*BLa^- = blood lactate concentration; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; *P<0.05; ES = Effect Size; T = Trivial; S = Small;*

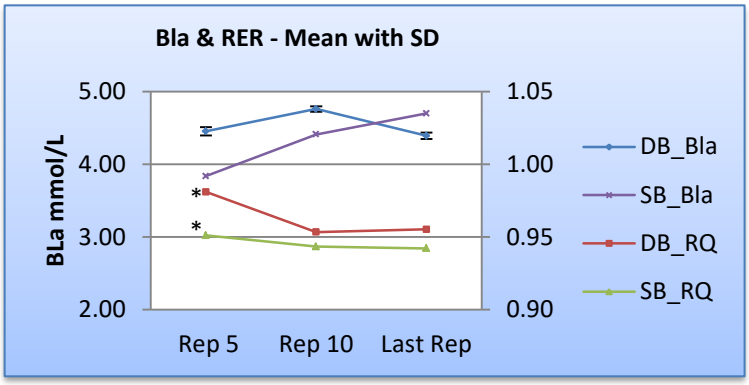
Figure 5-9(A) presents the results for DB and SB conditions. Figure 5-9(B) shows the mean difference between DB and SB conditions. Figure 5-9(C) compares BLa^- and RER in both SB and DB conditions.



(A) BLa^- – DB vs. SB



(B) BLa^- - DB vs.SB - Mean diff. (95% CI)



(C) BLa^- and RER comparison

Figure 5-9 BLa^- and RER comparison
Panels A and C show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panel B shows the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); There is a significant increase in BLa^- after Rep 5 only but a non- significant increase in respiratory exchange ratio (RER) although P=0.05. (*P<0.5)

5.4.6.8 HR

There was no significant difference in HR with DB and effect sizes were trivial. Table 5-17 summarises the results.

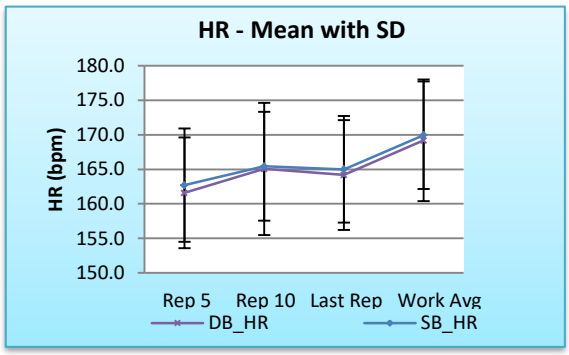
Table 5-17 HR (bpm) Results

	P	DB (BPM)	SB (BPM)	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.213	162 ± 8	163 ± 8	-1	-3	1	0.14	T
Rep 10	0.758	165 ± 10	165 ± 8	0	-3	2	0.04	T
Last Rep	0.509	169 ± 9	169 ± 8	-1	-2	1	0.07	T
Work_Avg	0.213	164 ± 8	165 ± 8	-1	-3	1	0.11	T

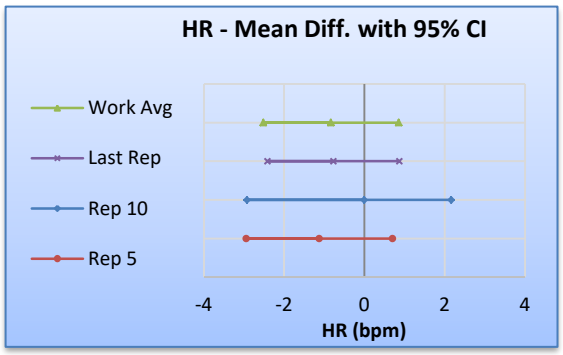
HR = Heart Rate; BPM Beats per minute; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery

intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial;

Figure 5-10(A) presents the results for DB and SB conditions. Figure 5-10(B) shows the mean difference between DB and SB conditions.



(A) HR – DB versus SB

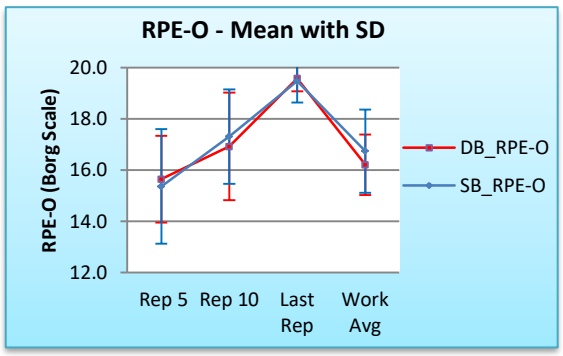


(B) HR - DB vSB - Mean diff. (95% CI)

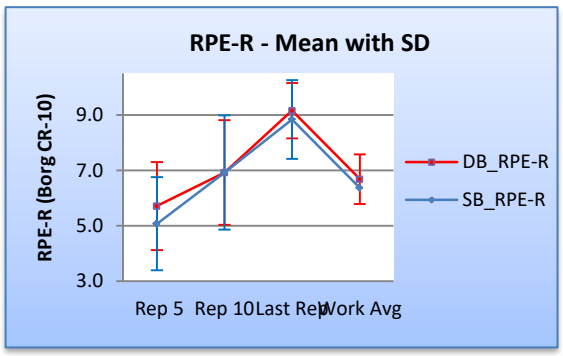
Figure 5-10 Heart Rate response
Panel A shows the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panel B shows the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); There is no significant difference in heart rate (HR) in any measure.

5.4.6.9 RPE-O & RPE-R

There was no significant difference in RPE-O at Rep 5 (P = 0.437; Trivial), Rep 10 (P = 0.463; Trivial) and Last Rep (P = 0.480; Trivial). There was no significant difference in RPE-R at Rep 5 (P = 0.111; Small), Rep 10 (P = 0.928; Trivial) and Last Rep (P = 0.429; Trivial). RPE does not meet parametric requirements for and was analysed using a Related-Samples Wilcoxen Signed Rank Test. **Error! Reference source not found.** Figure 5-11(A) presents the results for DB and SB conditions for RPE-O and Figure 5-11(B) presents the results for DB and SB conditions for RPE-R.



(A) RPE-O – DB vs. SB

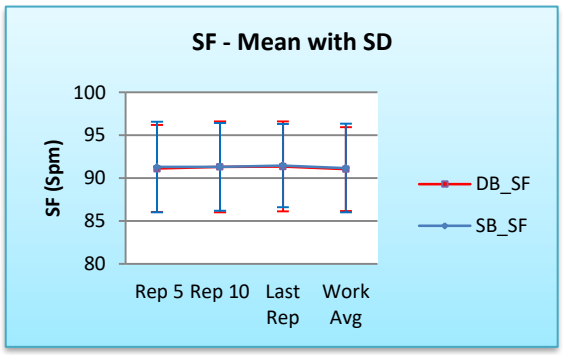


(B) RPE-R – DB vs. SB

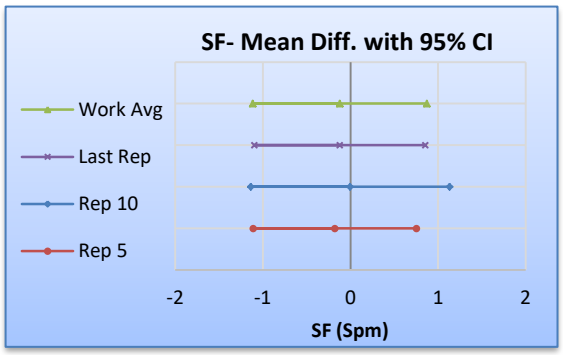
Figure 5-11 Ratings of perceived exertion
Panels shows the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3. Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); There is no significant difference in overall rating of perceived exertion (RPE-O) or respiratory rating of perceived exertion (RPE-R).

5.4.6.10 Locomotor Parameters

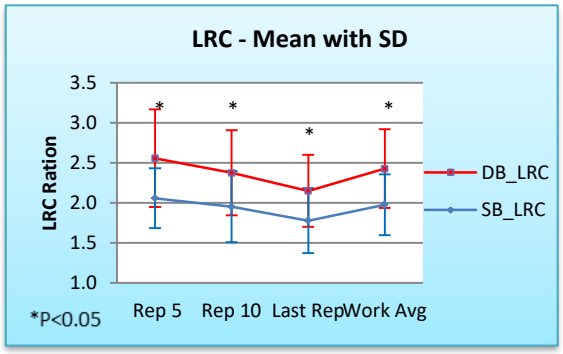
Stride frequency (SF) was measured and locomotor respiratory coupling (LRC) was calculated for both the SB and DB trials.



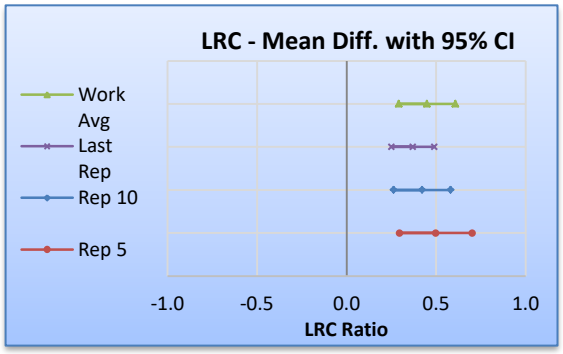
(A) SF – DB vs. SB



(B) SF - DB vs.SB - Mean diff. (95% CI)



(C) LRC – DB vs. SB



(D) LRC - DB vs. SB - Mean diff. (95% CI)

Figure 5-12 Locomotor parameters
Panels on the left show the mean response observed between the spontaneous breathing (SB) and deep breathing (DB) high intensity interval exercise (HIIE) trials of Study 3 while panels on the right show the mean difference with the 95% confidence interval (CI). Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (Error Bars); There is no significant difference in stride frequency (SF) (A&B). There is a significant difference in locomotor respiratory coupling (LRC) in all measures (C&D). (*P<0.05)

5.4.6.10.1 SF

Figure 5-12(A) presents the results for DB and SB conditions. Figure 5-12(B) shows the mean difference between DB and SB conditions. There was no significant difference in SF with DB and effect sizes were trivial. Table 5-18 summarises the results.

Table 5-18 SF Results

	P	DB (Spm)	SB (Spm)	Mean diff	95% CI		Cohen's d	ES
Rep 5	0.693	91 ± 5	91 ± 5	0	-1	1	0.03	T
Rep 10	1.000	91 ± 5	91 ± 5	0	-1	1	0.00	T
Last Rep	0.802	91 ± 5	91 ± 5	0	-1	1	0.02	T
Work_Avg	0.805	91 ± 5	91 ± 5	0	-1	1	0.02	T
Rec_Avg	0.693	91 ± 5	91 ± 5	0	-1	1	0.03	T

SF = Stride Frequency; Spm = Strides per minute; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; Results are mean ± Standard Deviation (SD); CI = Confidence Interval; ES = Effect Size; T = Trivial;

5.4.6.10.2 LRC

There was a significant increase in LRC ratio with DB for Rep 5, Rep 10, Last Rep Work_Avg and Rec_Avg with large effect sizes. Table 5-19 summarises the results.

Table 5-19 LRC Results

	P	DB	SB	Mean diff	95% CI		Cohen's d	ES
Rep 5	**0.000***	2.6	2.1	0.5	0.3	0.7	0.99	L
Rep 10	**0.000***	2.4	2.0	0.4	0.3	0.6	0.87	L
Last Rep	**0.000***	2.1	1.8	0.4	0.3	0.5	0.87	L
Work_Avg	**0.000***	2.4	2.0	0.5	0.3	0.6	1.03	L

*LRC = Locomotor Respiratory Coupling; Rep 5 = interval 5; Rep 10 = interval 10; Last Rep = the last interval; Work Avg = the average for all work intervals; Rec Avg = the average for all recovery intervals; DB = Deep Breathing; SB = Spontaneous Breathing; CI = Confidence Interval; ES = Effect Size; L = Large; *P<0.001;*

Figure 5-12(C) presents the results for DB and SB conditions. Figure 5-12(D) shows the mean difference between DB and SB conditions.

5.5 Discussion

High intensity interval exercise is a potent training stimulus (Billat, 2001a, 2001b; Buchheit & Laursen, 2013a, 2013b; Tschakert & Hofmann, 2013) that is used by athletes, non-athletes and clinical populations (Costigan et al., 2015; De Nardi et al., 2018; Gosselin et al., 2012). It is however highly demanding, stressing the cardiopulmonary system maximally with resultant high levels of perceived exertion and negative affective feelings that may prevent its use (Kilpatrick et al., 2015; Seiler & Sjursen, 2004). In athletes the ability to perform higher volumes of this time of training and/or with reduced perception of overall session effort could provide and increased physiological and psychological benefit to athletes and their subsequent performance. A definitive picture of respiratory limitation is lacking, however, various possible mechanisms have been suggested including, gas exchange inefficiency, metaboreflex mediated blood flow limitation and expiratory flow limitation (Wagner, 1992). We hypothesised that deep breathing might ameliorate some of the negative, metabolic, physiological and affective limitations to HIIE and thereby improving the ability to perform HIIE, reducing perceived effort and potentially improve performance indirectly via the ability to increase training stimuli.

It had been observed previously in incremental exercise (Study 2) that changes in breathing pattern were eliminated as intensity increased towards maximal levels. Therefore we first analysed breathing pattern to assess if DB was possible during HIIE. Results showed significant differences in breathing pattern, increased V_T and decreased RR with DB, at all time points, and when averaged across all work and all recovery intervals. V_T was significantly higher at all time points and over averaged work and recovery intervals (~0.4L). There was however a decline in V_T for both SB and DB from Rep 5 to rep 10 to the Last Rep suggesting possible fatigue in the respiratory muscle. An alternative possibility of expiratory flow limitation manifesting as the test progressed is supported by the reciprocal increase in RR and changes in secondary indices. RR rate increase in both SB and DB condition, with the exercise hyperpnea response was maintained, however significant differences existed. Even though RR rose in both SB and DB conditions, RR was significantly lower in DB and maximum RR for the Last Rep for DB was lower than Rep 5 under SB (Rep 5 (37 ± 9 vs. 45 ± 9); Last Rep (44 ± 9 vs. 52 ± 12)). There was also a reduction in Ti, Te and Ttot as the test progressed with a concomitant rise in V_E which resulted in increased MEFR while Peak Flow remained relatively stable. There was a non-significant difference between SB and DB conditions however, DB had higher Peak Flow across all measure but lower MEFR at Rep 5 and Rep10, with no difference by the Last Rep. AS we did no measure EFL we cannot confirm this observation but a higher V_T was maintained with DB and the decrease in V_T in SB was EFL related then DB may have ameliorated some of the effects by reducing MEFR. Interestingly duty cycle (Ti/Ttot) remained stable (~0.48) across all time points and averaged measures, with no difference between SB and DB.

This data shows that DB can be used during intermittent high intensity exercise, the intermittent nature and short duration allowing for breathing pattern to be altered before being supressed by powerful metabolic stimuli. In agreement with our previous work we observed increases in all temporal indices of the breath cycle nut the Ti/Ttot remained constant, the medullary rhythmic control maintained in the face of altered breathing pattern. The advantageous increase in Te:Ti seen in elite cyclists (Lucia et al., 2001) was not observed possible due to difference in ventilation patterns know to occur with different modes of exercise, namely running versus cycling Salazar-Martinez et al., 2016).

HIIE performance was assessed by comparing the number of repetitions completed. We found no significant difference between DB and SB but considerable individual variation with one subject completing 10 more repetitions under DB conditions while other decresed

under DB. In addition to the crude metric of number of completed reps we also assessed cardiopulmonary stress by quantifying the time spent at or near VO_2peak and it has been suggested that time accumulated at high intensities (>T90%) are necessary to attain maximal or near-maximal cardiac output and optimally signal cardiac and oxidative muscle fiber adaptation (Buchheit & Laursen, 2013b). They were extended to include time above 80% VO_2max (T80%) and 85%VO_2max (T85%) and used in conjunction with number of completed repetitions to assess overall HIIE performance. There was no significant difference between conditions suggesting that DB was neither beneficial nor harmful to HIIE performance. There was no significant change in any of our performance measures, however, there was very high variability in the response and perhaps an individual response that needs to be further explored.

Analysis of gas exchange parameters revealed a significant decrease in ventilation (VE) while VCO_2 did not change and therefore we observed significant improvements in ventilatory efficiency with DB. $P_{ET}CO_2$ an indirect marker of arterial PCO_2, was significantly higher at all three time points and work average during DB, with a similar decline from Rep 5 to Last Rep for both DB and SB, and more significant respiratory alkalosis in SB. This data suggests higher blood acidity with DB throughout the test and perhaps improved O_2 uptake in the tissue via a possible rightward shift of the oxy-hemoglobin dissociation curve. Unfortunately blood gases were not analysed in this study to confirm this or if hypoxemia had occurred as a result of either pattern to identify EIAH or differences in either pattern.

The metabolic stress of HIEE places higher or lower emphasis on oxidative and glycolytic fibres and energy pathways depending on the protocol, with greater emphasis on glycolytic pathways with higher intensity (Buchheit & Laursen, 2013b; Tschakert & Hofmann, 2013). Acid-base changes evident via increases in $P_{ET}CO_2$ and a possible metabolic shift suggested by increased BLa^- (4.5 ± 2.3 vs. 3.8 ± 1.7 mmol/L) at rep 5 only, and a non-significant rise in RER, suggest a possible change in substrate use and increase in carbohydrate (CHO) metabolism. BLa^- was also non-significantly higher at Rep 10 (4.8 ± 2.1 vs. 4.4 ± 1.8 mmol/L) and interestingly non-significantly lower at Last Rep This earlier reliance on CHO metabolism didn't result in any positive or negative effect on overall performance.

Despite evidence that athletes have improved interoception and more accuracy anticipatory response to perceived breathlessness, Faull (2016) suggested that some athletes may be

more susceptible to breathing anxiety either due to lower respiratory muscle endurance or higher ventilatory sensitivity, and at increased risk for performance limitation and benefit from psychophysiological interventions. We assessed both RPE-O and RPE-R to assess overall and respiratory effort and once again found no positive or negative effect of DB. We did not measure anxiety either trait or perceived respiratory anxiety which could have acted as a confounding factor, anxiety may have affected the ability to DB. It is well established that dyspnea and anticipatory or perceived breathlessness have a negative effect on performance and the acute change to respiratory pattern could interact with individual sensitivity. Further work is needed to ascertain if this is a factor in the individual variability observed.

Alterations in respiration may also have indirect effects on exercise performance by producing changes in locomotion which may affect mechanical efficiency of movement as the locomotor-respiratory coupling (LRC) is bidirectional. Therefore we also looked for changes in locomotor efficiency and LRC. We observed no difference in SF, and it was relatively constant throughout the duration of the test for both SB and DB. It would appear that the high intensity places strict biomechanical requirements and is the dominant stimulus in dictating the SF. However, due to reduced RR we seen significant higher LRC ratios with DB. It has been suggested that recoupling to higher integer ratios can occur but it unclear whether the level of change in the altered RR was influenced by the biomechanically imposed SF (Bramble and Carrier, 1983; Lafortuna et al., 1996; Siegmund et al., 1999; Rabler and Kohl, 2000). As we did not assess efficiency and not performance benefit was observed we cannot conclude if any benefit occurred from this altered LRC.

5.6 Conclusion

In male endurance athletes DB doesn't improve HIIE performance but neither does it impair it, however, there is considerable heterogeneity in the individual response. Research identifies the individuality of breathing pattern and the exercise hyperpnoiec response, and the diverse physiological and psychological inputs that influence it. It is therefore suspected that the ability to breathe more deeply during exercise, the primary way in how it is achieved (abdominal vs. thoracic), and therefore the potential to improve may also be highly individual. It is clearly necessary to investigate this individual pattern in future research to identify links between pattern type and the scope to increase depth of breath and exercise performance.

It is unclear whether RPE measures are sensitive enough to detect changes in perceived exertion and other affective responses need to be considered. DB has a significant effect on LRC and certain gas exchange parameters that indicate an earlier and increased reliance on carbohydrate metabolism and acid-base balance but the significance of these changes is unknown and there is no apparent performance improvement observed in the current study.

5.7 Limitations and future work

Due to technical limitations in our laboratory we were unable to measure certain parameters. We were unable to ascertain or categorise subject breathing patterns as abdominal or thoracic either under SB or DB conditions. This might be particularly useful in identifying individual differences. We were unable to measure blood gases, either arterial or capillary, and instead relied on the indirect measure of P_aCO_2 from $P_{ET}CO_2$. In light of the change in $P_{ET}CO_2$ with deep breathing, arterial blood gases would have allowed confirmation of this as it is possible the altered breathing pattern and change measured at the mouth may not accurately reflect arterial partial pressure changes. While clearly identified in the literature as a limiting factor, expiratory flow limitation was not measured.

We did not assess the effect of DB at peak or approaching peak intensity as it was not possible for subjects to DB. Locomotor efficiency could have been calculated using the methods of Zamparo et al. (2015).

Finally, we did not assess psychological parameters which may have influenced SB pattern and the ability to deep breathe, such as trait anxiety or respiratory anxiety. Also, because subjects must consciously increase the depth of breath it constitutes a dual-task performance which has been shown to affect physiological parameters and is therefore a possible confounding factor. The RPE measures used may not have been sensitive enough to measure subtle changes in perceived exertion.

Future work to measure these parameters would deepen our understanding considerably to probe the individual basis for breathing and those susceptible to limitation and possibly more amenable to improvement from DB. The acute change in breathing pattern was necessitated to assess the effect of pattern alone as it is established that the respiratory muscles when trained can influence performance and also the neuro-respiratory centres can undergo both modulatory and plastic responses that may benefit performance. However such an acute change could trigger individual respiratory anxiety levels to increase, interfering with perceived exertion and/or ability to deep breathe. A chronic intervention which trained subjects to deep breathe in isolation or in conjunction with respiratory

muscle training needs to be explored. The ability to DB and to maintain the pattern may be limited by respiratory muscle endurance and respiratory control circuits. Increased endurance may facilitate DB and chronic training may allow for modulatory and plastic responses to occur which might allow DB to fully benefit subjects.

6. Discussion

The aim of this thesis was to investigate if adopting a self-regulated, deep breathing pattern during exercise could improve exercise performance. Our hypothesis was that at higher intensities, when fatigue-limiting symptoms increase and limit exercise and performance, a deep breathing pattern may alleviate or counteract them via improved gas exchange and/or increased efficiency, either respiratory and/or locomotor. To achieve this we devised three studies which would examine both the heavy and severe intensity domains, with domain specific tests selected based on physiological and performance constraints. The heavy intensity domain enabled steady state assessment of gas exchange and efficiency parameters while in the severe domain non-steady state conditions were evaluated. The first study examined constant work rate (CWR) heavy intensity exercise and cast a wide net recruiting a heterogeneous group of healthy males and females, both untrained and trained. The specific purpose was to examine, under the most stable conditions (steady state), the effects of deep breathing and also if group-specific effects existed. However, steady state doesn't occur in the severe domain so domain specific protocols to test performance and replicate exercise training were the basis of Study 2 and Study 3 respectively. Despite the more widespread use of severe intensity exercise as a training stimulus to improve health in non-athletic populations, it was decided to focus specifically on male endurance trained subjects and running performance. Study 2 assessed endurance running and Study 3 examined high intensity interval exercise (HIIE) performance via a lab-based, treadmill interval running test to exhaustion. The analysis of each study condition was twofold, firstly, to what level were subjects able to achieve deep breathing, a pre-requisite to allow for its study and secondly, if achieved what effect on performance did it have.

6.1 Deep breathing

Research identifies the individuality of breathing pattern and the exercise hyperpnoiec response, and the diverse physiological and psychological inputs that influence it. It is therefore suspected that the ability to breathe more deeply during exercise, the primary way in how it is achieved (abdominal vs. thoracic), and therefore the potential to improve may also be highly individual. Performance of dual tasks such as a motor task and a cognitive task (e.g. consciously deep breathing) can results in a decrement in the performance of either or both (Schott and Klotzbier, 2018, Grassmann et al., 2016). The effects are referred to as dual task effects (DTE) and are the results of competition for our

limited attentional resources. Grassmann et al. (2016) examined the alterations in breathing associated with cognitive load and concluded that cognitive load caused overbreathing, resulting in decreased end-tidal CO_2 ($P_{ET}CO_2$) and increased VO_2 and VCO_2. It is therefore possible that the interaction between the consciously deep breathing may have a negative impact or at least represent a confounding factor in our results.

For deep breathing (DB) to be effective is must be achievable with reduced or minimal extra cost. Failure to synchronise the respiratory musculature can increase the cost of breathing and therefore negatively affect performance (Aliverti, 2008a, Hopkinson et al., 2010). Efficient breathing, especially efficient DB, requires the abdominal musculature to relax and not to oppose the diaphragm, as to do so will increase the cost of breathing and can also result in a sub-optimal thoracic breathing pattern. It is therefore crucial to identify how DB is accomplished, primarily via either abdominal or thoracic expansion. It is a major limitation of our study that we can only describe DB in terms of increased V_T and cannot make this differentiation. We must therefore interpret our findings with caution as this is a possible confounding factor and may have impacts on the effectiveness of DB.

Benchetrit (2000) identified the highly individual nature of breathing pattern while Masoaka and Homma (2001, 2004, 2004, 2008) have highlighted the role of both cognitive and emotional state and trait anxiety as factors that influence breathing pattern. These factors may influence who may benefit from DB or be able to effectively achieve such a pattern.

Dempsey et al., (2006) have shown that reductions in respiratory muscle work improve endurance exercise performance. It remains to be seen if DB reduces respiratory work or in fact increases respiratory work by increased activation of the diaphragm which has been shown to compete with locomotor musculature for blood supply.

The ability to deep breathe requires room for expansion of tidal volume (V_T). Our results reveal that the ability to deep breathe is not only intensity-specific but also subject to temporal constraints. Exercise hyperpnea follows a typical response, with increases in depth of breath until a plateau occurs either as the result of functional or mechanical constraints. Therefore an upper limit for tidal volume expansion exists that would be expected to be achieved in the severe exercise domain if sufficient time is spent at a given intensity or if increasing intensity, as in an incremental test. Subjects were able to increase V_T to varying degrees during heavy-intensity CWR exercise in Study 1 and for the duration of the HIIE in Study 3. However, in Study 2, with incremental exercise there

reached a point where V_T was not different between SB and DB and at peak exercise there was no significant difference with DB in any indices of breathing pattern or gas exchange parameters. The fact that V_T increase was achievable during HIIE at 100% vVO_2peak but not during incremental exercise supports the temporal component role in achieving the V_T limit and not simple intensity. This suggests either two potential limits with DB, one the physical limit which cannot be tackled and two, the temporal component which may induce fatigue that reduces the ability to deep breathe. Based on the literature examining respiratory muscle fatigue and resultant performance limitation, it is to be expected that the increased respiratory effort to deep breathe in an acute setting such as ours could induce fatigue and limit its achievement. In Study 3 a decline in V_T was observed from Rep 5, to Rep 10 to Last Rep and a reciprocal rise in RR and V_E. This may be as a result of respiratory muscle fatigue and the increased V_E demands due to less efficient gas exchange, i.e. greater deadspace ventilation due to decreased V_T. This decline was evident in both SB and DB conditions. It is however possible that the increased metabolic disturbance as the test progressed increased ventilatory demands that drove a tachypnoeic response and a resultant decrease in V_T. As V_E did not increase significantly between Rep 10 and the Last Rep for SB we would contend that this response is fatigue related. In relation to the temporal indices of breathing pattern, deep breathing resulted in significantly longer inspiration and expiration when RR was reduced. The most interesting finding was that when breathing was self-paced, duty cycle was tightly maintained with no difference in T_I/T_{TOT} between SB and DB, reflecting the tight regulation of breathing rhythm by medullary control centres. (Besleaga et al., 2016) highlights the individuality of breathing pattern however suggests the autonomic control preserves duty cycle. It had been thought that the lower RR might increase T_E and reduce flow rates but it appears that the concomitant rise in in V_T with T_E and the maintenance of duty cycle prevented this. The advantageous increase in T_E:T_I seen in elite cyclists (Lucia et al., 2001) was not observed, possibly due to difference in ventilation patterns know to occur with different modes of exercise, namely running versus cycling (Salazar-Martinez et al., 2016). In respect to flow rates, no significant difference was seen in either maximum expiratory flow rate (MEFR) or Peak Flow across all three studies though some non-significant reductions were measured under DB conditions. Without the evaluation of expiratory flow limitation (EFL) which we were unable to measure, the significance of these findings is unknown.

Interacting with this is the considerable individual variability that exists and/or the presence of dysfunctional breathing patterns which unfortunately we were unable to assess. For example, if an individual naturally breathes deeply there is less room to increase

towards this upper limit or if an individual adopts a paradoxical breathing pattern, the inward movement of the abdomen with inspiration would decrease the abdominal cavity space required for diaphragmatic expansion and increase the cost of respiration with excessive demands placed on thoracic expansion that would not be able to compensate. Across the three studies individual variability was observed: the ability to expand V_T was very different despite exercising at the same relative metabolic intensity in Study 1 and Study 3. Interestingly, we did not observe any difference in gender in Study 1 but untrained subjects increased V_T significantly more when asked to deep breathe. We cannot explain the reasons behind this phenomenon but it is likely that this is an artefact of the differences in exercise modalities between the two groups. However, we cannot discount the possibility of a difference between the two groups: perhaps the adaptations known to occur in athletes and the tighter matching of respiratory control to exercise demands have an influence. While not assessed, it may be possible that the untrained group increased V_T above optimal levels while the trained subjects did not. To examine if such a difference exists, further studies matching exercise modality and metabolic intensity are required.

6.2 Performance outcomes

Due to the inability of subjects to significantly increase V_T in the later stages of Study 2, our analysis of the effects of (DB) is limited in this study. We can however confirm its achievement in Study 1 and Study 3 and truly evaluate its effects in these studies. The questions are therefore:

Study 1: Does DB improve CWR exercise in the heavy intensity domain?

Study 2: Does DB in the early stages of a 3-minute incremental running test to exhaustion improve running performance?

Study 3: Does DB during HIIE improve HIIE capacity?

The primary performance metrics differed between studies with some overlap. In all three studies we examined changes to SF and LRC that might have resulted from DB and in study 1 and 2 it was possible to examine locomotor efficiency which is a performance determining factor.

6.2.1 Locomotor Efficiency

Alterations in respiration may also have indirect effects on exercise performance by producing changes in locomotion which may affect mechanical efficiency of movement. Therefore we also looked for changes in locomotor pattern, LRC and locomotor efficiency.

There was a significant decrease in stride frequency (SF) in Study 1 and Study 2 but not in Study 3. It would appear that the high intensity of HIIE places strict biomechanical requirements and is the dominant stimulus in dictating the SF. For a change in the LRC to occur, a disproportionate decrease in SF and RR or a decrease in RR with SF maintained was required to effect an increase in the stride frequency to respiratory rate ratio (SF:RR). There was a significant effect on LRC across all three studies but only during some of the submaximal stages in Study 2. It has been suggested that recoupling to higher integer ratios can occur but it is unclear whether the level of change in the altered RR was influenced by the biomechanically imposed SF (Bramble and Carrier, 1983; Lafortuna et al., 1996; Siegmund et al., 1999; Rabler and Kohl, 2000). The magnitude of change in SF is not proportional to the change in RR and would suggest that while a mutual attraction may exist, locomotion is the dominant force in the relationship. McDermott et al. (2003) have shown that increasing mechanical and metabolic work affects both processes and we suggest that this may impose an overriding and limiting effect on the magnitude of this reverse coupling. Further to this, the magnitude of this effect is most likely constrained by the intensity and gradient of the exercise undertaken as well as mechanical limitations such as individual leg length constraining the ability to increase stride length to decrease SF.

6.2.1.1 Cost of locomotion

Locomotor efficiency was calculated as unit cost of oxygen (ml/kg/km) and unit cost energy (kcal/kg/km) in both Study 1 (CWR) and the sub-maximal stages of Study 2 (vVO_2peak) only. There was significant difference between trained and untrained in both conditions. The untrained group had significantly poorer efficiency as might be expected, however, due to the different types of locomotion, uphill walking in the untrained versus 1 % gradient running in the trained, a direct comparison is not possible. Interestingly, females were significantly more efficient than males, both in the trained group and the untrained group. Our interest was in the effect of DB and no there was no effect of gender on the reductions in O_C. E_C was also higher in the untrained who were the only group to show a significant improvement with DB. From the differences in these two measures it appears than while O_C is reduced, an increase in RER influenced E_C in trained subjects such that no significant energy saving was made. In the untrained subjects it is difficult to interpret whether this is advantageous or not and requires further study as to the interaction between reduced O_C and E_C and the impact on total energy expenditure in an exercise bout. If the main objective for some individuals is to maximise energy expenditure this would have a negative impact unless the increased efficiency was accompanied by counteracting

affects. These might include the ability to tolerate exercise in this domain that would not be otherwise be achievable or the ability to exercise longer in this domain that would result in a higher overall energy expenditure. Contrary to the findings in Study 1 during CWR exercise, in Study 2 when we looked at the same efficiency measures during the early sub-maximal stages which traverse the moderate intensity domain, no significant difference was observed. We speculate that it may be the case that stage duration plays a role in this finding as the alterations in breathing pattern with DB may alter gas exchange kinetics and delay steady state being achieved. Further work looking at the changes in kinetics is warranted to fully elucidate the effects of DB.

6.2.2 **Endurance running performance**

There was no difference in vVO_2peak or VO_2peak between SB and DB and while this indicated that DB didn't improve running performance neither did it have any negative effect. While vVO_2peak provides one of the most valid and reliable indirect methods of evaluating endurance running performance (Machado et al., 2013b, McLaughlin et al., 2010, Midgley et al., 2007c), the results from our study showing the inabilty to change breathing pattern at higher intensities and therfore limits our ability to assess the effect DB on peak performance. It was observed in this study that intensity plays a significant role in the ability to deep breathe: at higher intensities in a 3-minute incremental running test DB was not achieved and as such our tests failed to compare SB and DB except at lower submaximal intensities. While vVO_2peak is one of the best predictors of endurance running performance, the respiratory demands of incremental exercise differ from the constant work load of endurance running performance and so therefore may not be the most suitable test to evaluate DB. Other constant work load tests such as time trials and time to exhaustion tests, despite their questionable reliability, might be more suitable, mimicking the respiratory demands of performance more accurately.

6.2.3 **HIIE capacity**

High intensity interval exercise is a potent training stimulus (Tschakert and Hofmann, 2013, Buchheit and Laursen, 2013b, Billat, 2001a, Billat, 2001b, Buchheit and Laursen, 2013a) that is used by athletes, non-athletes and clinical populations (Costigan et al., 2015, Gosselin et al., 2012, De Nardi et al., 2018). It is however highly demanding, stressing the cardiopulmonary system maximally with resultant high levels of perceived exertion and negative affective feelings that may prevent its use (Kilpatrick et al., 2015, Seiler and Sjursen, 2004). In athletes, the ability to perform higher volumes of this type of training

and/or with reduced perception of overall session effort could provide an increased physiological and psychological benefit to athletes and their subsequent performance. In addition to the crude metric of number of completed reps we also assessed cardiopulmonary stress by quantifying the time spent at or near VO_2peak and it has been suggested that time accumulated at high intensities (>T90%) are necessary to attain maximal or near-maximal cardiac output and optimally signal cardiac and oxidative muscle fibre adaptation (Buchheit and Laursen, 2013b). They were extended to include time above 80% VO_2max (T80%) and 85%VO_2max (T85%) and used in conjunction with the number of completed repetitions to assess overall HIIE performance. We hypothesised that deep breathing might ameliorate some of the negative metabolic, physiological and affective limitations to HIIE and thereby improve the ability to perform HIIE, reducing perceived effort and potentially improve performance indirectly through increase training stimuli. There was no significant change in any of our performance measures, however there was very high variability in the response and perhaps an individual response that needs to be further explored.

6.2.4 **Ventilatory and metabolic response**

Ventilation, V_E, is primarily controlled by metabolic rate (Haouzi, 2006). At intensities below LT, V_E is dynamically coupled with VCO_2 to maintain arterial PCO_2, while at intensities above LT, V_E is linked to the maintenance of arterial pH in the face of metabolic acidosis (Ward, 2007). All three studies placed subjects above LT in the heavy and severe exercise domains so the main driving force was therefore metabolic. No change in V_E was observed in Study 2 either during submaximal stages or at peak. The significant reduction in V_E in both Study 1 (CWR) and Study 3 (HIIE) suggests possible improvement in gas exchange efficiency. The reduction in V_E was not matched by a reduction in VCO_2, and despite a non-significant decrease in VCO_2, V_E/VCO_2 decreased significantly with DB due the disproportionate decrease in V_E thereby positively impacting ventilatory efficiency. There was a significant increase in $P_{ET}CO_2$ in both Study 1 and Study 3, reflecting an increase in arterial PCO_2 and disturbance to the acid-base balance. While not measured as part of the study, alterations to blood pH could affect a rightward shift is the oxyhaemoglobin disassociation curve possibly enhancing O_2 unloading.

In Study 1, the greater decrease in VO_2 relative to VCO_2 resulted in a non-significant increase in the RER, reflecting a change in substrate use and increase in carbohydrate metabolism. While there was no significant increase in BLa^-, there was a non-significant

increase with DB, corroborating a greater reliance on glycolytic metabolism supported by the RER findings. In Study 3, acid-base changes were also evident via increases in $P_{ET}CO_2$ and a possible metabolic shift was suggested by increased BLa^- (4.5 ± 2.3 vs. 3.8 ± 1.7 mmol/L) at Rep 5 only, and a non-significant rise in RER. This also suggests a possible change in substrate use and increase in carbohydrate (CHO) metabolism. BLa^- was also non-significantly higher at Rep 10 (4.8 ± 2.1 vs. 4.4 ± 1.8 mmol/L) and interestingly non-significantly lower at Last Rep This earlier reliance on CHO metabolism didn't result in any positive or negative effect on overall performance. The metabolic stress of exercise places higher or lower emphasis on oxidative and glycolytic fibres and energy pathways depending on the protocol, with greater emphasis on glycolytic pathways with higher intensity (Buchheit and Laursen, 2013b, Tschakert and Hofmann, 2013).

Despite clear evidence of the powerful modulatory effect of DB on heart rate via RSA (Cysarz and Büssing, 2005), heart rate (HR) did not change significantly between DB and SB. The intensity of exercise and metabolic stimuli most likely prevent a reduction in HR due to the metabolic demand for blood flow. Therefore, the same cardiac output coupled with a change in ventilation may suggest that an alteration to ventilation/perfusion matching (V_A/Q) may be a possible mechanism underlying the observed improvement in economy. This is supported by the work of Giardino et al. (2003) and Hayano et al. (1996) which demonstrated improvements in gas exchange and V_A/Q matching with deep slow breathing.

6.2.5 **Percevied exertion**

Despite evidence that athletes have improved interoception and a more accurate anticipatory response to perceived breathlessness, Faull (2016) suggested that some athletes may be more susceptible to breathing anxiety either due to lower respiratory muscle endurance or higher ventilatory sensitivity placing tham at increased risk for performance limitation and they may benefit from psychophysiological interventions. We assessed both RPE-O and RPE-R to assess overall and respiratory effort and found no positive or negative effect of DB in Study 2 or Study 3, however in Study 1, during heavy intensity CWR exercise, DB resulted in a significant reduction in RPE-O for the female group but not in RPE-R. DB did not result in any significant changes in perceived exertion (RPE-O) or dyspnoea (RPE-R) for males. Significant research into gender differences identifying dysynapsis and increased susceptibility to EFL in females (Harms and Rosenkranz, 2008a) may be a possible reason why females may have benefited more from

DB. Tong et al. (2004) have shown that dyspnoea may be an exercise limiting factor and Bernardi et al. (2002) have proposed that slow breathing may delay the onset of dyspnoea, however no significant results to support this were found in this study. Also the large inter-individual variability in our subjects supports the findings of Bernasconi and Kohl (1993) who found that, with comparatively smaller manipulation of respiratory pattern via paced breathing some subjects expressed annoyance with forced pattern, a feeling echoed by many of our subjects despite non-significant changes in perceived exertion. Such feelings are understandable due to the autonomic control of respiration and the various complex and poorly understood physiological control mechanisms underpinning respiration. We did not measure anxiety, either trait or perceived, which could have acted as a confounding factor. It is well established that dyspnoea and anticipatory or perceived breathlessness have a negative effect on performance and the acute change to respiratory pattern could interact with individual sensitivity.

7. Conclusion

Deep breathing during exercise, its applicability and effects, depends on whether exercise is constant, incremental or intermittent and the interaction between intensity of exercise and duration at that intensity. It can be concluded from Study 1 that the adoption of deep breathing pattern results in increased locomotor efficiency as measured by a significant reduction in VO_2, O_2 cost and energy cost during CWR heavy intensity exercise in healthy males and females that are either trained or untrained. DB also effects a change in the LRC relationship, however the proposed decoupling of locomotion from respiration is not definitive and the possibility of a re-coupling and a more optimum coupling ratio is suggested as a possible mechanism explaining the improvements observed. These improvements were demonstrated in both trained and untrained males and females with a wide age range.

DB results in no significant improvement in running performance in well trained endurance runners, contrary to our hypothesis. Neither did we find any difference in submaximal or maximal gas exchange parameters that might suggest improved gas exchange or ventilatory efficiency. No changes were found in ratings of perceived exertion. While we found changes in SF and LRC at both submaximal and at peak (SF only), these changes didn't impact performance. While vVO_2 is one of the best predictors of endurance running performance the respiratory demands of incremental exercise differ from the constant work load of endurance running performance and so therefore may not be the most suitable test to evaluate DB. Other constant work load test such as time trials and time to exhaustion tests, despite their questionable reliability, might be more suitable, mimicking the respiratory demands of performance more accurately

DB doesn't improve HIIE performance but neither does it impair it. It is unclear whether RPE measures are sensitive enough to detect changes in perceived exertion and other affective responses such as anxiety, both trait and respiratory, need to be considered. DB has a significant effect on LRC and gas exchange parameters but the significance of these changes is unknown and there is no apparent performance improvement in the current study

8. Limitations and future work

Due to technical limitations in our laboratory we were unable to measure certain parameters. We were unable to ascertain or categorise subject breathing patterns as abdominal or thoracic either under SB or DB conditions. This might be particularly useful in identifying individual differences. We were unable to measure blood gases, either arterial or capillary, and instead relied on the indirect measure of P_aCO_2 from $P_{ET}CO_2$. In light of the change in $P_{ET}CO_2$ with deep breathing, arterial blood gases would have allowed confirmation of this as it is possible the altered breathing pattern and change measured at the mouth may not accurately reflect arterial partial pressure changes. While clearly identified in the literature as a limiting factor, expiratory flow limitation was not measured. We did not assess the effect of DB at peak or approaching peak intensity as it was not possible for subjects to breathe deeply. Finally, we did not assess psychological parameters which may have influenced SB pattern and the ability to deep breathe, such as trait anxiety or respiratory anxiety. The RPE measures used may not have been sensitive enough to measure subtle changes in perceived exertion.

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