PHYSIOLOGICAL CONSEQUENCES OF THE WORK OF BREATHING AND OF INSPIRATORY MUSCLE TRAINING

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PUBLICATIONS

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
A_{tot}^-	Weak acids
$[A_{tot}^-]$	Total concentration of weak acids
ATP	Adenosine tri-phosphate
a-vO_2diff	Arterial - venous oxygen difference
BE_{ECF}	Base excess of the extracellular fluid
BMI	Body mass index
BPNS	Bilateral phrenic nerve stimulation
BSA	Body surface area
BTPS	Body temperature, pressure and saturated
Ca^{2+}	Calcium
CaO_2	Arterial oxygen content
Cl^-	Chloride
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variation
EELV	End expiratory lung volume
EIAH	Exercise induced arterial hypoxaemia
EILV	End inspiratory lung volume
ELITE	ELaboratore di Immagini TElevisive
EMG	Electromyography
EMT	Expiratory muscle training
ETC	Electron transport chain
FAD	Flavin adenine denuclotide
F_EO_2	Fractional concentration of expired oxygen
F_ECO_2	Fractional concentration of expired carbon dioxide

FEV_1	Forced expiratory volume in 1 second
FFM	Fat free mass
F_IO_2	Fractional concentration of inspired oxygen
F_ICO_2	Fractional concentration of inspired carbon dioxide
FM	Fat mass
f_R	Breathing frequency
FRC	Functional residual capacity
FRL	Flow resistive loading
FVC	Forced vital capacity
H^+	Hydrogen
Hb	Haemoglobin
HCO_3^-	Bicarbonate
He	Helium
HFF	High frequency fatigue
H_2O	Water
HR	Heart rate
HSD	Honestly significantly different
IMT	Inspiratory muscle training
ITL	Inspiratory muscle pressure threshold loading
K^+	Potassium
lac^-	Lactate
$[lac^-]_B$	Blood lactate concentration
$[lac^-]_M$	Muscle lactate concentration
LDH	Lactate dehydrogenase
LFF	Low frequency fatigue
MCT	Monocarboxylate transporter
MEP	Maximal expiratory mouth pressure

MIP	Maximal inspiratory mouth pressure
MLSS	Maximal lactate steady state
MRPD	Maximal rate of pressure development
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
MVV_n	Maximal voluntary ventilation ($_n$, duration in s)
Na^+	Sodium
NHLBI	National heart, lung and blood institute
NAD^+	Nicotinamide adenine-dinucleotide
NADH	Reduced nicotinamide adenine-dinucleotide
O_2	Oxygen
OH^-	Hydroxyl
P_0	Mouth pressure at zero inspiratory flow
*P*ab	Abdominal pressure
PAP	Post-activation potentiation
PAV	Proportional assist ventilation
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
PCO_2	Partial pressure of carbon dioxide
PDH	Pyruvate dehydrogenase
*P*di	Diaphragm pressure
PEF	Peak expiratory flow rate
PFK	Phospho-frucktose kinase
*P*ga	Gastric pressure
pH	Negative logarithm of $[H^+]$
P_i	Inorganic phosphate
PIF	Peak inspiratory flow rate
pK	Dissociation constant in water

PaO_2	Partial pressure of oxygen in the arterial blood
PO_2	Partial pressure of oxygen
*P*oe	Oesophageal pressure
*P*oe/*P*di	Relative chest wall muscle recruitment
$\dot{P}_{opt}$	Optimal inspiratory muscle pressure
*P*m	Mouth pressure
*P*pl	Pleural pressure
PPr^-	Plamsa protein
*P*rc,e	Pressure generated by expiratory rib cage muscles
*P*rcm,i	Pressure generated by inspiratory rib cage muscle
*P*tp	Transpulmonary pressure
$\dot{Q}$	Cardiac output
RER	Respiratory exchange ratio ($\dot{V}CO_2 / \dot{V}O_2$)
RMT	Respiratory muscle training
RPD	Ratings of perceived dyspnoea
RPE	Ratings of perceived exertion
r.p.m.	Revolutions per minute
RV	Residual volume
SaO_2	Oxygen saturation in the arterial blood
SD	Standard deviation
SEE	Standard error of the estimate (σ_{est})
SID	Strong ion difference
SpO_2	Estimated oxygen saturation in the arterial blood
$SRCa^{2+}$	Sarcoplasmic reticulum calcium
STPD	Standard temperature, pressure and dry
SV	Stroke volume

T_2	Force of a paired non-volitional stimulation
TIRE	Test of Incremental Respiratory Endurance
T_I/T_{tot}	Inspiratory time / total breath duration (duty cycle)
TLC	Total lung capacity
T_{lim}	Time to the limit of volitional tolerance
Vab	Abdominal volume
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_2 max$	Maximal oxygen consumption
$\dot{V}_{opt}$	Optimal inspiratory flow rate
$\dot{V}CO_2$	Carbon dioxide production
VC	Vital capacity
$\dot{V}_E$	Minute ventilation
$\dot{V}_E max$	Maximal minute ventilation
$\dot{V}_E / \dot{V}O_2$	Ventilatory equivalent of oxygen
$\dot{V}_E / \dot{V}CO_2$	Ventilatory equivalent of carbon dioxide
$\dot{V}_I$	Inspiratory flow
VIH	Voluntary isocapnic hyperpnoea
$\dot{V}max$	Maximal inspiratory flow rate
Vrc,a	Volume of the rib cage apposed to the diaphragm
Vrc,p	Volume of the rib cage apposed to the lung
V_T	Tidal volume
$\dot{W}_I$	Inspiratory muscle power
$\dot{W}_I max$	Maximal inspiratory muscle power
$\dot{W}max$	Maximal power

ABSTRACT

A reduced blood lactate concentration ($[lac^-]_B$) is commonly observed during whole-body exercise following inspiratory muscle training (IMT). However, whether the inspiratory muscles are, in part, the source of these reductions remains unknown. Accordingly, this thesis investigated: (I) the contribution of the respiratory muscles to the systemic $[lac^-]_B$ and (II) the effects of IMT upon inspiratory muscle lactate exchange and clearance. In addition, the thesis also evaluated the determinants of inspiratory muscle strength (maximal inspiratory mouth pressure; MIP). All subjects were healthy, active and free of pulmonary and respiratory muscle disease.

Under resting conditions, 10 min intense volitional hyperpnoea at 85% of maximal exercise minute ventilation ($\dot{V}_E$max) increased $[lac^-]_B$ by 0.96 mmol·L^{-1}. This was attenuated by 25% following 6 wks IMT. 8 min volitional hyperpnoea at 90% $\dot{V}_E$max imposed upon exercise at the maximal lactate steady state (MLSS) increased $[lac^-]_B$ by 0.99 mmol·L^{-1}. Following 6 wk IMT, the steady state and hyperpnoea-mediated increase in $[lac^-]_B$ were lower by 8 and 26%, respectively. Relative to pre-IMT, loading the trained inspiratory muscles using a low-intensity pressure threshold resistance (15 cmH_2O) immediately following maximal exercise accelerated both lactate exchange and clearance capacities by ~70%. Collectively these findings support the notion that the respiratory muscles are capable of net lactate production and are the first to suggest that IMT increases their capacity for lactate clearance. This thesis also demonstrates that the respiratory muscles are responsible, in part, for the reductions observed in $[lac^-]_B$ during whole-body exercise following IMT.

Finally, baseline MIP was positively correlated with the strength of the chest wall inspiratory muscles. The IMT-mediated increase in MIP was negatively correlated with the relative increase in chest wall muscle strength. Therefore, these findings are the first to demonstrate that the lower the initial strength of the chest wall inspiratory muscles, the lower the MIP and the greater the improvement in global inspiratory muscle strength following IMT.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 7

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

CHAPTER 1

GENERAL INTRODUCTION

1.1 THE RESPIRATORY SYSTEM

1.1.1 INTRODUCTION

The respiratory system comprises the *lung parenchyma* across which gases are exchanged between the alveoli and the pulmonary capillaries and the *respiratory pump* (which includes the rib cage [also referred to as the thorax] and the respiratory muscles) which powers pulmonary ventilation. The primary function of the respiratory system is to preserve the partial pressure of arterial blood gases including oxygen (O_2: PO_2) and carbon dioxide (CO_2: PCO_2) and thus a large proportion of acid-base homeostasis. At rest and during even heavy exercise, the pulmonary system achieves this with exceptional precision despite a reduction in the transit time of the red blood cell in the pulmonary capillary and the marked increase in venous PCO_2 and decrease in PO_2. This unique function requires the co-ordination of central motor output from the autonomic nervous system to the inspiratory and expiratory respiratory muscles to match breathing pattern with the ever changing feedforward (motor output) and feedback (afferent discharge) stimuli. However, all of this must take place with a minimal energy cost to the body (Dempsey et al. 2006a).

1.1.2 THE LUNG PARENCHYMA

Although the lung is not the focus of the thesis a brief summary of its structure and function are essential for a holistic understanding of the pulmonary system in the healthy human. The lungs are elastic structures and comprise approximately 300 million small sacks of tissue called alveoli (Figure 1.1). Within the alveoli gases are exchanged by passive diffusion across the blood-gas barrier into the myriad of pulmonary capillaries. The total surface area of the alveoli is approximately 50 to 100 m^2 (West 2000) and since the blood gas barrier is extremely thin (around 0.3 μm) and the diameter of a pulmonary capillary is similar to that of a red blood cell (~10 μm), the alveoli wall may be described as a sheet of blood exposed to alveolar gas.

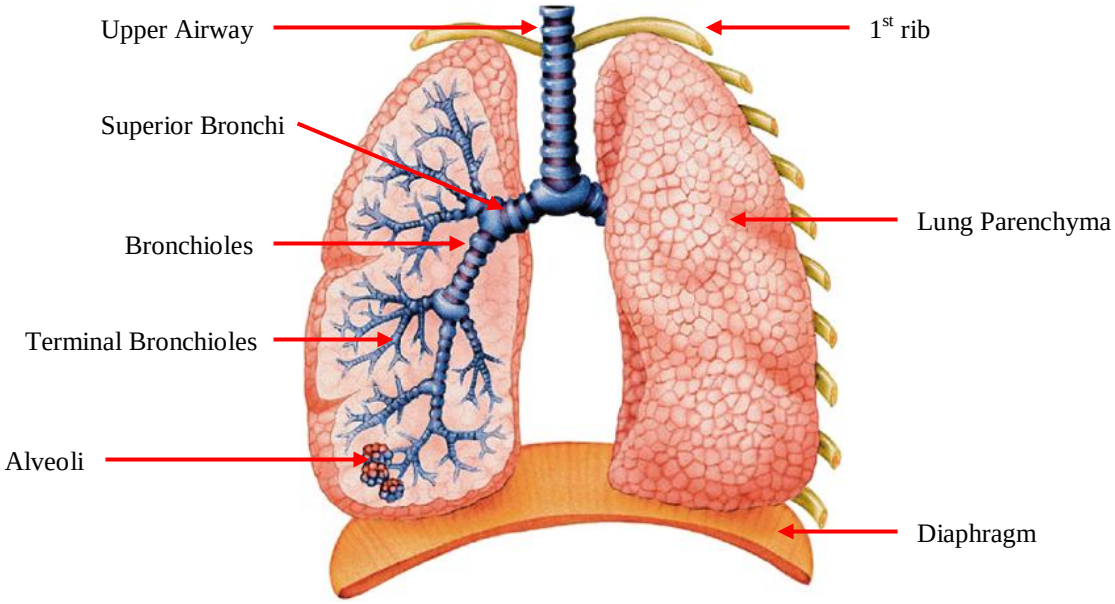

Figure 1.1 Cross sectional illustrations of the lungs and airways which terminate at the alveoli; it is here where gas exchange occurs by passive diffusion (adapted from Griesenbach et al. 2004)

Typically in healthy exercising humans, the pulmonary system does not limit maximal oxygen consumption ($\dot{V}O_2$max; Demspey 1986). However, with chronic endurance training, almost all components of the O_2 transport and metabolic systems (cardiac muscle, circulation and skeletal muscle) demonstrate progressive functional adaptation whereas the lung does not. Interestingly, the lung only demonstrates plasticity during hypoxia immediately after birth or with surgical denervation of up to 50% of the lungs (Wagner 2005). As a consequence, many other systems overtake the capacity of the pulmonary system. Therefore, in this instance the pulmonary system may occupy a critical link in the O_2 transport system (Dempsey 1986; Wagner 2005).

1.1.3 THE THORAX

The thorax is a semi-rigid elastic structure to which the respiratory muscles attach permitting changes in lung volume through an increase or decrease in thoracic volume (Cappello and De Troyer 2002). The chest wall and lung are separated by the pleural space which contains the pleural fluid (Lai-Fook 2004). The pleural fluid acts as a lubricant which facilitates the slide of the lung against the posterior surface of the chest wall and the conduction of intra-thoracic pressure changes which permit lung expansion (Agostoni 1986). At functional residual capacity (FRC) the elastic recoil of the lung and the tendency for rib cage expansion balance one another and thus pleural pressure (*P*pl) remains constant at approximately -5 cmH_2O.

1.1.4 FUNCTIONAL ANATOMY OF THE THORAX

The thorax comprises 12 pairs of ribs, which articulate with the thoracic vertebrae and the sternum; this is known as the costochondral junction (Figure 1.2). The 1^{st} rib is relatively flat and articulates with the manubrium sterni in an immovable cartilaginous joint. Ribs 2 to 10 are relatively fixed during the breath cycle at their origin: the vertebrae (costovertebral joints) and transverse processes (costotransverse joints) such that movement of these ribs occur through rotation of the long axis of its neck (De Troyer et al. 2005). Ribs 11 and 12 are known as floating ribs and interact directly with the inner surface of the abdominal wall.

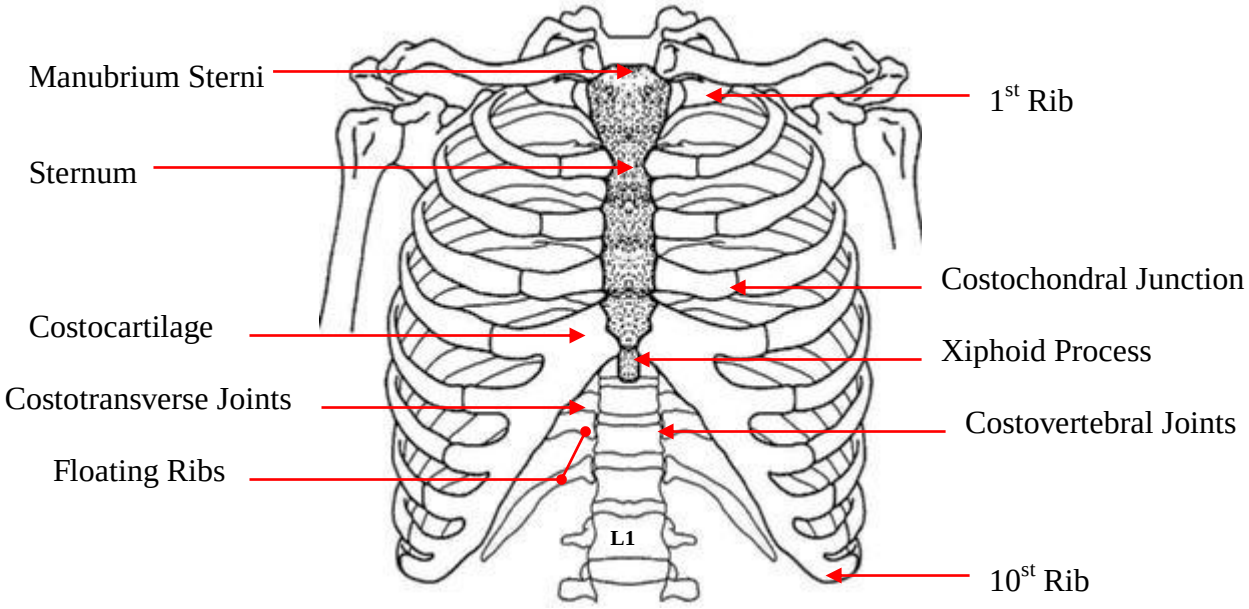

Figure 1.2 Anatomical illustration of the thorax (adapted from Stone and Stone 2000)

During inspiration, the displacement of individual ribs varies. The upper ribs are more rigid due to their connection with the sternum and throughout inspiration they become more horizontal, the anterior ends move up and outward with minimal lateral displacement. These ribs follow the direction of the sternum; this is known as the pump handle action (Figure 1.3A). The hinging of the middle ribs carries them more lateral and cranial; this is referred to as the bucket handle action (Figure 1.3B). Finally, the lower ribs are susceptible to rib-cage distortion because they have a freely moveable, cartilaginous connection with the sternum; this is known as the calliper-like action (Figure 1.3C).

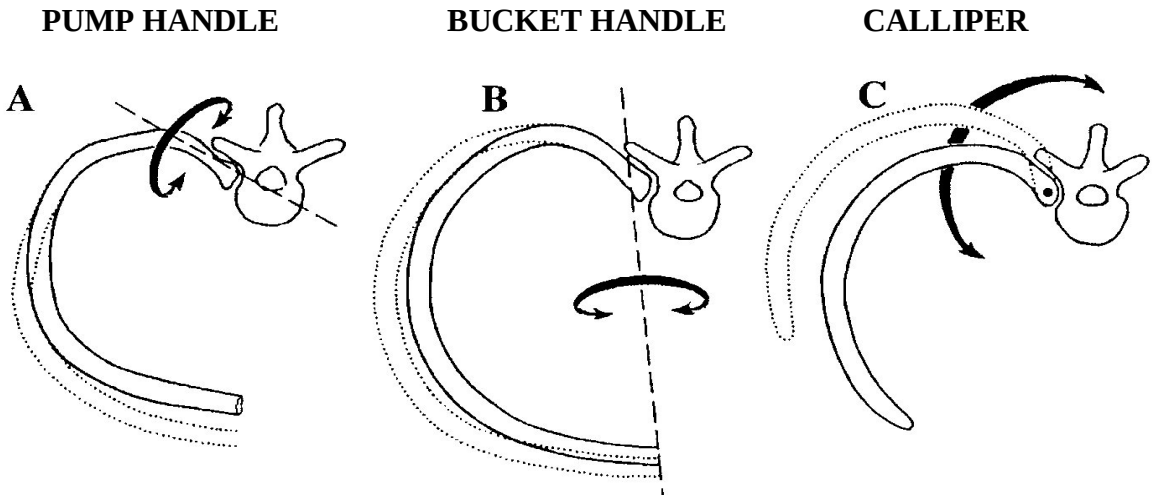

Figure 1.3 The functional action of the ribs showing A) pump handle action of the upper ribs; B) bucket handle motion of the middle ribs and C) calliper-like motion of the lower ribs (De Troyer et al. 2005).

1.2 THE RESPIRATORY MUSCLES

Respiratory muscle mass is approximately 3% of total body mass (Robertson et al. 1977) and although small relative to other skeletal muscles (e.g. quadriceps: ≥50% of total body mass) they are active throughout life and are considered the *only essential* skeletal muscles (Poole et al. 1997). As a consequence, their functional demands are uncompromised even during and possibly following intense exercise. Primary inspiratory muscles are active during quiet breathing and accessory inspiratory muscles are recruited to achieve high inspiratory flow rates. Primary expiratory muscles are recruited at the onset of exercise (Aliverti et al. 1997) whereas accessory expiratory muscles are recruited when very high expiratory flow rates are required. The precise co-ordination and recruitment of inspiratory and expiratory muscles in response to systemic metabolic demand is fundamental to arterial blood gas homeostasis, whole-body energy expenditure and potentially, exercise performance.

1.2.1 DIAPHRAGM

The physical characteristics of the human diaphragm were investigated in 70 human cadavers (age range: 16 - 91 yr). Mean mass of the diaphragm was 283 ± 53 g which represented approximately 0.5% of total body mass. The mass and thickness of the diaphragm was also proportional to both the height and body mass of the cadaver with the thickness greater in those that performed manual / physical tasks throughout their life (Arora and Rochester 1982).

The diaphragm is an elliptical cylinder capped by a dome: similar to a parachute. It separates the thoracic and abdominal cavities and is solely innervated by the phrenic nerves. The diaphragm has a non-contractile central tendon that projects to the anterior contractile costal hemidiaphragm and the posterior crural hemidiaphragm. Figure 1.4A illustrates the cross section of a rat diaphragm which is anatomically similar to the human diaphragm (Figure 1.4B).

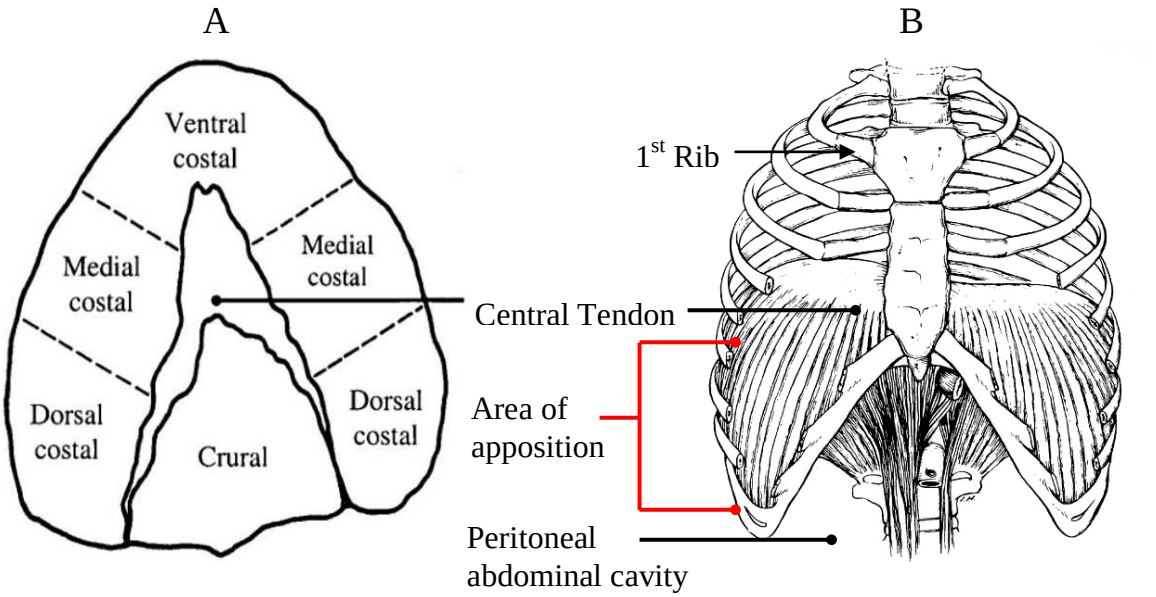

Figure 1.4 A) Schematic of the rat diaphragm (Poole et al. 1997); B) the human diaphragm at FRC (Rochester et al. 1981). The costal margin inserts in to the xiphoid process and the upper margins of the lower six ribs. The crural hemidiaphragm inserts in to the first three and two lumbar vertebrae on the left and right sides of the vertebral column, respectively (De Troyer and Estenne 1988).

At rest, the muscle fibres of the costal diaphragm run cranially such that during resting breathing approximately 30% of the lower rib cage is directly apposed to the diaphragm; this is known as the area of apposition (Figure 1.4B). During a quiet inspiration at rest, the zone of apposition is reduced, as the costal muscle fibres shorten (De Troyer and Estenne 1988). Here, the diaphragm may account for up to 90% of the total change in lung volume (Poole et al. 1997).

1.2.2 SCALENES

The scalenes anterior, medial and posterior (Figure 1.5) are often overlooked as primary inspiratory muscle (e.g. Sheel et al. 2002). Their total mass is approximately 0.1% total body mass (Legrand et al. 2003). Using computed topographic scanning and needle EMG, it has been shown that the scalenes contract in synchrony with the diaphragm (De Troyer and Estenne 1984; Gandevia et al. 1996; Legrand et al. 2003). Scalene muscle activation displaces the sternum cranially through rotation of the long axis of the ribs' neck (De Troyer and Kelly 1984) and apposes the dorsal displacement of the upper ribcage throughout inspiration (Legrand et al. 2003).

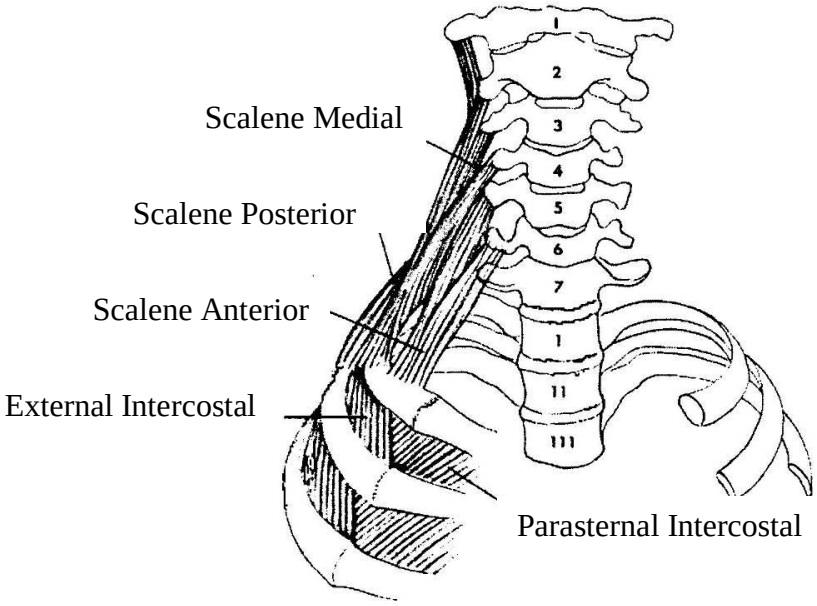

Figure 1.5 Anterior view of the upper rib cage and the anterior, medial and posterior scalenes (De Troyer and Estenne, 1988). The scalenes originate at the transverse processes of C1 to C6 and insert on to the upper surface of the first (scalenes anterior and medial) and second ribs (scalene posterior).

1.2.3 PARASTERNAL INTERCOSTALS

The parasternal intercostals (Figure 1.6) are located ventrally between the lateral borders of the sternum and the costochondral junctions (De Troyer et al. 2005) and have a mass <0.1% total body mass (De Troyer et al. 1998). Parasternal shortening causes rotation of the chondrosternal junction and elevates the ribs (De Troyer and Estenne 1998), this action occurs in synchrony with the scalenes and the diaphragm (Gandevia et al. 1996).

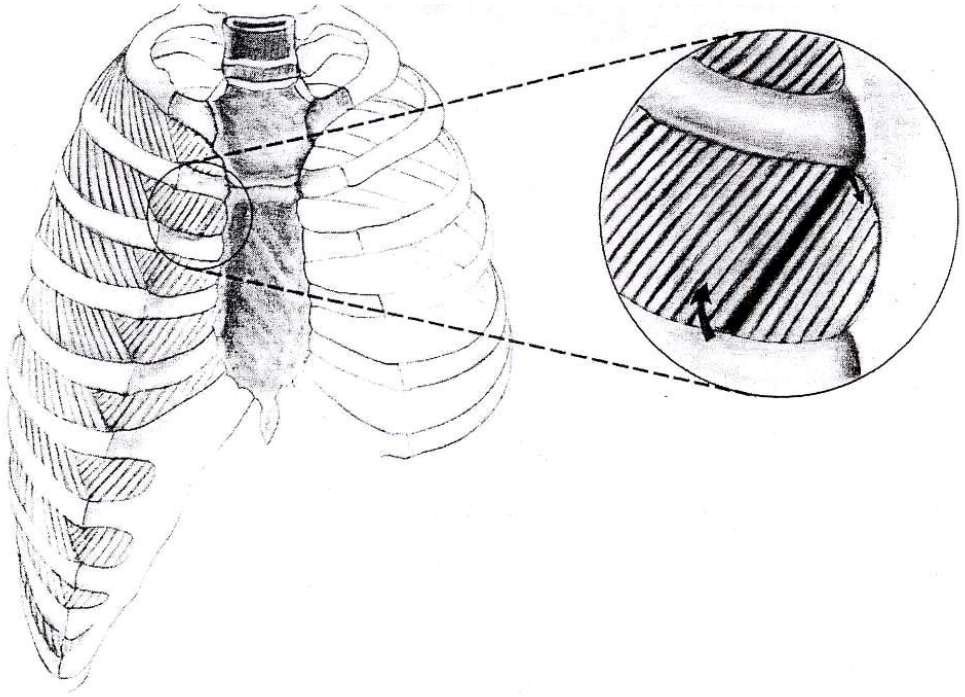

Figure 1.6 Functional anatomy of the parasternal intercostals which are located between the lateral borders of the sternum and the chondrosternal junctions (De Troyer and Estenne, 1988).

1.2.4 ACCESSORY INSPIRATORY MUSCLES

During exercise, tidal volume (V_T) plateaus at approximately 50 to 60% of vital capacity (VC) and the elastic resistance of the thorax becomes increasingly restrictive (Wetter and Dempsey 2000). Therefore, further increases in minute ventilation ($\dot{V}_E$) are brought about by an increase in breathing frequency (f_R; Sheel 2002). The increase in f_R is achieved by recruiting accessory respiratory muscles (and expiratory muscles). Some of these accessory respiratory muscles have an extra-thoracic origin and may only assist breathing during exercise where near maximal flow rates are generated. These include the pectoralis major and minor, trapezius, serratii and muscles of the upper airway. The external intercostals, sternocleidomastoids and the levator costae are directly involved in the cranial and ventral displacement of the rib cage and are considered the most important accessory respiratory muscles in humans (De Troyer and Estenne 1998), thus a brief synopsis of their function follows.

The muscle fibres of the external intercostals are orientated obliquely in the caudal-ventral direction from the rib above to the rib below (De Troyer et al. 2005; Figure 1.7). The total muscle mass of the external intercostals is <0.01% total body mass and is reduced from the 2nd to the 8th intercostal space in a dorsal-ventral (back to front) direction. Wilson et al. (2001) demonstrated that due to the differences in rib rotation mechanics (See Figure 1.3) toward the top and back of the rib cage the external intercostals are inspiratory and toward bottom and front of the rib cage they are expiratory. Despite this, evidence for their direct respiratory function is limited since they are activated following the onset of inspiration when the gain of parasternal and scalene activation is increasing.

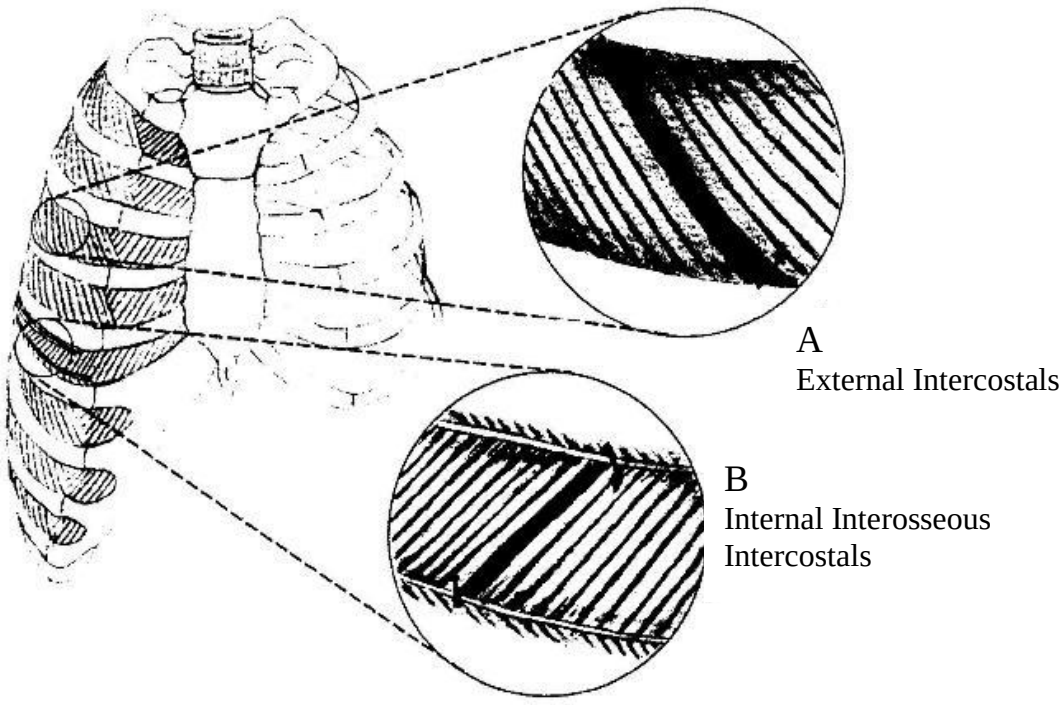

Figure 1.7. Functional anatomy of (A) external intercostals and (B) internal interosseous intercostals (De Troyer and Estenne, 1988). The muscle fibres of the external intercostals extend from the dorsal tubercles of the ribs to the ventral costochondral junctions. The internal interroseous intercostals extend from the sternocostal junctions to the tubercles of the ribs.

The sternocleidomastoids run parallel to the scalenes (Figure 1.8). Limited research has focused on this muscle group in humans. In anaesthetised dogs, electrical stimulation of the sternocleidomastoid indicates that it facilitates the pump-handle cranial displacement of the upper ribs (De Troyer and Kelly 1984) and increases the dorso-ventral diameter of the rib cage (Legrand et al. 2003).

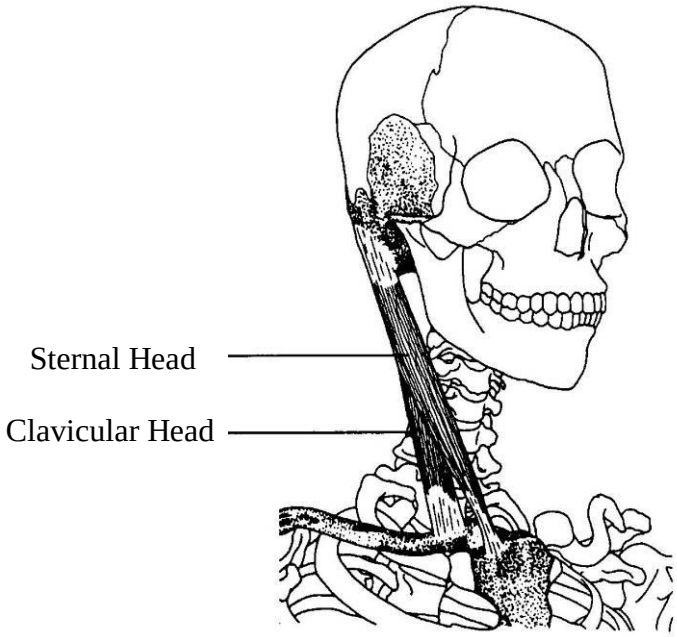

Figure 1.8 Anterior view of the sternocleidomastoids (Stone and Stone 2000). The sternocleidomastoids insert proximally in to the mastoid process of the occipital bone and originates distally at the manubrium sterni, and the clavicle.

The levator costae (Figure 1.9) are thin triangular shaped muscles which elevate the ribs. However they are recruited secondary to the parasternal intercostal (De Troyer and Farkas 1989). The levator costae are located in the dorsal intercostal spaces adjacent to the transverse processes of the vertebrae (De Troyer et al. 2005).

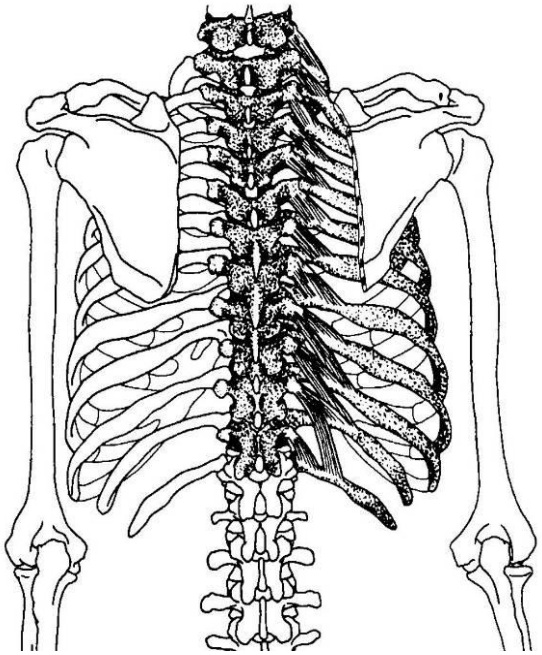

Figure 1.9 Posterior view of the levator costae (Stone and Stone 2000). The levator costae is a thin triangular shaped muscle that originates from both sides of the transverse processes of the vertebrae (C7 to T11) and inserts into the caudal portion of the ribs adjacent to the external intercostal.

1.2.5 ABDOMINAL MUSCLES

The primary expiratory muscles are the rectus abdominals, the transverse abdominals and the internal and external obliques (Figure 1.10). Activation of the rectus abdominals reduces the anterior-posterior diameter of the rib cage and also decreases the distance between the pubis and the xiphoid process (Mier et al. 1985). The transverse abdominis is recruited ahead of the superficial muscles of the abdominal cavity due to its shape and therefore role in compressing the abdominal contents (De Troyer and Estenne 1988; De Troyer et al. 1990). The internal and external obliques may also serve to compress the abdominal viscera (De Troyer and Estenne 1988).

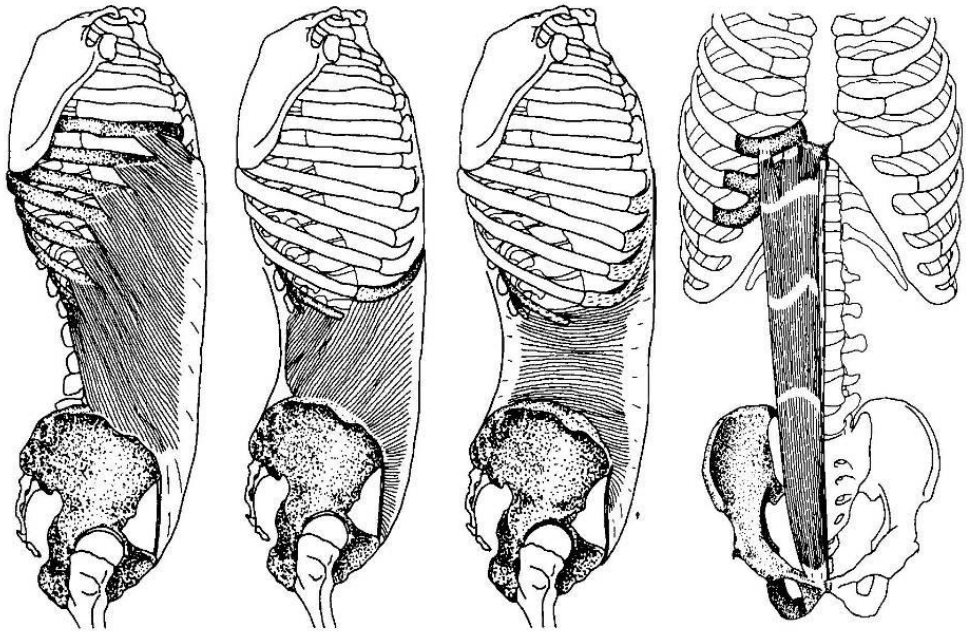

Figure 1.10 Muscles of the anterior abdominal wall (Stone and Stone 2000): A) external oblique which originates at the lower 8 ribs and the external surfaces of ribs 5 to 12 and inserts into the linea alba, pubic tubercle and the anterior half of iliac crest; B) internal oblique which originates at the thoracolumbar fascia, the anterior two thirds of the iliac crest, and the lateral half of the inguinal ligament and inserts to the costal margin and inferior borders of ribs 10 to 12, the linea alba, and the pubic pecten; C) transverse abdominus whose origin is the internal surfaces of the 7^{th} to 12^{th} costal cartilages, thoracolumbar fascia, iliac crest, and lateral third of the inguinal ligament and inserts in to the linea alba and the aponeurosis of the internal oblique as well as the pubic crest and pecten pubis and D) rectus abdominus which originates at the pubic symphysis and pubic crest and inserts in to the xiphoid process and 5th-7th costal cartilages.

1.2.6 ACCESSORY EXPIRATORY MUSCLES

Of the accessory expiratory muscles, the internal interosseous intercostal muscles and the triangularis sterni are considered the most important. The internal interosseous intercostal muscles are located in the ventral intercostal spaces (Figure 1.7B). The fibres run in a caudal-dorsal direction from the rib above to the rib below (De Troyer et al. 2005) and are superficial to the external intercostals. The total muscle mass of the internal interosseous intercostals is approximately 0.05% of total body mass. They are preferentially recruited from the bottom to the top of the chest wall.

The triangularis sterni (Figure 1.11) lies deep to the parasternal intercostals and the sternum. They may be activated in concert with the abdominal muscles below FRC which may promote the passive ascent of the chest wall during subsequent inspiration (De Troyer and Estenne 1988).

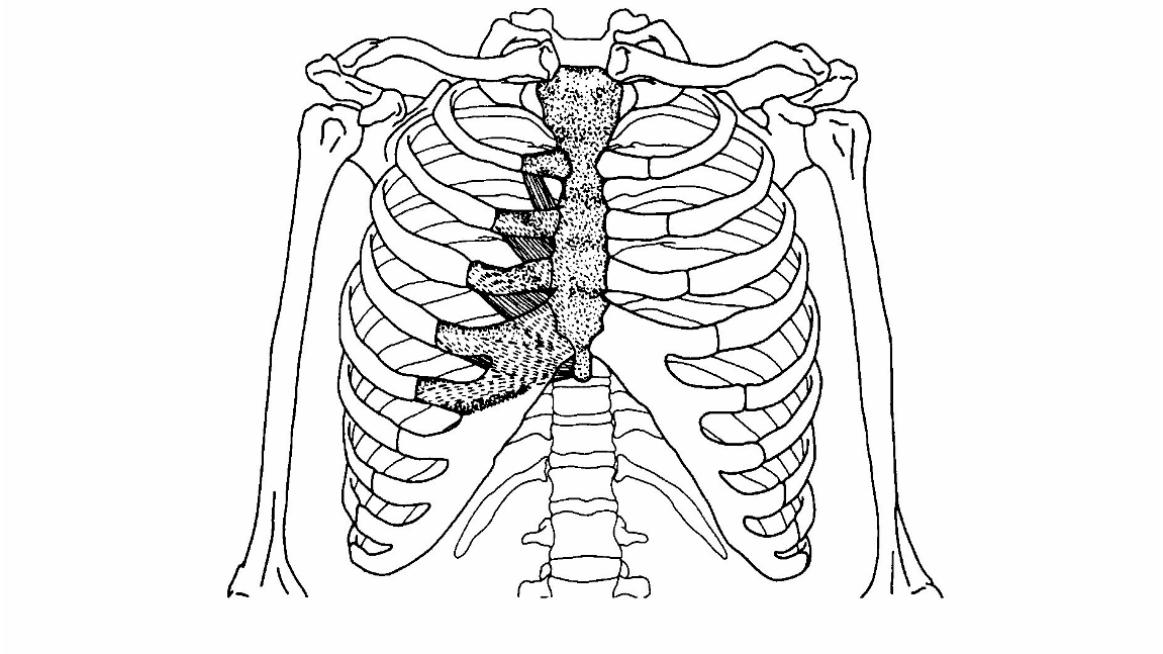

Figure 1.11 Anterior view of the trunk illustrating the triangulairs sterni (Stone and Stone, 2000). The triangularis sterni originates at the dorsal aspect of the caudal half of the sternum and inserts into the inner surface of the costal cartilages of ribs 3 to 7.

1.3. PHYSICAL CHARACTERISTICS OF THE RESPIRATORY MUSCLES

1.3.1 RESPIRATORY MUSCLE MORPHOLOGY

Few studies have determined the morphology of human respiratory muscles. Muscle fibre type distribution determined in male human cadavers indicate 60% type I muscle fibres in the internal and external intercostals and 49% in the diaphragm (Mizuno and Secher 1989; Figure 1.12). This is similar to findings recently observed in the healthy living human diaphragm (Nguyen et al. 2000). Type IIa distribution was similar between the internal intercostals and diaphragm (~30%) yet only 17% in the external intercostals. In contrast, Type IIx was similar between the external intercostals and diaphragm (~25%) and only 1% in the internal intercostals. It is interesting to note that the distribution of myosin heavy chain isoforms is remarkably similar to that of the vastus lateralis (Figure 1.12).

Indeed, it appears that the superior oxidative capacity of the diaphragm is not explained by muscle architecture, but rather its vascular supply, enzyme activity and mitochondrial density. These additional morphological adaptations are not present in the accessory respiratory muscles.

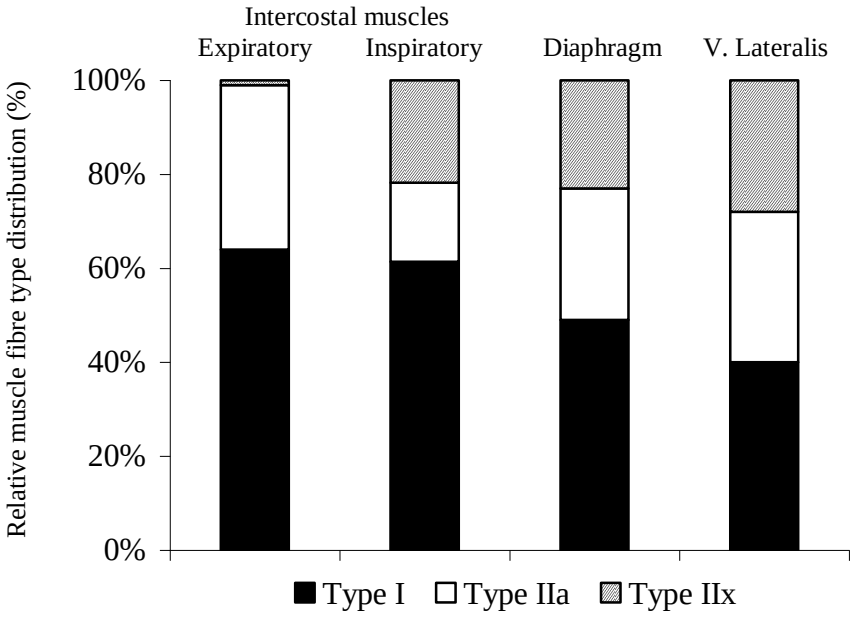

Figure 1.12 Relative distribution of human slow twitch (type I), fast twitch a (type IIa) and fast twitch x (type-IIx) muscle fibres for the expiratory and inspiratory intercostals, the costal diaphragm and the vastus lateralis (Adapted from Mizuno and Secher 1989).

1.3.2 RESPIRATORY MUSCLE VASCULATURE

Data from ponies shows that diaphragm blood flow increases from 11.5 ± 2.8 $ml \cdot min^{-1} \cdot 100g^{-1}$ at rest to 265 ± 35.9 $ml \cdot min^{-1} \cdot 100g^{-1}$ during maximal exercise which was greater than all other respiratory and non-respiratory skeletal muscles (Manohar 1986). Harms et al. (1998) estimated that during maximal exercise 14 to 16% of total cardiac output ($\dot{Q}$) was directed to the respiratory muscles. Using the thermodilution technique, Harms and colleagues measured locomotor muscle blood flow during maximal cycling exercise (control condition) and also when the respiratory muscles were unloaded using a proportional assist ventilator (PAV). PAV forces air into the lungs during inspiration with a positive pressure, thus partially unloading or reducing the work of the inspiratory

muscles by 40 to 50%. Leg blood flow (20.3 ± 0.5 $L \cdot min^{-1}$) was unchanged when the respiratory muscles were unloaded yet total $\dot{Q}$ decreased. Therefore, the reduction in total $\dot{Q}$ with PAV was assumed to be equal to the respiratory muscle blood flow (4.2 ± 0.1 $L \cdot min^{-1}$); however, since the work of breathing was unloaded by only ~50%, this was likely to underestimate the true respiratory muscle blood flow. Of the total $\dot{Q}$ available during maximal exercise, 77% was directed to the locomotor muscles, 16% to the respiratory muscles and 7% to other metabolically active tissues.

The large blood flow to the respiratory muscles is achieved by the vast vascular supply which more closely reflects the vascular supply of the brain rather than other skeletal muscles (Comtois et al. 1987). Unlike locomotor skeletal muscles which have a single arterial supply, the diaphragm has multiple. These include the superior and inferior phrenic arteries, intercostal artery and internal mammary artery which originate from the thoracic branch of the descending aorta (Comtois et al. 1987). This multiple arterial supply provides an amazing protective arrangement against ischemia. Although the morphology of the diaphragm and the vastus lateralis are similar, the number of capillaries per muscle fibre in the human diaphragm is much greater. The area and circumference of diaphragm muscle fibres are also smaller than all other skeletal muscles (Mizuno and Secher 1989). This results in a significantly lower diffusion distance in the diaphragm and a 2 to 3 times larger capillary bed than that found in locomotor skeletal muscles (Mizuno and Secher 1989; Hoppeler et al. 1981). However, following specific strength training of the inspiratory muscles, an 8 to 12% increase in diaphragm thickness is observed (Chiappa et al. 2008a; Downey et al. 2007; Enright et al. 2008). Whether this may increase the diffusion distance and / or reduce the number of capillaries per muscle fibre is unknown.

1.4 RESPIRATORY MUSCLE MECHANICS DURING EXERCISE

1.4.1 BREATHING MECHANICS

Ward et al. (1992) proposed that the volumes of the chest wall are described by three distinct compartments including 1) the upper rib cage apposed to lung (Vrc,p) and therefore exposed to *P*pl, 2) the rib cage apposed to the diaphragm (Vrc,a, i.e. the zone of apposition) where *P*pl is equal to *P*ab and 3) the abdomen (Vab) which is equal to gastric pressure (*P*ga). Kenyon et al. (1997) used a geometric optical reference system ('ELITE', ELaboratore di Immagini TElevisive, Milan Polytechnic, Milan, Italy; see Figure 1.13) to quantify the pressure and volume characteristics of the different compartments during exercise. Four digital cameras tracked 86 surface hemispherical reflective markers (Cala et al. 1996) and provided 3D real-time changes in the volume of a respective chest wall compartment (Figure 1.13). Using this novel method, the authors demonstrated that during submaximal exercise of increasing intensity (0, 30, 50 and 70% $\dot{W}max$) the precise coordination of the respiratory muscles was shown to maximise chest wall compliance and minimise the elastic work of breathing.

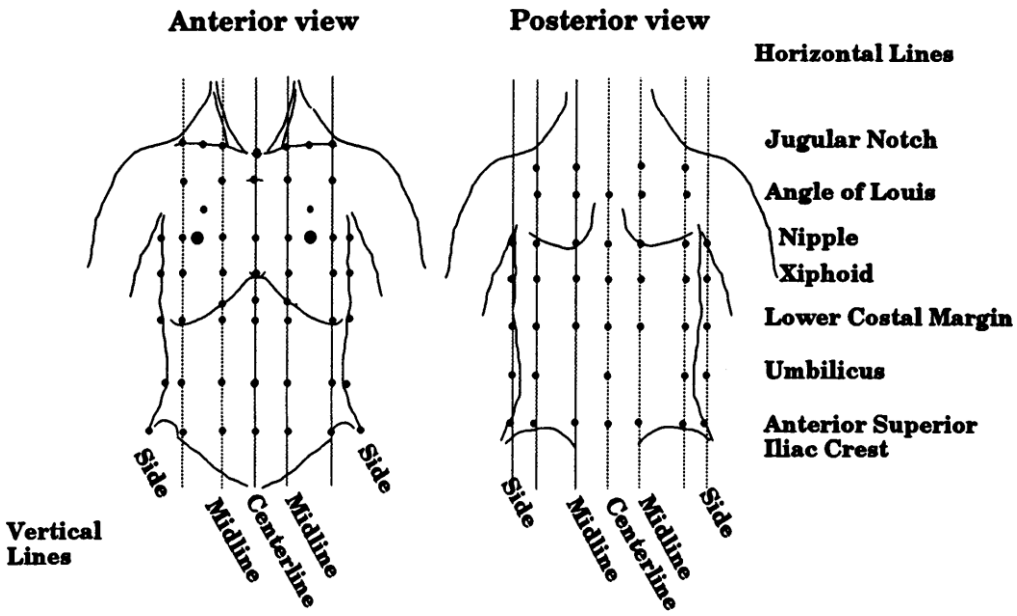

Figure 1.13 Placement of the 86 reflective markers on the thorax (Cala et al. 1996; Kenyon et al. 1997)

However, the relative contribution and interactions between the diaphragm, chest wall and abdominal muscles in achieving this co-ordinated action are less well understood. Aliverti et al. (1997) used the optoelectronic plethysmography motion analysis system to determine the relative contributions of the chest wall muscles, the diaphragm and the abdominal muscles to the changes in compartmental volumes and pressures at rest and sub-maximal cycle ergometry exercise (0, 30, 50 and 70% $\dot{W}max$). The contribution of each muscle to changes in force (pressure) and velocity (flow) were also determined. At rest, diaphragm shortening accounted for the majority of the increase in lung volume. This increased Vrc,p, Vrc,a and since the abdominal compartment is virtually incompressible, Vab also increased (Figure 1.14). At the onset of exercise and with increasing intensities, the increase in V_T was achieved by a large increase in end inspiratory Vrc,p (+1.0 L) and Vrc,a (+0.5 L). During expiration due to the increase in abdominal muscle recruitment a significant decrease in Vab was observed (-0.98 L; Figure 1.14). By utilising the inspiratory and expiratory reserve volumes the chest wall utilises the most compliant compartments of the system which reduces the elastic work of breathing and unnecessary forces to restore the geometry of the thorax (Kenyon et al. 1997). By increasing the end-inspiratory Vrc,p throughout exercise the parasternal intercostals are placed at a more efficient position on their length-tension curve since the optimal length of the parasternal intercostal is shorter than the diaphragm at FRC (Decramer and De Troyer 1984).

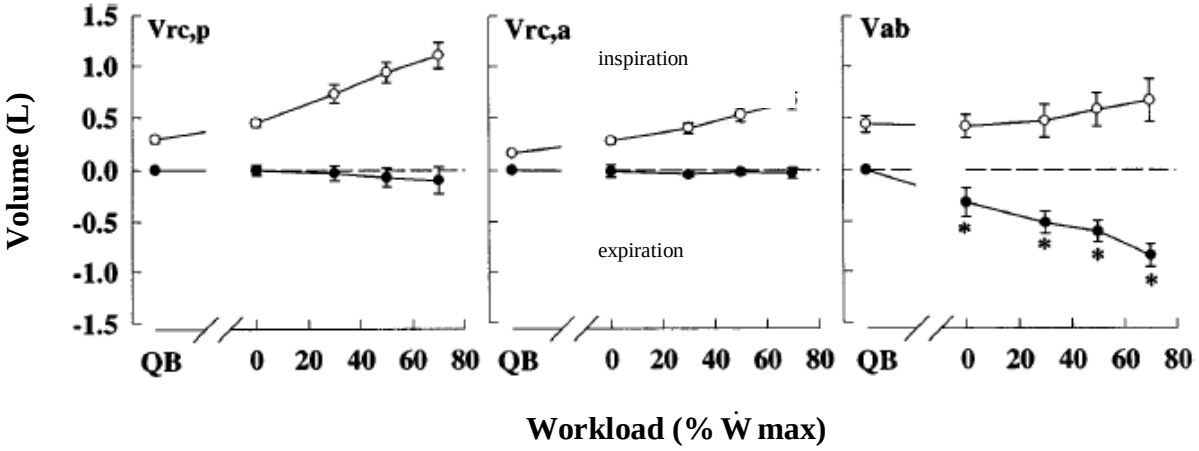

Figure 1.14 Changes in volume of the abdomen (Vab), the rib cage apposed to the diaphragm (Vrc,a) and the rib cage apposed to lung (Vrc,p) at rest with quiet breathing (QB) and during exercise (Aliverti et al. 1997).

Aliverti et al. (1997) demonstrated that as the exercise intensity increased up to 70% $\dot{W}max$, non-diaphragmatic respiratory muscle recruitment increased. This is demonstrated clearly in Figure 1.15A where the pressure developed by the diaphragm, the expiratory intercostals and the abdominal muscles are plotted as a fraction of the pressure developed by inspiratory intercostals. As exercise intensity increases, the diaphragm relative to the inspiratory rib cage muscles (illustrated by ■) contributed less over time to changes in inspiratory pressure. Figure 1.15B shows that this was because of an increase in the passive work performed by the diaphragm during inspiration (illustrated by Δ). The greater passive work performed by the diaphragm was achieved by a gradual relaxation of the abdominal muscles throughout inspiration which minimises the change in *P*di and is in stark contrast to resting breathing where *P*ga increases throughout inspiration. Thus during exercise the gradual relaxation of the abdominal muscles essentially unloads the diaphragm and permits it to achieve the high flow rates required during intense exercise. The total work of each muscle group was calculated as the area contained within the individual pressure-volume loops (see Figure 1.16B) and divided by time to calculate power. Since the pressure was known, the power output of each muscle group was partitioned into the relative contributions of force and velocity of shortening. Despite a similar increase in power output by the diaphragm, abdominal muscles and the rib cage muscles, in the diaphragm this was achieved primarily by an increased velocity of shortening. In contrast,

the increase in abdominal and rib cage muscle power output was achieved by large increases in pressure which act to displace the abdomen and rib cage (Aliverti et al. 1997).

In addition to minimising the change in *P*di throughout inspiration during exercise, the abdominal muscles also assist the function of the diaphragm in other ways. An increase in *P*ab reduces end-expiratory lung volume (EELV) to achieve a required V_T and prevents excessive shortening of the diaphragm, thus placing it at a more advantageous position on its length-tension curve (Martin et al. 1982). Also, by reducing EELV, the diaphragm is stretched, thus storing elastic energy. The abdominal muscles act as a fulcrum for subsequent diaphragm contraction (Dempsey et al. 1996) and at the onset of inspiration promotes an initial passive descent of the diaphragm.

The findings of Aliverti et al. (1997) and Kenyon et al. (1997) demonstrate that the diaphragm acts primarily as a flow generator during exercise and other inspiratory muscles generate pressure which displaces the chest wall. This action unloads the diaphragm and promotes high flows rates whilst minimising chest wall distortion. If this interaction was not present, *P*di would increase in order to displace the rib cage rather than produce changes in inspiratory air flow. This would significantly increase the work performed by the diaphragm during exercise and increase the likelihood of diaphragm fatigue.

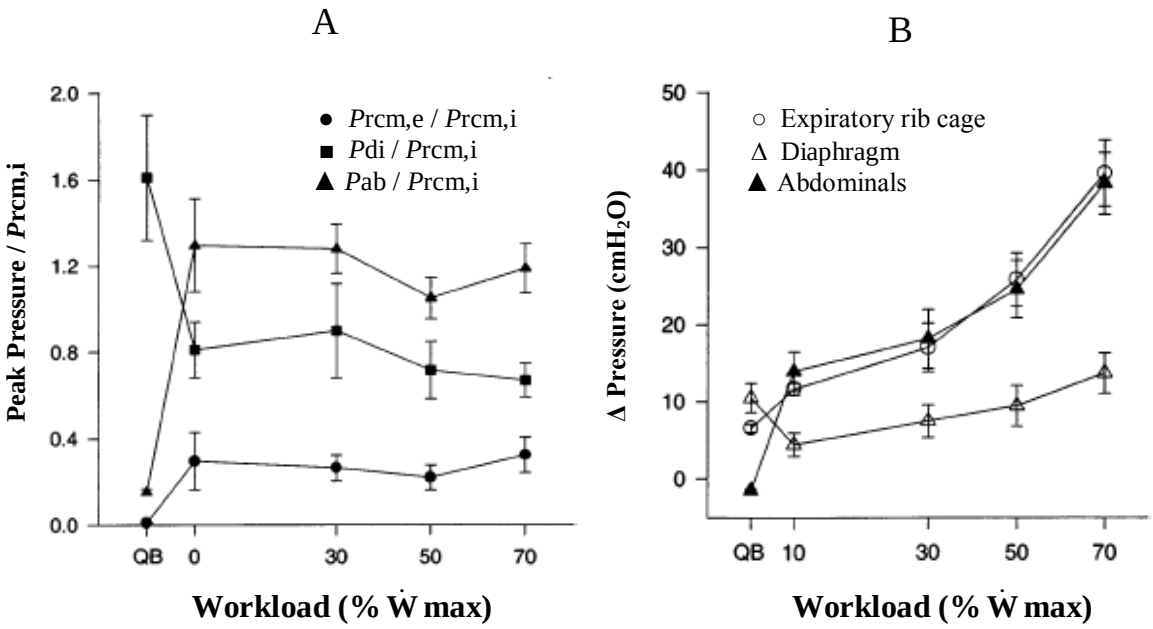

Figure 1.15 A) Peak pressure of the expiratory rib cage muscles (*P*rc,e), the diaphragm (*P*di) and the abdominal muscles (*P*ab) normalised to inspiratory rib cage muscle pressure (*P*rcm,i) and B) pressure generation in the inspiratory and expiratory rib cage muscles (○) and the diaphragm (Δ) from the start of inspiration to the end of inspiration and in abdominal muscles (▲) from end inspiration to the end of expiration. The change in *P*di is the difference between the passive *P*di at the start of inspiration and the active *P*di at the end of inspiration (Aliverti et al 1997).

1.4.2 THE WORK OF BREATHING

The work of breathing reflects the respiratory muscle metabolic and / or energetic cost of pulmonary ventilation. This can be determined *directly* by integrating the change in intra-thoracic pressure generated by the respiratory muscles for a given change in lung volume. The area subtended by the resultant pressure:volume loop represents the total work performed over a specific time interval (Figure 1.16B). *Indirectly* the work of breathing can be measured by calculating the difference between the whole-body $\dot{V}O_2$ with and without specific breathing tasks which mimic the breathing pattern and minute ventilation ($\dot{V}_E$) achieved during exercise (Milic-Emili 1991).

During exercise the respiratory muscles must perform elastic and resistive work. Elastic work is the energy required to expand the lung and chest wall and overcome the natural elastic recoil force; operating at a greater lung volume will elevate the elastic work done. Resistive work describes the energy required to increase inspiratory and expiratory flow rates (Dempsey et al. 2006a). Of these, the former is minimised during exercise as the

respiratory system typically follows the minimal work trajectory by utilising the most compliant part of the chest wall (Kenyon et al. 1997), however, the latter is increased during intense exercise when pulmonary air flow becomes turbulent and during expiration the internal diameter of the airways is reduced. Airway narrowing occurs because the intrathoracic pressure exceeds the critical expiratory pleural pressure (Wetter and Dempsey 2000). Elastic work may be further increased with exercise modalities where the caudal displacement of the diaphragm is restricted by the abdominal cavity such as the catch position of the rowing stroke or in the crouched position during a cycling time-trial (Steinacker et al. 1993).

Previous studies determined the work of breathing of dynamic whole-body cycling exercise at 70 and 100% $\dot{V}O_2$ max by mimicking the transpulmonary pressure (*P*tp):V_T loop, EELV and f_R achieved during exercise under resting conditions. *P*tp was used as an estimate of the total respiratory muscle work required to expand the chest wall and lungs (where *P*tp = oesophageal pressure [*P*oe] – mouth pressure [*P*m]) and EELV was measured using inductance plethysmography to control respiratory muscle length (Aaron et al. 1992a, b). The rigorous control of these parameters is fundamental to accurate work of breathing assessment during volitional hyperpnoea since a self-selected spontaneous breathing pattern to attain a given $\dot{V}_E$ is more costly and the respiratory muscle $\dot{V}O_2$ can be up to 25% greater than that of exercise with an identical breathing pattern (Coast et al. 1993). The higher $\dot{V}O_2$ throughout volitional hyperpnoea is likely due to a faster f_R (tachypnoea) and recruitment of less efficient accessory (chest wall) respiratory muscles (Coast et al. 1993), which may also affect the dynamic EELV.

Respiratory muscle $\dot{V}O_2$ was calculated as the change in pulmonary $\dot{V}O_2$ for a given change in $\dot{V}_E$ ($\Delta\dot{V}O_2 / \Delta\dot{V}_E$) during the mimic trial (Aaron et al. 1992a). During volitional hyperpnoea, the O_2 cost of breathing was 1.8 ml O_2 per L·min^{-1} of $\dot{V}_E$ at 70% $\dot{V}O_2$ max which increased to 2.9 ml O_2 per L·min^{-1} of $\dot{V}_E$ at 100% $\dot{V}O_2$ max (Aaron et al.

1992a). This equated to 4.6 ± 0.4% and 10.0 ± 0.5% of the total whole-body $\dot{V}O_2$ at 70 and 100% $\dot{V}O_2$ max , respectively (Aaron et al. 1992b). In subjects where $\dot{V}_E$ exceeded 150 L·min^{-1} during exercise, which from anecdotal evidence is common in healthy active males, the O_2 cost of breathing increased substantially to 15.4% of the total $\dot{V}O_2$ (Aaron et al. 1992b). These data suggest that the energetic demands of the respiratory muscles can be profound during exercise when a high $\dot{V}_E$ is attained. Given that the increase in *P*di during exercise is achieved by a large increase in the velocity of shortening suggests that the substantial increase in $\dot{V}O_2$ is likely due to the increased recruitment of accessory muscles which illustrates that these muscles are far less economical. Thus delaying the recruitment of these muscles and / or improving their functional efficiency may serve to reduce the work of breathing and improve exercise tolerance.

1.4.3 DETERMINANTS OF THE WORK OF BREATHING

The work of breathing is not solely attributable to the absolute $\dot{V}_E$ attained during exercise but rather the proportion to which the inspiratory pressure developed and expiratory flow rates encroach upon the boundaries of individual maximal flow:volume and pressure:volume loops determined under resting conditions (Figure 1.16A and B; Klas and Dempsey 1989). The respiratory muscles display both force:velocity (pressure:flow) and length:tension (lung volume:pressure) relationships similar to other skeletal muscles (Hyatt and Flath 1966). For example, the pressure generating capacity is reduced with an increase in EILV (greater inspiratory muscle length) and when the velocity of shortening is increased (high inspiratory flow rates). Thus, it appears that the work of breathing during exercise is dependent upon the proportion to which the tidal pressure and flow characteristics approach / converge with the boundaries of their maximal capacities.

Conceptually, it is difficult to appreciate the mechanical limits imposed upon exercise hyperpnoea and how this may exacerbate the work of breathing. During exercise inspiratory muscle length is dependent upon a number of factors which include antagonist shortening, expiratory flow rates and the magnitude of expiratory flow and expiratory pressure generation (i.e. the *P*ga). For example, the transition from rest to light exercise increases expiratory muscle recruitment, *P*ga and expiratory flow rates. As a result, EELV is reduced and V_T increases (Henke et al. 1988). With increasing exercise intensities EELV is further reduced until the expiratory flow rate approaches or exceeds the maximal capacity to produce expiratory flow (Figure 1.16A: grey area); here, any further increases in expiratory pressure generation (Figure 1.16B, grey area) fail to increase expiratory flow and results in dynamic airway compression as the critical pressure of the airways is exceeded (Klas and Dempsey 1989). To avoid expiratory flow limitation, the operating lung volume is increased. This is achieved by increasing EELV and EILV (see also: Figure 1.15, specifically Vrc,a and Vab) and subsequently shifts the V_T to a greater percentage of TLC; a process known as dynamic hyperinflation. Thus, despite maintaining expiratory flow rates and $\dot{V}_E$, increasing EILV places the diaphragm at a greater (more inefficient) operating length and reduces the inspiratory pressure generating capacity (Figure 1.16B: red area). This series of events throughout exercise is known to increase the sensations of breathlessness during incremental cycle ergometry (Kayser et al. 1997) possibly due to an increase in f_R (McClaran et al. 1999). The increase in expiratory intrathoracic pressure swings may also affect both cardiac output and O_2 delivery to the working muscles (Aliverti et al. 2005). The heart and the vessels of the within the thorax are exposed to such pressure swings. In the face of expiratory flow limitation and increased expiratory pressures, ventricular transmural pressure is reduced, thereby reducing decrease the rate of ventricular filling during diastole; this reduces both cardiac output and stroke volume (Dempsey et al. 2008). Furthermore, venous return is reduced despite an active locomotor

muscle pump when positive expiratory pressures are generated within the abdominal compartment (Miller et al. 2005).

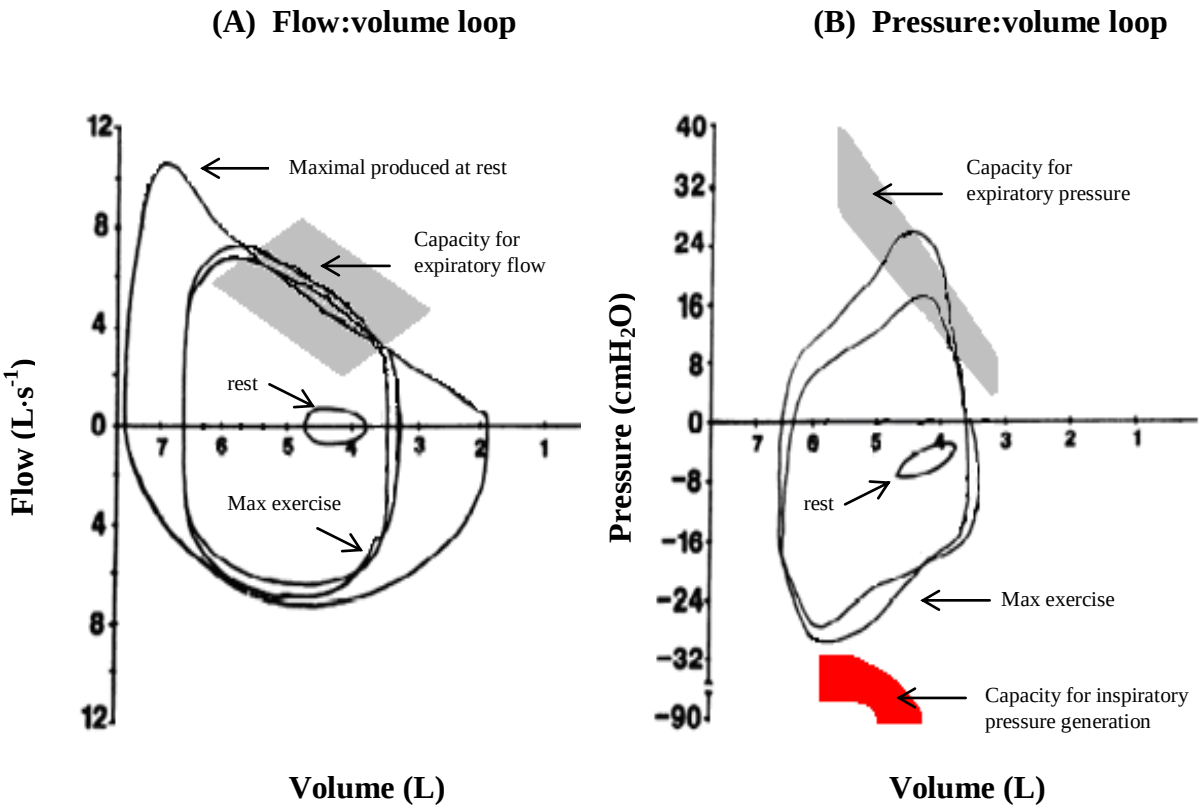

Figure 1.16 (A) Flow:volume loop. Grey area: portion of tidal breathing meeting the maximal limits for expiratory flow, indicating expiratory flow limitation; (B) Pressure:volume loop. Grey and red areas: the maximal capacity to generate expiratory and inspiratory pressure for a given lung volume, respectively.

The magnitude to which the flow:volume and pressure:volume characteristics of the tidal breath encroach upon their maximal limits determines the severity of expiratory flow limitation, dynamic hyperinflation and thus the work of breathing. This hypothesis was supported by Johnson et al. (1992) who described the flow and pressure:volume responses during maximal exercise in well trained endurance athletes. Eight healthy males ($\dot{V}O_2$ max : 73.0 ± 1.0 ml·kg^{-1}·min^{-1}) performed an incremental exercise test followed by repeated 3 min bouts of maximal exercise on a motorised treadmill. During sub-maximal exercise ($\dot{V}_E$: 117.3 ± 6.7 L·min^{-1}) expiratory flow rates reached the limits of the maximal flow:volume loop only towards the very end of expiration and inspiratory pressure

development was sub-maximal. During maximal exercise however ($\dot{V}_E$: 168.7 ± 5.4 $L \cdot min^{-1}$), up to 61 and 46% of the tidal volume encroached on the maximal capacity for expiratory flow and pressure, respectively. Furthermore, the inspiratory muscles utilised up to 89% of the maximal pressure generating capacity; this was due to the increase in EILV and inspiratory flow rates. Thus dynamic whole-body exercise weakens the inspiratory muscles by reducing their pressure generating capacity (LeBlanc et al. 1988).

These findings were supported by McClaran et al. (1999) who prevented expiratory flow limitation during maximal exercise in six well trained male cyclists ($\dot{V}O_2$ max : 65.0 ± 8.0 $ml \cdot kg^{-1} \cdot min^{-1}$) by substituting the inspired air with a less dense gas mixture comprised of helium (He) and O_2 (heliox; F_IO_2: 0.26-balance He). Inspiring heliox reduces the pressure generated to forcefully expire a given volume of air since airway resistance is attenuated, thereby increasing the maximal flow-volume loop performed at rest. Relative to the control trial (F_IO_2: 0.26-balance N_2) when breathing heliox EILV was not different. However, maximal expiratory flow rates and V_T were increased and EELV was reduced. Consequently, flow limitation was also reduced from 43% to less than 10% of the V_T and the reduced capacity of the inspiratory muscles to generate pressure was attenuated. It is interesting to note that inspiratory flow limitation does not occur since increases in inspiratory pressure would serve to increase rather than decrease the internal diameter of the airways. Collectively, the findings of Johnson et al. (1992) and McClaran et al. (1999) show the importance of expiratory flow limitation in determining EELV and the pressure generating capacity of the inspiratory muscles. Although expiratory flow limitation inhibits the mechanical advantage of the inspiratory muscles, without such an evolutionary mechanism and progressive hyperinflation, excessive intrathoracic pressures would be routinely developed to overcome the narrowing of airways in order to preserve expiratory flow rates. As a consequence, the severity of flow limitation would exacerbate respiratory motor output for a given expiratory flow rate which may intensify the sensations of dyspnoea. It has also been shown that an increase in thoracic pressure resulting from

expiratory flow limitation reduces venous return (Miller et al. 2005) by cuffing the inferior abdominal vena cava which exceeds the driving pressure from locomotor skeletal muscle pump and recoil pressure generated by distension of the locomotor veins. A reduction in venous return attenuates cardiac output by reducing the transmural pressure gradient across the walls of the heart and inhibits left ventricular preload (Miller et al. 2007). These mechanisms are important since they reduce limb muscle perfusion and O_2 delivery which attenuates endurance performance (Miller et al. 2006), since locomotor force output is particularly sensitive to even small changes in SaO_2 (Romer et al. 2006b).

Whole-body endurance training does not affect the maximal flow:volume loop (Johnson et al. 1992), thus a mechanism related directly to this is unlikely to attenuate expiratory flow limitation. Recent studies have illustrated that short-term whole body training (exercise, 2 sessions·day^{-1} for 7 days) enhances vasorelaxation of porcine pulmonary arteries due to acute augmented sheer-stress on the vessel walls (Johnson et al. 2001). Such an adaptation may promote greater pulmonary blood flow, O_2 exchange and delivery to the working muscle which may well improve locomotor tolerance (Romer et al. 2006b). However, given the occurance of pulmonary shunt and ventilation-perfusion inequality (West 2000) that exists during human whole-body exercise, questions the ergogenic effect of such an adaptation, particularly in light of expiratory flow limitation. Notwithstanding this, following whole-body endurance training for a given absolute exercise intensity and thus $\dot{V}_E$, the work of breathing may be attenuated as respiratory compensation is typically reduced. Although this is unlikely to influence the work of breathing for a given relative exercise intensity (Babcock et al. 1996). It is important to note that the occurrence of flow limitation was highly variable: despite having a similar endurance training status, some participants in the study by Johnson and colleagues (1992) experienced severe flow limitation whilst for a similar $\dot{V}_E$ others did not. This was despite similar pulmonary function and is likely due to inter-individual differences in the exercise ventilatory response to feedback (chemical and mechanical) and feedforward stimuli

(peripheral chemoreflex sensitivity). Recently however, studies have reported a genetic link between chronic aerobic training and pulmonary adaptation during exercise. In a series of elegant studies, Kirkton et al. (2009) and Howlett et al. (2009) reported that 15th generation rats selective bred for either their high or low endurance running ability in the same environmental conditions demonstrated markedly different pulmonary and cardiovascular responses during exercise. The endurance selective group showed around a 50% increase in $\dot{V}O_2max$ (measured in absolute units; Howlett et al. 2009) attributed largely to an increased conductive O_2 transport and ~30% increase in alveolar ventilation during maximal exercise (Kirkton et al. 2009). Such unique genetic adaptations achieved in rats does lend support to the notion that endurance athletes may well have an advantage over their sedentary counterparts (assuming their ancesters were also well trained). Such selective pulmonary adaptations may well pre-dispose an athlete to a lower $\dot{V}_E$ at maximal exercise and thus attenuate the potential debilitating effects of flow limitation on exercise tolerance.

It is unlikely that expiratory muscle training would attenuate flow limitation since increasing the pressure generating capacity of this muscle group would merely exacerbate dynamic airway compression. Whether increasing the strength of the inspiratory muscles would reduce the work of breathing is unknown. Increasing the strength of the inspiratory muscles would increase their maximal pressure generating capacity and possibly reduce the absolute pressure generated during exercise for a given $\dot{V}_E$. Specific inspiratory muscle training (IMT) may also alter the duty cycle (T_I/T_{tot}), by speeding inspiration. The reduction in duty cycle would prolong expiratory duration, reducing mean expiratory flow rates and possibly flow limitation. This may offset the transient increase in EELV and EILV. Whether such a mechanism is present or would have a detectable effect upon exercise performance and breathing mechanics is unknown. However, it was reported that following IMT, during maximal incremental exercise despite a similar relative exercise intensity, a non-significant increase in V_T was observed which was achieved by an increase

in EILV (Romer et al. 2002c) suggesting that flow limitation or indeed hyperinflation was attenuated.

1.5 PHYSIOLOGICAL CONCEQUENCES OF THE WORK OF BREATHING

1.5.1 RESPIRATORY MUSCLE FATIGUE: VOLITIONAL MEASURES

Fatigue can be defined as "a condition in which there is the loss in the capacity of a muscle for developing force and / or velocity, resulting from muscle activity under load and which is reversible by rest" (NHLBI 1990). One of the most common measurements used by respiratory physiologists to assess global respiratory muscle fatigue (McConnell and Romer 2004a) is the maximal inspiratory and / or expiratory mouth pressure (MIP and MEP respectively). MIP and MEP are quasi-static efforts which reflect the global pressure generating capacity of the respiratory muscles contracting in synergy; they are, however, dependent on participant motivation and task learning. Such measurements also lack specificity and fail to provide an indication to the underlying mechanism(s) of fatigue. Furthermore fatigue of an individual inspiratory muscle, for example, the diaphragm, is not always reflected by volitional measures (Johnson et al. 1993).

Decreases in MIP and MEP have been reported following different exercise intensities and durations. Loke et al. (1982) reported significant reductions in MIP and MEP following a 3 hr and 24 min marathon (MIP rest: 166 ± 11 cmH_2O, post-exercise: 139 ± 8 cmH_2O, $P<0.05$; MEP rest: 240 ± 20 cmH_2O, post-exercise: 173 ± 23 cmH_2O, $P<0.05$). Similar data are reported for a simulated marathon on a motorised treadmill with reductions in MIP from 118 ± 20 cmH_2O at rest to 100 ± 22 cmH_2O. No changes were observed however in MEP (Ross et al. 2008). In agreement with this, Chevrolet et al. (1993) also observed a 25% decrease in MIP but not MEP following a marathon.

Inspiratory and expiratory muscle fatigue has been observed following high-intensity, short duration exercise. Bye et al. (1984) observed a significant reduction in volitional *P*di max during a maximal inspiratory effort following 5.9 ± 1.3 min of

exhaustive cycling exercise at 80% $\dot{W}$max. Similar reductions in MIP (~10%) were observed following a 6 min all out rowing ergometer test (Volianitis et al. 2001), a 200 m race pace swim (29%; Lomax and McConnell 2003) and following maximal incremental exercise (17%; Ozkaplan et al. 2005). Reductions in MIP are also noted after 20, 25 and 40 km cycling time trials (12 to 18%; Johnson et al. 2007; Romer et al. 2002c). In contrast to these findings, Perret et al. (1999) detected no evidence of inspiratory or expiratory muscle fatigue assessed by MIP and MEP measurements, respectively, following whole-body exercise to volitional failure at 85% $\dot{V}O_2$max. Measurements in this study were performed 5 min post-exercise whereas in previous studies measurements were performed within 2 to 3 min of end exercise (Johnson et al. 2007; Romer et al. 2002c). This suggests that subjects are either unable to perform the manoeuvre correctly immediately post-exhaustive exercise likely due to post-exercise hyperventilation and the overwhelming drive to breathe and / or that the generation of volitional force recovers very quickly (< 5 min). Despite this, these findings suggest that volitional measures of inspiratory and less frequently, expiratory muscle strength, are reduced following intense exercise. This is in stark contrast to non-volitional measures of respiratory muscle fatigue which can be depressed for up to 70 ± 4 min following exhaustive exercise (Johnson et al. 1993). Differences in post-exercise reductions in MIP and MEP are not explained by training status as MIP is not different between athletes and non-athletes (Coast et al. 1990; Eastwood et al. 2001). However, McConnell et al. (1997) illustrated that the reduction in MIP following exhaustive exercise was greater in participants with a lower baseline MIP.

Reductions in respiratory muscle endurance have also been observed following exercise. This may reflect a reduction in central respiratory muscle motor drive. Loke et al. (1982) report a 10% reduction in maximal voluntary ventilation (MVV) following a marathon. Boussana et al. (2001) observed a lower inspiratory pressure threshold (75% MIP) breathing time following 20 min intense mixed cycle-run exercise. Similarly, following whole-body exercise to volitional failure at 85% $\dot{V}O_2$max, inspiratory resistive

breathing time at 79 ± 9% MIP was reduced from 364 ± 88 s at rest to 219 ± 122 s post-exercise (Perret et al. 1999). These latter findings were also confirmed following exercise to volitional tolerance at 65, 75, 85, and 95% $\dot{V}O_2max$ (Perret et al. 2000). Changes in post-exercise respiratory muscle endurance may however be affected by endurance training status. Martin and Stager (1981) indicate that ventilatory endurance (MVV_{12} to exhaustion) is greater in athletes vs. non-athletes. Also 20 wk endurance training has been shown to improve ventilatory function (maximal sustainable ventilation for 20 min; Robinson and Kjeldgaard 1982). Evidence also suggests that training status affects respiratory muscle endurance and the breathing pattern adopted during a progressive inspiratory pressure threshold loading test. Relative to their untrained counterparts, endurance trained participants (marathon runners training 10 ± 6 $h \cdot wk^{-1}$) achieved a greater peak inspiratory pressure at the cessation of the test (90% vs. 78% MIP) which was achieved through a larger V_T, lower f_R and T_I/T_{tot} yet longer inspiratory and expiratory durations (Eastwood et al. 2001). Therefore, the breathing pattern adopted by untrained subjects may well increase the fraction of each breath the vessels of the inspiratory muscles are occluded (Bellemare et al. 1983). This may influence oxygen delivery and metabolite washout from the respiratory muscles, ultimately affecting respiratory muscle endurance. Thus relative to the untrained subject, studies recruiting well trained athletes may report smaller reductions in respiratory muscle endurance. In summary, volitional measures of respiratory muscle strength and endurance are reduced following exercise. However, the extent to which these are attributed to changes in central drive and / or peripheral mechanisms is unknown. Furthermore these techniques are fundamentally dependent upon subject motivation and task learning, thus their use as a quantitative measure of respiratory muscle fatigue is limited.

1.5.2 RESPIRATORY MUSCLE FATIGUE: NON-VOLITIONAL MEASURES

Non-volitional techniques have been developed where a supramaximal electrical or magnetic charge stimulates all of the nerves that innervate a muscle. For the respiratory muscles, the change in force output is measured by inserting balloon catheters per-nasally into the stomach (*P*ga) and lower one third of the oesophagus (*P*oe). By measuring the difference in pressure within the two compartments, the force generated by the diaphragm can be measured (*P*di = *P*ga - *P*oe). Bilateral phrenic nerve stimulation (BPNS) has been used to quantify *P*di prior to and following whole-body exercise (Johnson et al. 1993). Similarly, stimulation of the cervical (Similowski et al. 1998; Verges et al. 2006) and thoracic nerve roots (Taylor et al. 2006; Taylor and Romer 2008) has been performed to activate the chest wall and abdominal muscles, respectively. These techniques provide reliable measurements of inspiratory and expiratory muscle fatigue (within-day reproducibility ~5 to 10%; Taylor and Romer 2009; Johnson et al. 1993) and have the ability to distinguish between central and peripheral origins of respiratory muscle fatigue.

1.5.3 EXERCISE INDUCED PERIPHERAL DIAPHRAGM FATIGUE

Peripheral or metabolic fatigue is defined as a reduction in muscle force due to processes at or distal to the neuromuscular junction (Allen et al. 2008; Fitts 1994). Prior to the early 1990s, it was generally accepted that the diaphragm did not fatigue during exercise based largely on the interpretation of spectral shifts in the surface diaphragm EMG (Bye et al. 1984). However, in 1993, exercise-induced diaphragm fatigue was reported for the first time using BPNS following intense exercise in well trained athletes (Johnson et al. 1993). This was followed up swiftly by Mador et al. (1993) in healthy, untrained subjects.

In the study by Johnson and colleagues (1993), 12 well trained subjects ($\dot{V}O_2$max: 61 ± 4 ml·kg·min^{-1}) performed exercise at ~85% $\dot{V}O_2$max and ~95% $\dot{V}O_2$max until volitional tolerance. Prior to and following exercise, *P*di was measured using BPNS. The

stimulation protocol included a single electrical pulse (twitch) and tetanic stimulation (400 ms) at 10 and 20 Hz. Stimulating the muscle for 400 ms at frequencies of 10 and 20 Hz was used to ensure treppe; this refers to the summation of single twitches and more closely reflects the contraction characteristics of a muscle during exercise. Single twitch *P*di was significantly reduced after exercise at ~85% $\dot{V}O_2$max. Following exercise at ~95% $\dot{V}O_2$ max, twitch *P*di and the response to tetanic stimulation at 10 and 20 Hz was also reduced by 21 ± 3 and 13 ± 2% ($P<0.05$), respectively. Interestingly, in all trials, the work performed by the chest wall muscles (shown by the integration of *P*oe over the period of inspiratory flow multiplied by the f_R; ∫*P*oe) and diaphragm (∫*P*di) increased from rest but following approximately 40% of the trial ∫*P*di reached a plateau whereas ∫*P*oe continued to increase. This action facilitated a continual time-dependent increase in $\dot{V}_E$ and was achieved by increased rib cage muscle recruitment. As a consequence, despite the presence of diaphragm fatigue, there was no sign of respiratory failure (i.e. a plateau or reduction in $\dot{V}_E$). Johnson et al. (1993) suggest that the change in respiratory muscle motor output (i.e. an increase in ∫*P*oe/∫*P*di) may indicate the onset of diaphragm fatigue since motor-output to the diaphragm is known to be reduced in the presence of peripheral diaphragm fatigue (Bellemare and Bigland-Ritchie 1987). Interestingly, the ∫*P*di attained during exercise was positively correlated with the reduction in evoked *P*di post-exercise ($r = 0.80$, $P<0.01$) suggesting that the magnitude of diaphragm fatigue may be a consequence of the work performed by the diaphragm during exercise.

Mador et al. (1993) observed similar reductions in twitch *P*di following intense exercise lasting 8.2 ± 4.1 min in healthy untrained subjects ($\dot{V}O_2$max: 35.6 ml·kg·min^{-1}). Twitch *P*di at rest was 28.9 ± 3.7 cmH_2O and decreased to 23.9 ± 5.1 cmH_2O following exercise (decrease ~17%; $P<0.05$) which was similar to Johnson et al. (1993) at both 10 and 20 Hz. In agreement with Johnson et al. (1993), Mador and colleagues observed a plateau in the work performed by the diaphragm and a continual rise in the work of

accessory inspiratory muscles. In both studies *P*di was lower than rest for up to 70 min post-exercise, after which it was similar to baseline. Collectively, these findings demonstrate that the reduction in evoked *P*di was caused by low frequency fatigue (LFF). LFF is characterised by reductions in force at low stimulation frequencies (single twitch, 10 and 20 Hz) and can last hours or even days (Laghi et al. 1995). The aetiology of LFF may be twofold: firstly by damage to the sarcomere and / or secondly, by disruption to the sarcoplasmic reticulum Ca^{2+} pump ($SRCa^{2+}$ pump; Jones 1996). Damage to the sarcomere is unlikely to have accounted for the reduction in *P*di following exercise in these studies since *P*di recovered within about one hour. Rather it appears that during exercise there was disruption to the $SRCa^{2+}$ pump. Data from exercising rats lends support to this notion. Matsunaga et al. (2002) reported that following high-intensity exercise lasting 4.79 min, diaphragm $SRCa^{2+}$ pump activity was reduced with a 22 and 24% decrease in Ca^{2+} release and uptake, respectively. These reductions have been ascribed to either increased concentrations of intracellular inorganic phosphate ($[P_i]$) which binds to Ca^{2+}, enters the SR and inhibits $SRCa^{2+}$ release, or possibly due to structural changes in the ATP or phosphorylation bindings sites within the $SRCa^{2+}$ pump (Matsunaga et al. 2002).

Exercise-induced diaphragm fatigue has also been observed following intense exercise at much higher stimulation frequencies suggesting that LFF is not solely responsible for diaphragm fatigue (50, 70 and 100 Hz; Babcock et al. 1998). Tetanic stimulation at these frequencies is intolerable for the subject (Polkey et al. 1997), therefore to avoid this a technique known as paired stimulation or ‘doublet’ is used where one twitch is followed rapidly by a second twitch. The peak force generated by the first twitch is subtracted from the second and the amplitude of the resultant force is recorded (the resultant force is called T_2; Yan et al. 1993). Measuring a reduction in T_2 at high stimulation frequencies which returns to baseline within approximately 30 min denotes the presence of high frequency fatigue (HFF).

In well trained participants ($\dot{V}O_2$max: 56.9 mL·kg^{-1}·min^{-1}), the T_2 response to paired BPNS was measured prior to and following 9.9 ± 0.5 min maximal exercise at 95% $\dot{V}O_2$max (Babcock et al. 1998). In agreement with previous findings (Johnson et al. 1993) V_T reached a plateau whereas f_R continued to increase which caused a gradual rise in $\dot{V}_E$ until exercise termination. The increase in $\dot{V}_E$ was achieved by a continual increase in $\int Poe/\int Pdi$ (~0.70 to ~0.85). Following exercise, reductions in the T_2 response were observed at 50, 70 and 100 Hz by 23.9 ± 0.9%. After 30 min of recovery, the T_2 response was not different to baseline. HFF is characterised by a reduction in the amplitude and slowing of the resultant twitch M-wave in conjunction with a reduced force output (Jones 1996). This is caused by interference in the propagation of the action potential along or throughout the t-tubule system. Babcock et al. (1998) did not observe any changes in M-wave amplitude or duration but a reduction in force was present. This suggests that the HFF was a result of impaired action potential transport through the t-tubule system likely due to an increased extracellular [K^+]. An increase in extracellular [K^+] may influence resting membrane potential, causing a depolarisation block and prolonging repolarisation due to Na^+-K^+ ATPase pump inhibition (Lindinger et al. 1995; Nielsen and Overgaard 1996).

1.5.4 PHYSIOLOGICAL INTERACTIONS DURING WHOLE-BODY EXERCISE WHICH LEAD TO DIAPHRAGM FATIGUE

Although exercise induced-diaphragm fatigue is correlated with the increase in *P*di during exercise, the work of the diaphragm during exercise may not be solely responsible. Babcock et al. (1995) measured *P*di in response to BPNS when the exercise *P*di and breathing pattern at 85 to 90% $\dot{V}O_2$max were mimicked at rest. Subjects mimicked the V_T, f_R, $\int Pdi$ and the T_I/T_{tot} achieved over the final third of maximal exercise for the same duration as the entire exercise bout (13.2 ± 2.0 min). Prior to and following the exercise

and mimic trials, BPNS was performed at 1, 10 and 20 Hz and twitch stimulation of a non-respiratory muscle was also performed (first dorsal interosseous muscle; between the thumb and first finger in the hand). In a sub-group of participants, a subsequent supra-mimic trial was performed at 150% of the exercise *P*di. As expected, following exercise *P*di was reduced at all stimulation frequencies by approximately 25%. Following the mimic trial, *P*di was not different to rest and only in the supra-mimic condition was *P*di reduced (~22%). The force generated by stimulating the first dorsal interosseous was not different before or after exercise in any trial. These findings demonstrate that diaphragm fatigue occurs due to a unique interaction between the work performed by the diaphragm and the systemic environment of whole-body intense exercise.

These findings were supported by Babcock et al. (2002) who measured *P*di at rest and following maximal exercise at 85% $\dot{V}O_2$max using BPNS (twitch, 10, 20, 50 and 100 Hz and T_2 at 50, 70 and 100 Hz). A control trial was performed for 9.6 ± 0.6 min. In another trial exercise was performed for the same duration but the respiratory muscles were unloaded by 40 to 50% using PAV. Immediately after the control trial, *P*di was reduced at all stimulation frequencies. However, following exercise with PAV, *P*di remained unchanged from rest. A previous study from the same group indicated that exercise-induced diaphragm fatigue does not occur at lower exercise intensities (75% $\dot{V}O_2$ max) performed both with and without PAV (Wetter et al. 1999). Therefore, these findings demonstrate that exercise-induced diaphragm fatigue is most likely caused by fatiguing diaphragm contractions and the competition with the exercising locomotor muscles for the available $\dot{Q}$. During intense exercise, the competition for blood flow between respiratory and locomotor muscles may decrease O_2 utilisation within and metabolite clearance from the diaphragm, increasing the reliance upon anaerobic processes and the potential for fatigue.

These findings are also supported by studies that measured respiratory muscle blood flow during exercise and quantified the effects of the F_IO_2 on diaphragm fatigue. Vogiatzis et al. (2008) measured external intercostal and vastus lateralis muscle blood flow using near-infrared spectroscopy and indocyanine green dye during 5 min exercise in normoxia (F_IO_2: 0.21), hypoxia (F_IO_2: 0.13) and hyperoxia (F_IO_2: 1.00). Femoral arterial oxygen content (CaO_2) was 196.6 ± 4.4, 150.9 ± 4.3 and 219.5 ± 6.2 ml·L^{-1} in normoxia, hypoxia and hyperoxia, respectively. The corresponding arterial oxygen saturation was 95, 72 and 100%, respectively. In each trial the exercise intensity was adjusted to ensure a similar work of breathing. Following each bout of exercise, twitch *P*di was significantly reduced, however, the magnitude of this reduction in hypoxia (33%) was greater than that in normoxia and hyperoxia (~25%). This reduction was attributed to the failure of intercostal muscle blood flow (and possibly the diaphragm, although not measured) and O_2 delivery to significantly increase during exercise in hypoxia (53.6 ± 8.5 ml·100 ml·min^{-1}) relative to normoxia / hyperoxia (~51 ml·100 ml·min^{-1}). These findings tentatively support the concept that exercise-induced diaphragm fatigue manifests due to a substantial diaphragm work combined with the competition for $\dot{Q}$ with the locomotor muscles (Dempsey et al. 2006b). However, the limitations to near-infrared spectroscopy such as the depth of blood flow measurement (~2 cm) and failure to measure diaphragm blood flow suggests this requires further investigation.

1.5.5 DIAPHRAGM FATIGUE AND THE POST-EXERCISE SHIELDING HYPOTHESIS

Previous studies suggest that diaphragm fatigue occurs during exercise most likely when the ratio of $\int P$oe/$\int P$di increases. However, recent evidence suggests that diaphragm fatigue does not develop *during* exercise but rather only *after* exercise. This process has been termed the 'post-exercise diaphragm shielding hypothesis' (Kabitz et al. 2007, 2008a, b; Figure 1.17).

In a series of studies, Kabitz et al. (2007, 2008a, b) measured for the first time evoked *P*di during exercise using BPNS. The findings of these studies are illustrated in Figure 1.17. In this Figure (A and B), each grey shaded vertical bar represents a 90 s phase of the exercise bout. The exercise intensity of each phase is relative to 85% $\dot{V}O_2$max (which in the Figure is the highest bar and defined as 100%). Figure 1.17A and B illustrate that as the exercise intensity increases, *P*di increases (closed circles: •). This also occurs when the exercise breathing pattern is mimicked at rest (Figure 1.17B; grey triangles: ▲). However, despite a continual rise in twitch *P*di, at the cessation of exercise only, *P*di was significantly reduced below baseline (Figure 1.17A, B; closed circles: •). In their final study, Kabitz et al. (2008b) illustrate that despite twitch *P*di increasing throughout exercise and decreasing below baseline after exercise, in a subsequent bout, *P*di increased once more to a similar level as the previous bout (Figure 1.17C; open circles: ○).

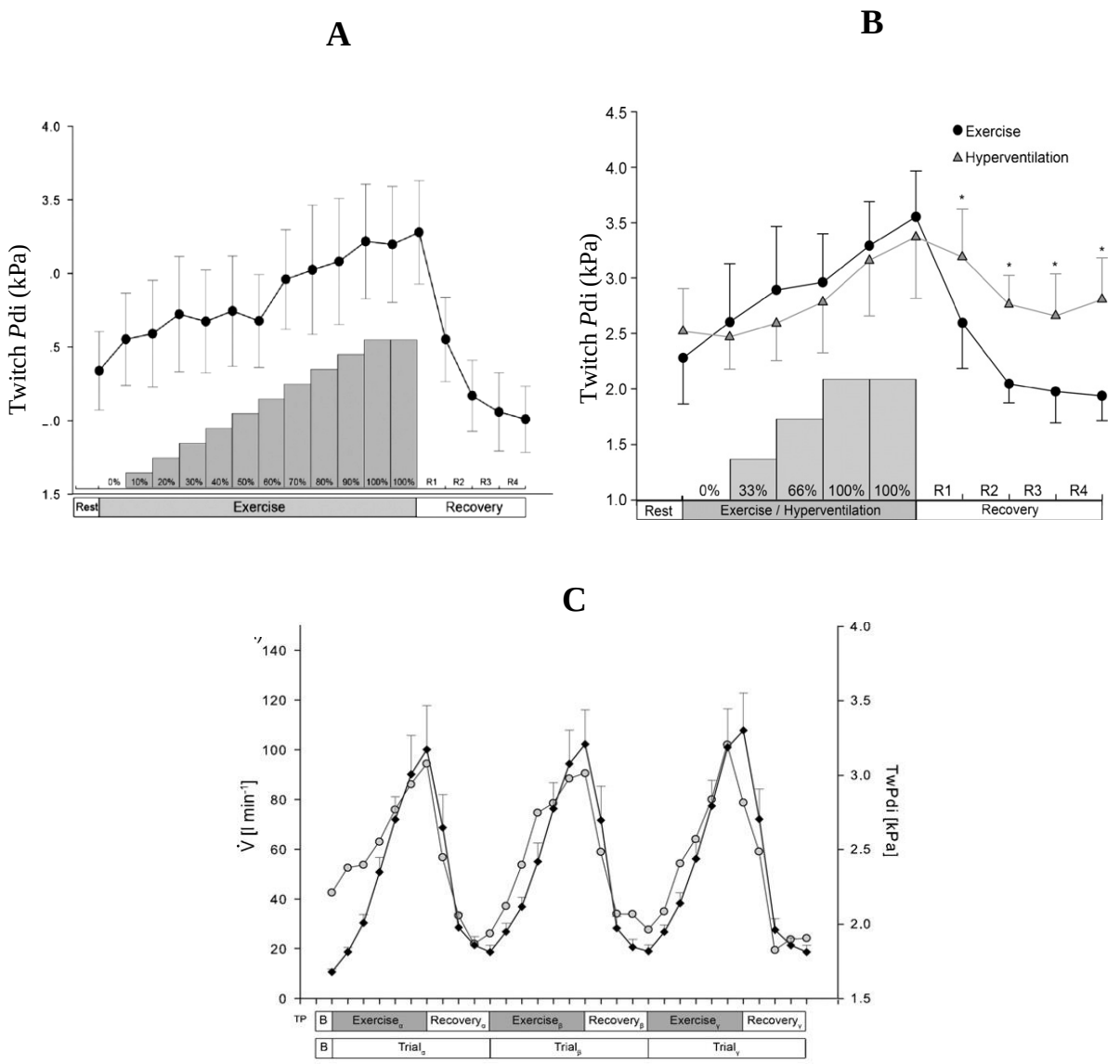

Figure 1.17 (A) Twitch *P*di in response to bilateral phrenic nerve stimulation during exercise and recovery; (B) during and following exercise and a voluntary mimic trial with an identical breathing pattern as that achieved during exercise and (C) throughout repeated bouts of exercise and recovery (Kabitz et al. 2007, 2008a, b, respectively)

Collectively these findings suggest that diaphragm fatigue must not develop *during* exercise, as this would attenuate the rise in *P*di in the subsequent bout. The rise in *P*di throughout exercise was attributed to increased diaphragm excitability. Kabitz and colleagues suggest that diaphragm motor output (and thus twitch *P*di) is reduced only *after* exercise when a centrally regulated control limits diaphragm excitability in order to allow the diaphragm to "recover without actually resting" (Kabitz et al. 2008b, pg. 236). Therefore, these findings suggest that (I) diaphragm fatigue does not develop during exercise but rather after exercise; (II) diaphragm fatigue does not limit *P*di during

subsequent exercise bouts; (III) does not affect subsequent performance and (IV) post-exercise shielding acts to protect the diaphragm, by allowing it to recover without resting. Such a mechanism would protect a fatigued muscle from excessive and / or deleterious muscle contractions which may preserve cellular integrity. However, as shown in Figure 1.17C, post-exercise diaphragm shielding does not actually shield the diaphragm since subsequent, intense contractions which manifest in diaphragm fatigue are achieved in subsequent exercise. Furthermore, if a post-exercise shielding were achieved, the recruitment of non-diaphragmatic respiratory muscles (Jonville et al. 2005) would increase which may increase either $\dot{V}_E$ (which was similar across subsequent bouts; see Figure 1.17C, ♦) or modify the breathing mechanics (neither the ratio $\int Poe/\int Pdi$ nor f_R were reported).

Recent evidence however argues against this hypothesis (Renggli et al. 2008). In this study, subjects (n = 14) were required to perform 8 min isocpanic volitional hyperpnoea (71 ± 10% MVV) followed by 6 min rest and rerpeated this protocol until task failure (subjective exhaustion or 3 continous breaths below the target). Renggli and colleagues reported a reduction in twitch *P*di following as little as 8 min. Furthermore, there was a temporal increase in pressure generation of the inspiratory chest wall muscles (*P*oe) (Figure 1.18). Thus this study contradicts that of Kabitz et al. By illustrating a rapid and sustained depression of twitch *P*di during volitional hyperpnoea with no evidence of *P*di recovery between bouts.

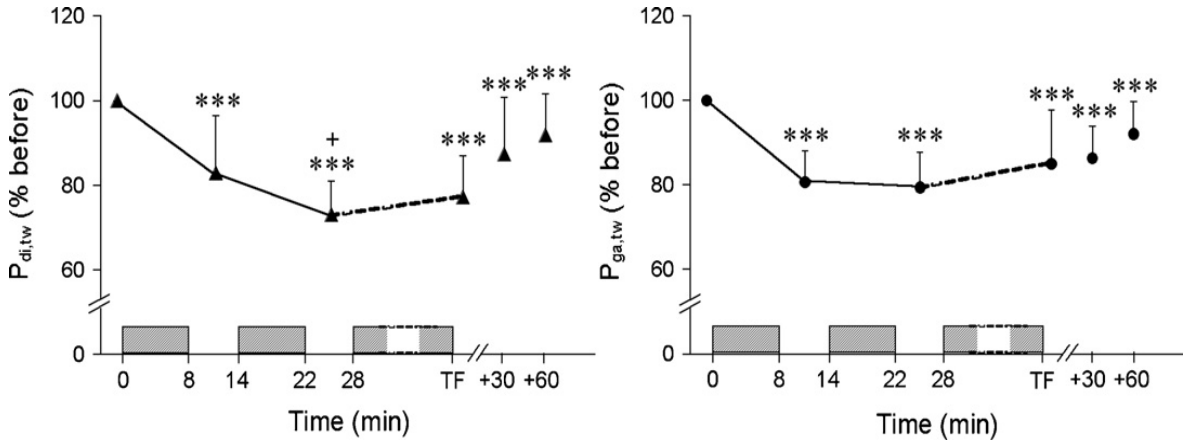

Figure 1.18 Reduction in twitch *P*di ($P_{di,tw}$; diaphragm fatigue) (Left) and gastric pressure ($P_{ga,tw}$; abdominal fatigue) (Right) relative to rest during repeated bouts of 8 min isocapnic volitional hyperpnoea with 6 min recovery.

These conclusions are also in stark contrast to the hypothesis of Johnson et al (1993) and Babcock et al. (1995, 1998). They are also contrary to those published by Romer et al. (2007a). In this latter study, both potentiated and unpotentiated *P*di evoked at 10 to 100 Hz were unchanged following maximal incremental cycling exercise. The incremental cycling protocol was similar to that used in the studies by Kabitz and colleagues. Romer et al. (2007a) suggest that exercise-induced diaphragm fatigue was prevented due to the brevity (4.3 min) at which exercise was performed above the critical intensity for diaphragm fatigue. Interestingly, this duration was markedly greater than that of Kabitz et al. (2007, 2008a) where subjects exercised for only 3 min at or above this threshold (i.e. two 90 s phases). Therefore, rather than demonstrating post-exercise shielding of the diaphragm, the findings of Kabitz et al. (2007, 2008a, b) are more likely explained by several methodological shortcomings which are explained below.

The methodological shortcomings include firstly, the protocol used to measure the resting and post-exercise twitch *P*di and secondly, the lung volume at which *P*di was measured. During intense exercise twitch *P*di increases due to increased motor-unit excitability (Rassier and MacIntosh 2000). The increased twitch force occurs due to post-activation potentiation (PAP). PAP is caused by the phosphorylation of the regulatory myosin light chains as a direct consequence of the work performed in the previous contraction(s) (Folland et al. 2008). In the studies by Kabitz and colleagues, the precise

measurement of *P*di and accounting for PAP before, during and after exercise was fundamental to the post-exercise shielding hypothesis. In their studies, to control for PAP throughout exercise, the resting twitch was fully potentiated by performing 5, 5 s MIP efforts immediately prior, subsequently the first two twitch *P*di responses are discarded since *P*di is still rising (Romer et al. 2007a); this method is known to maximally potentiate the subsequent twitch response up to 52% (Guleria et al. 2002; Mador et al. 1994; Wragg et al. 1994). However, a sustained maximal Müeller manoeuvre will recruit all motor units of the diaphragm, including both fast and slow twitch muscle fibres (Bellemare and Bigland-Ritchie 1987). Since PAP is fibre-type specific, the potentiated twitch will increase the force generating capacity of both type I and II fibres (Sale 2002). In the studies by Kabitz and colleagues the peak exercise and recovery $\dot{V}_E$ was ~100 and ~20 $L \cdot min^{-1}$, respectively. At these ventilations, breathing is sub-maximal. Furthermore, since diaphragm muscle fibre recruitment follows the size principle (Sieck and Fournier 1989) only the smaller motor units would be active. Therefore, it would appear that the PAP conditions employed at rest i.e. following a 5 s MIP and during and following exercise were extremely different. Thus it is likely that the reduction in twitch *P*di observed following exercise: the so called 'post-exercise shielding' may simply reflect an un-potentiated twitch which is lower than that produced at rest.

The second limitation is the lung volume at which twitch pressure was measured. Throughout exercise from onset to termination, functional residual capacity shifts. This is because of a decrease in EELV and subsequent increase in EILV which facilitates an increase in V_T and expiratory flow rates. The diaphragm is known to express functional length-tension relationships (Braun et al. 1982; Johnson et al. 1993; LeBlanc et al. 1988) where an increase in lung volume lengthens the inspiratory muscles and reduces their force generation capacity. In the studies by Kabitz and colleagues, prior to each twitch, participants were instructed to breathe in and out slowly and then hold their breath, twitch *P*di was then measured at the muscle length specific to that breath and ventilation. The

increase and decrease in *P*di during and following exercise, respectively, may simply reflect the transient change in EELV and thus diaphragm length. The change in diaphragm length would affect diaphragm force generating capacity. These methods are in contrast to those of Johnson et al. (1993), Babcock et al. (2002) and Romer et al. (2007a) where measurements of twitch *P*di at a lung volume or *P*oe ± 10% of that at resting FRC were excluded from subsequent analysis.

In summary, the recently advocated post-activation shielding hypothesis appears to be fundamentally flawed by methodological error. Further study is therefore required to identify whether such a mechanisms is indeed present following exercise. Also, why post-exercise shielding would occur despite *P*di increasing in subsequent exercise should be investigated. It would seem reasonable to suspect that if the diaphragm were to be shielded following exercise, subsequent breathing mechanics would be affected. Finally, if a centrally regulated shielding is initiated following exercise, how is this identified by stimulating the motor nerve roots in the vertebrae where the central controller is essentially bi-passed? Consequently, owing to the limitations of the post-exercise shielding hypothesis, the remainder of this review focuses on the traditional hypothesis by Johnson et al. (1993) that diaphragm fatigue manifest during and not following exercise.

1.5.6 SUPRASPINAL DIAPHRAGM FATIGUE

The origin of supraspinal fatigue is the central nervous system or higher brain centres. Central fatigue results in decreased voluntary muscle activation due to a reduction in the motor output from the brain to the motor-neuron pool (Gandevia 2001). Central diaphragm fatigue was first reported by Bellemare and Bigland Ritchie (1987). In this study *P*di was measured during repeated maximal voluntary inspiratory efforts with a twitch superimposed upon this effort. Using this technique a further increase in *P*di above that generated volitionally indicates the magnitude of central activation by the motor cortex. Central activation and peripheral fatigue was assessed during and between

inspirations, respectively, during a fatiguing flow-resistive loading task at 75% *P*di max. At rest, no increase in *P*di was observed during an MIP with a superimposed twitch, demonstrating that activation of the diaphragm was maximal. However, at task failure, the central and peripheral components of fatigue were 39 ± 8% and 61 ± 8%, respectively. Throughout the first 20 to 40% of the trial, peripheral fatigue (i.e. decrease in twitch *P*di) accounted for a 25% reduction in voluntary *P*di which therefore remained constant. A marked increase in the superimposed twitch (or a decrease in central activation) was subsequently observed. Bellemare and Bigland Ritchie (1987) demonstrated that during fatiguing inspiratory work, a level of peripheral fatigue was developed and following this, central activation of the diaphragm was reduced limiting central motor output, preventing the development of further peripheral failure. Therefore in the latter stages of the breathing test, a reduced central motor output accounted for the reduction in voluntary *P*di. Interestingly, the reduction in motor output was specific to the diaphragm as a rise in accessory respiratory muscle EMG was observed. These findings are similar to and supported by those recently observed during whole-body cycling exercise where the magnitude of peripheral locomotor muscle fatigue was regulated by a reduction in central motor output (Amann et al. 2008b, 2009).

Guleria et al. (2002) studied central fatigue of the diaphragm and the quadriceps during two exercise trials which targeted specifically each muscle group. Diaphragm loading was achieved by inspiratory pressure threshold loading which began at 30% MIP and increased by 10% every 3 min. Quadriceps loading was achieved using isometric contractions initiated at 10% MVC and increased by 10% every 3 min. Stimulation of the diaphragm (BPNS) and quadriceps (femoral nerve) was performed in each protocol, respectively, during each contraction to indicate central activation and following each trial to quantify peripheral fatigue. At task failure the reduction in quadriceps twitch force was ~20% less and central activation was ~18% greater than the diaphragm. These findings suggest, similar to those of Bellemare and Bigland Ritchie (1987) that central motor output

to the diaphragm is reduced during exercise to a greater extent than other skeletal muscles, which limits contraction failure following exercise. The modified quadriceps response was suggested to suit evolutionary needs such as when hunting or escaping from predators. Similar findings were reported by Verin et al. (2004) following maximal incremental treadmill exercise. Transcranial magnetic stimulation of the motor cortex indicated significant reductions in both diaphragm and quadriceps motor evoked potentials (~60%). Following 20 min of recovery, motor evoked potentials were 45% of baseline for the diaphragm but had increased to 74% of baseline in the quadriceps. This suggests a supraspinal process within the cortex which provides a substantially greater protection of the diaphragm following exercise, which limits diaphragm fatigue during and following exercise and supports the notion that the respiratory muscles are the only essential skeletal muscle.

Therefore, when considering both peripheral and central fatigue together, the plateau in the work of the diaphragm relative to the chest wall muscles (Johnson et al. 1993; Babcock et al. 1998) may be a result of peripheral fatigue and possibly a subsequent reduction in central motor output to the diaphragm. As a consequence, motor output to the chest wall and accessory respiratory muscles is increased (Bellemare and Bigland-Ritchie 1987). The sub-conscious redistribution of respiratory motor-output may provide a protective mechanism against excessive peripheral diaphragm fatigue (Guleria et al. 2002; Verin et al. 2004). Despite this protective mechanism, a reduction in diaphragm motor output and increased accessory muscle recruitment would elevate the work of breathing and the sensations of respiratory discomfort. However, it appears that this increase in the work of breathing is more favourable than excessive diaphragm fatigue and possible respiratory failure. Indeed, accessory muscle recruitment and the large increase in perceived breathlessness and breathing discomfort may serve as an important protective signal for volitional termination of exercise.

1.5.7 EXERCISE INDUCED PERIPHERAL ABDOMINAL MUSCLE FATIGUE

At rest abdominal muscle recruitment is minimal as the chest and lung elastic recoil facilitates expiration. However, almost immediately during exercise, abdominal muscle recruitment is increased (Aliverti et al. 1997). Abdominal muscle recruitment facilitates inspiration in many ways but predominantly by reducing EELV which increase V_T and increases diaphragm length (Abbrecht et al. 1991). Despite this important function, the expiratory muscles have received much less attention than their inspiratory counterparts.

By stimulating the thoracic nerve roots which innervate the abdominal muscles and measuring the subsequent *P*ga, abdominal muscle contractile fatigue can be assessed. Verges et al. (2006) reported a significant 13 ± 7% reduction in twitch *P*ga following exercise to volitional tolerance at 85% $\dot{W}$max with no change in abdominal muscle M-wave. Abdominal muscle force was not different to rest following 30 min of recovery. Similarly, Taylor et al. (2006) measured *P*ga in response to supramaximal stimulation of the abdominal nerve roots prior to and following intense exercise at 90% $\dot{V}O_2$max (14.2 ± 4.2 min). Following the cessation of exercise, *P*ga at 1 through 25 Hz (1 s train) was reduced by 25 ± 4% which persisted for 30 min post-exercise with no changes observed in M wave. Both of these findings provide evidence for LFF of the abdominal muscles following exercise; HFF has yet to be assessed. Given the important role for the abdominal muscles in supporting diaphragm work, the implications of expiratory muscle fatigue on inspiratory muscle function have not been documented during whole body exercise although recent findings shows that fatiguing expiratory loading promotes both expiratory and inspiratory muscle fatigue (Taylor and Romer 2009). Fatigue of the expiratory muscle prior to or during exercise may attenuate the decrease in EELV, causing excessive flow limitation and dynamic hyperinflation. These actions would increase the operating length of the inspiratory muscles throughout the breath cycle reducing their mechanical efficiency. This may have the potential to accelerate inspiratory muscle fatigue by reducing the capacity for inspiratory pressure development, increasing accessory muscle recruitment

and therefore the inspiratory work of breathing. Furthermore, increasing the pressure required by the inspiratory muscles may also intensify the sensations of breathlessness. However, this theorectival notion was recently contested by Taylor and Romer (2008) and Taylor and Romer (*In Press*) where following expiratory muscle loading which significantly reduced *P*ga EELV remained unchanged throughout exhaustive exercise relative to a control trial without prior expiratory muscle loading.

1.5.8 CARDIO-RESPIRATORY INTERACTIONS DURING INTENSE EXERCISE

During intense-exercise in healthy adults the respiratory muscles can demand up to 10% of the total cardiac output and oxygen consumption and up to 15% in well trained athletes. A consequence of the work of breathing is exercise-induced respiratory muscle fatigue which is mediated both centrally and peripherally. It is likely that a level of peripheral fatigue is developed beyond which central respiratory motor outflow is curtailed limiting further, possibly damaging muscle contractions. The development of exercise-induced diaphragm fatigue can have significant implications for both locomotor blood flow and exercise tolerance.

Fatiguing diaphragm and abdominal muscle contractions elicited by inspiratory resistive loading at 60% MIP increase locomotor muscle sympathetic nerve activity (MSNA) measured from the peroneal nerve (St Croix et al. 2000; Derchak et al. 2002). In addition, fatiguing inspiratory muscle loading causes a time-dependent reduction in limb blood flow (decreased vascular conductance) secondary to an increased limb vascular resistance. These effects are not observed with non-fatiguing inspiratory resistive loading (Sheel et al. 2002). The increased locomotor MSNA and subsequent limb vascular resistance was attributed to the discharge of chemo-sensitive type III and IV afferent fibres located in the fatiguing diaphragm muscle fibres. The diaphragm has a rich supply of afferent nerve endings and their firing rate increases with fatigue (Hill 2000, 2001). Type III and IV afferent fibres are also highly sensitive to metabolites; in particular, lactate is

known to significantly increase the discharge of afferent fibres in the cat (Graham et al. 1986; Rotto and Kaufman 1988; Sinoway et al. 1993). The discharge of diaphragm afferents increases sympathetic efferent discharge to the locomotor muscles which reduces limb blood flow. Although the sympathetic efferent response is general to many vascular beds, diaphragm perfusion may well be protected since the α_1-adrenergic receptors of the second order diaphragm arterioles appear to be less sensitive to vasoconstrictive stimuli (Aaker and Laughlin 2002a). This has been termed the respiratory muscle metaboreflex (Figure 1.19).

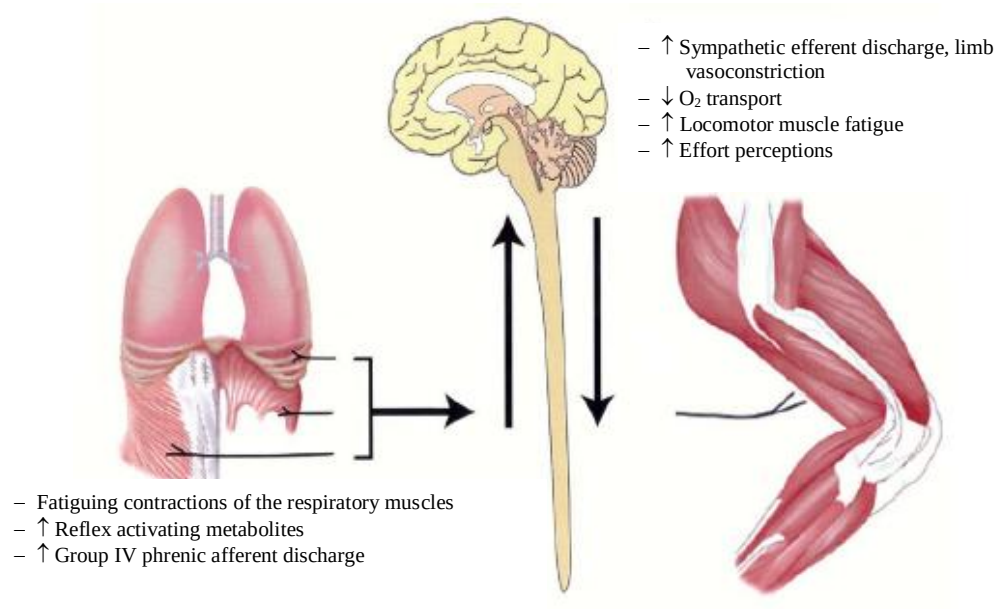

Figure 1.19 Schematic of the origin and consequences of the respiratory muscle metaboreflex (Dempsey et al. 2006b).

Harms et al. (1997) reported that during maximal whole-body exercise to the limit of volitional tolerance, increasing inspiratory muscle work by adding mesh screens to the inspiratory circuit (5 $cmH_2O \cdot L \cdot s^{-1}$) reduced limb blood flow (measured by the thermodilution technique) by ~0.9 $L \cdot min^{-1}$ due to an increase in limb vascular resistance. Limb O_2 extraction remained unchanged, therefore, limb $\dot{V}O_2$ was reduced by 0.4 $L \cdot min^{-1}$. When the inspiratory muscles were partially unloaded using PAV, the opposite effects were observed. A significant correlation was revealed between the work of breathing and

limb blood flow (r = 0.84 to 0.9, P<0.05) and limb $\dot{V}O_2$ (r = 0.77, P<0.05; Figure 1.20). Significant correlations were also reported between the increase in leg vascular resistance and nor-adrenaline spillover in the limb. These physiological responses were not observed during sub-maximal exercise at 50 and 75% $\dot{V}O_2$max (Wetter et al. 1999). This demonstrates a unique interaction between the work of breathing and locomotor blood flow. The increase in limb vascular resistance was correlated with nor-adrenaline spillover within the locomotor muscles. Nor-adrenaline spillover is reflective of sympathetic outflow which further suggests suggest that the respiratory muscle metaboreflex may be a sympathetically mediated response.

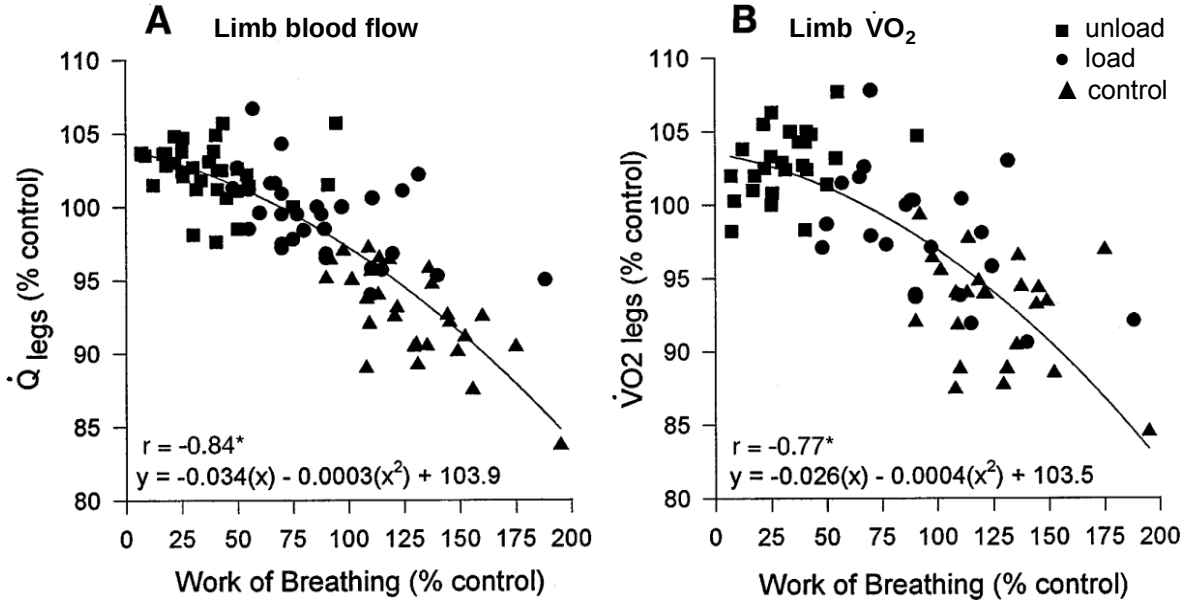

Figure 1.20 Effects of the work of breathing on A) limb blood flow ($\dot{Q}$legs) and B) limb $\dot{V}O_2$ ($\dot{V}O_2$legs ; Harms et al. 1997).

Compelling evidence for the metaboreflex has been provided by animal studies. Rodman et al. (2003) injected a bolus of lactic acid in to the phrenic and deep circumflex arteries which supply the diaphragm and abdominals muscles, respectively. The injections were performed at rest and during 2 to 3 min sub-maximal steady state exercise (3.5-5.5 km·h^{-1}). Both at rest and during exercise, lactic acid infusion increased mean arterial blood pressure and reduced cardiac output (due to subsequent increases in systemic vascular

resistance) by 21 and 6%, respectively. Limb perfusion was reduced by 20% which was caused by an increased locomotor MSNA. These effects were not observed when the experiment was repeated with pharmacological sympathetic blockade of the sympathetic nervous system (adrenergic receptors) using injections of phentolamine and propranolol.

Skeletal muscle blood flow is determined by blood vessel diameter. Vessel diameter is affected by both vasodilator and vasoconstrictor stimuli. Therefore, the balance of these ensures that the resultant perfusion pressure and vascular tone is matched to the systemic metabolic demand. However, amazingly the increase in sympathetic output mediated by nor-adrenaline spillover (Harms et al. 1997) does not appear to affect diaphragm perfusion. Aaker and Laughlin (2002a) reported that the α_1-adrenergic receptors of diaphragm second order arterioles are less responsive to the vasoconstrictive effects of nor-adrenaline. The internal diameter of these vessels was unchanged with progressively large doses of nor-adrenaline infusion into the individual arterioles. Diaphragm arterioles also responded in a dose-dependent manner to adenosine infusion (a potent vasodilator; Aaker and Laughlin 2002b) although evidence from ponies suggests that the vasodilator capacity of the diaphragm is maximal during intense exercise (Manohar 1986). Whether the respiratory muscle metaboreflex promotes / increases respiratory muscle blood flow or diaphragm O_2 extraction has yet to be confirmed. Since the diaphragm vasodilator capacity is maximised (Manohar 1986) during intense exercise the metaboreflex may serve to either preserve diaphragm perfusion in light of the increasing metabolic demand or act to increase accessory respiratory muscle perfusion since the activation of the latter exceeds the former during intense exercise (Johnson et al. 1993). Furthermore, whether the respiratory muscle metaboreflex would be activated during athletic competition is unknown since previous studies in humans and animals have lacked ecological validity.

1.5.9 THE WORK OF BREATHING AND EXERCISE TOLERANCE

Some studies have pre-fatigued the respiratory muscles prior to exercise to determine the effects of this muscle group upon performance. Prior respiratory muscle fatigue has been induced by a number of methods. 150 min volitional hyperpnoea (separated by a 4 min break every 15 min) at the maximal sustainable ventilation (58 to 82% MVV_{12}; Martin et al. 1982), 10 min volitional hyperpnoea at 80% $\dot{V}_E$ max (Dodd et al. 1989) and 60% MVV to exhaustion in which twitch *P*di was depressed up to 120 min post-hyperpnoea (Mador et al. 1996). Flow resistive loading at 80% MIP has also been used (Mador and Acevedo 1991a, b; Sliwinski et al. 1996) until twitch *P*di fell by 20% relative to rest (Verges et al. 2007a) has also been employed. With the exception of a few studies who investigated the effects of prior respiratory muscle work on diaphragm contractile properties *per-se* (Mador et al. 1996; Sliwinski et al. 1996; Verges et al. 2007a), all of aforementioned studies reported a significant reduction in incremental exercise performance time (Martin et al. 1982) and constant power time to the limit of tolerance at 85% (Dodd et al. 1989; Verges et al. 2006) and 90% $\dot{W}$ max (Mador and Acevedo 1991a, b). Interestingly, when isocapnic volitional hyperpnoea performed at 52% MVV for 15 min, there were no changes in volitional measures of respiratory muscle strength and endurance performance (78% $\dot{W}$max , ~lactate threshold; Spengler et al. 2000).

Prior-fatigue of the abdominal muscles has also been shown to limit exercise tolerance. Following expiratory muscle loading that caused a 20% fall in MEP, the distance covered and the average speed during a 12 min run were decreased by 85 m and 0.13 $m \cdot s^{-1}$, respectively, relative to a control (Verges et al. 2007a). Recently, using magnetic stimulation of the abdominal nerve roots and the femoral nerve, the effects of prior expiratory muscle fatigue on exercise tolerance and locomotor muscle fatigue were assessed (Taylor and Romer 2008). Prior-abdominal muscle fatigue was imposed by expiratory resistive loading at 40% *P*ga max. The expiratory loading task reduced twitch

*P*ga by 27 ± 5%. Relative to a control trial, performance time was reduced by -33% and quadriceps fatigue was greater (28% vs. 12%).

These studies clearly indicate that the respiratory muscles may indeed limit exercise tolerance. However, all of these studies observed a significant change in breathing pattern during the exercise trial, specifically an increased f_R with no change in V_T (rapid but not shallow breathing) and a heightened sensation of both leg and breathing discomfort (dyspnoea). An increase in f_R caused by an increased accessory muscle recruitment results from an increased central motor output (St. Croix et al. 2000; Sheel et al. 2001). The conscious awareness of central motor output via corollary discharge from the motor cortex to the sensory cortex increases the perceptions of breathing effort (Gandevia et al. 1981; McConnell and Romer 2004b) which are both important determinants of exercise tolerance (Jones and Killian 2000; Presland et al. 2005). Therefore it is unclear whether the impaired performance is a consequence of the greater sense of dyspnoea or the alteration in breathing pattern and / or respiratory muscle fatigue.

To avoid these confounding effects, Harms et al. (2000) investigated the effects of fatiguing diaphragm work on whole-body exercise tolerance and Romer et al. (2006a) quantified the effects of this on locomotor muscle fatigue using magnetic stimulation of the femoral nerve. Both studies used PAV to reduce the inspiratory muscle work of breathing that normally occurred during intense exercise therefore eliminating the potentially confounding effects of prior-fatigue on the responses to the subsequent exercise bout. In the study by Harms et al. (2000) well trained cyclists ($\dot{V}O_2$max: 63 ± 5 ml·kg^{-1}·min^{-1}) exercised at 90% $\dot{V}O_2$max to volitional exhaustion (control trial) and repeated this in two subsequent trials. One trial was performed with PAV and one with an increased inspiratory resistance provided by mesh screens. Unloading and loading the inspiratory muscles increased and decreased performance time by +1.3 and -1.0 min, respectively (Figure 1.21). The changes in performance were associated with changes in $\dot{V}O_2$ (increased with

loading and decreased with unloading) and correlated with changes in both leg and respiratory muscle discomfort. These findings illustrate a significant interaction between changes in respiratory muscle work with locomotor muscle performance and the effects upon both the sensations of respiratory and limb discomfort.

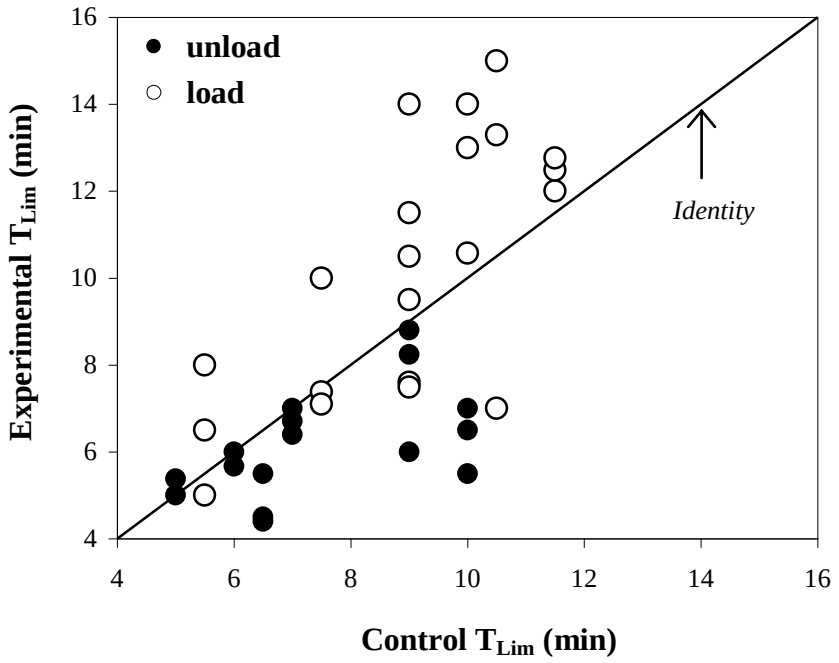

Figure 1.21 Relationship between time to exhaustion (T_{lim}) and the experimental conditions. Note the trend for the loaded breathing trial to fall below the line of identity (Harms et al. 2000).

Romer at al. (2006a) extended these findings by assessing the magnitude of limb muscle fatigue using magnetic stimulation of the femoral nerve following cycling exercise performed at 90% $\dot{V}O_2$max (292 ± 13 W) with PAV or loaded breathing (using mesh screens). In a control trial, exercise was performed to volitional exhaustion for 13.2 ± 0.9 min and evoked quadriceps force was reduced by 28 ± 5% (mean of 1 to 100 Hz) 2.5 min post-exercise. With PAV, exercise was terminated at the same time as the control trial, however the reduction in evoked force was reduced to 20 ± 5%. Inspiratory muscle loading is known to impair whole-body performance time relative to a control (Harms et al. 2000), thus an additional control trial was performed at the same exercise intensity (292 ± 13 W) but which reflected the exercise duration that could be sustained with additional inspiratory loaded breathing (7.9 ± 0.6 min). Following the control trial without loaded breathing

quadriceps muscle fatigue decreased by 12 ± 8%. During the experimental trial with loaded breathing, quadriceps muscle fatigue increased to 20 ± 7%. Increases and decreases in both respiratory and limb discomfort were also observed with loading and unloading the inspiratory muscles.

The reductions in locomotor muscle performance (Harms et al. 2000) and the exacerbated limb muscle fatigue (Romer et al. 2006a) when loading the inspiratory muscles was attributed to a reflex sympathetically-mediated vasoconstriction of the limb musculature (Sheel et al. 2001, 2002) which originated in the fatiguing inspiratory muscles. Therefore, during intense exercise, the respiratory muscle metaboreflex may have attenuated locomotor muscle blood flow and locomotor O_2 transport. A reduction in O_2 transport is known to have significant effects upon skeletal muscle force output. For example, recently it was demonstrated that quadriceps fatigue is inversely related to CaO_2 (F_IO_2 = 0.15 to 1.00; CaO_2: 17.6 to 24.4 $ml \cdot L^{-1}$) in trained male cyclists ($\dot{V}O_2$max: 63.3 $ml \cdot kg^{-1} \cdot min^{-1}$; Amann et al. 2006). Furthermore, Romer et al. (2006b) observed that attenuating the reduction in arterial oxygen saturation from 92% to 98% attenuated peripheral quadriceps fatigue by approximately 50%. That increasing the work of breathing reduced limb blood flow and limb O_2 delivery with no change in O_2 extraction, suggests that the respiratory muscle metaboreflex may have a detrimental effect on intense endurance performance to volitional tolerance. It is also clear that reducing the work of breathing which is normally incurred during intense exercise by using PAV prevents this response.

The work of breathing is considerable during intense exercise and may have important implications for locomotor muscle fatigue. However, the use of PAV during competition and training to reduce the work of breathing and improve performance is impractical. As a consequence, training the respiratory muscles independent to whole-body training to reduce the work of breathing during intense exercise may provide a unique alternative to potentially improve exercise tolerance.

1.6 RESPIRATORY MUSCLE TRAINING

1.6.1 BRIEF INTRODUCTION

Specific training of the respiratory muscle (RMT) was first developed in clinical populations by Delhez et al. (1966) where repeated 3 s Müeller manoeuvres were performed for 8 wk. Following RMT, MIP increased 37 ± 23%. Delhez and colleagues concluded that the increase in inspiratory muscle strength (which was a surprising finding at that time) may reduce the potential for the respiratory muscles to limit ventilatory work. Probably the first study to investigate whether RMT affects the strength and endurance of the respiratory muscles in healthy subjects was conducted by Leith and Bradley (1976). In their classic study, they hypothesised that known training principles would apply to the respiratory muscles. Following 5 wk voluntary hyperpnoea training a 19% increase in the maximal sustainable ventilatory capacity was observed and in another group, a 55% increase in MIP was observed following 5 wk RMT comprising repeated MIP efforts at 20% intervals over the VC range for 30 $min \cdot day^{-1}$. However, at the time of their study, neither respiratory muscle strength nor ventilatory endurance were known to limit exercise tolerance, consequently the authors suggested that the application of RMT to improve exercise performance was limited (Leith and Bradley 1976).

Despite these early perspectives, it should be recognised that breathing is a form of muscular exercise and the physiological consequences of the work of breathing may exacerbate locomotor muscle fatigue and intensify the sensations of both limb and respiratory discomfort. Therefore, the notion that specific training of the respiratory muscles may attenuate some or all of these and improve performance has received considerable attention.

1.6.2 RESPIRATORY MUSCLE TRAINING TECHNIQUES

RMT can be performed by voluntary isocapnic hyperpnoea (VIH), flow resistive loading (FRL) and pressure threshold loading. Due to the high pressure and slow velocity

of each breath performed with FRL, this method targets the force axis of the inspiratory muscle force-velocity curve. Given the very high flow rates performed with VIH, this mode of training specifically targets the velocity axis of the respiratory muscle force-velocity curve. Pressure threshold inspiratory muscle training (IMT) targets both axes (McConnell and Romer 2004a; Romer and McConnell 2003; Figure 1.22). This is important as the respiratory muscles are sensitive to specific training principles. It is also this reason which justifies the use of pressure threshold IMT in the experimental chapters of this thesis. For example, IMT which requires high inspiratory flow rates / pressures or a moderate intensity for both, results in protocol specific adaptations to the force-velocity characteristics of the inspiratory muscles (Romer and McConnell 2003; Tzelepis et al. 1994; Figure 1.22).

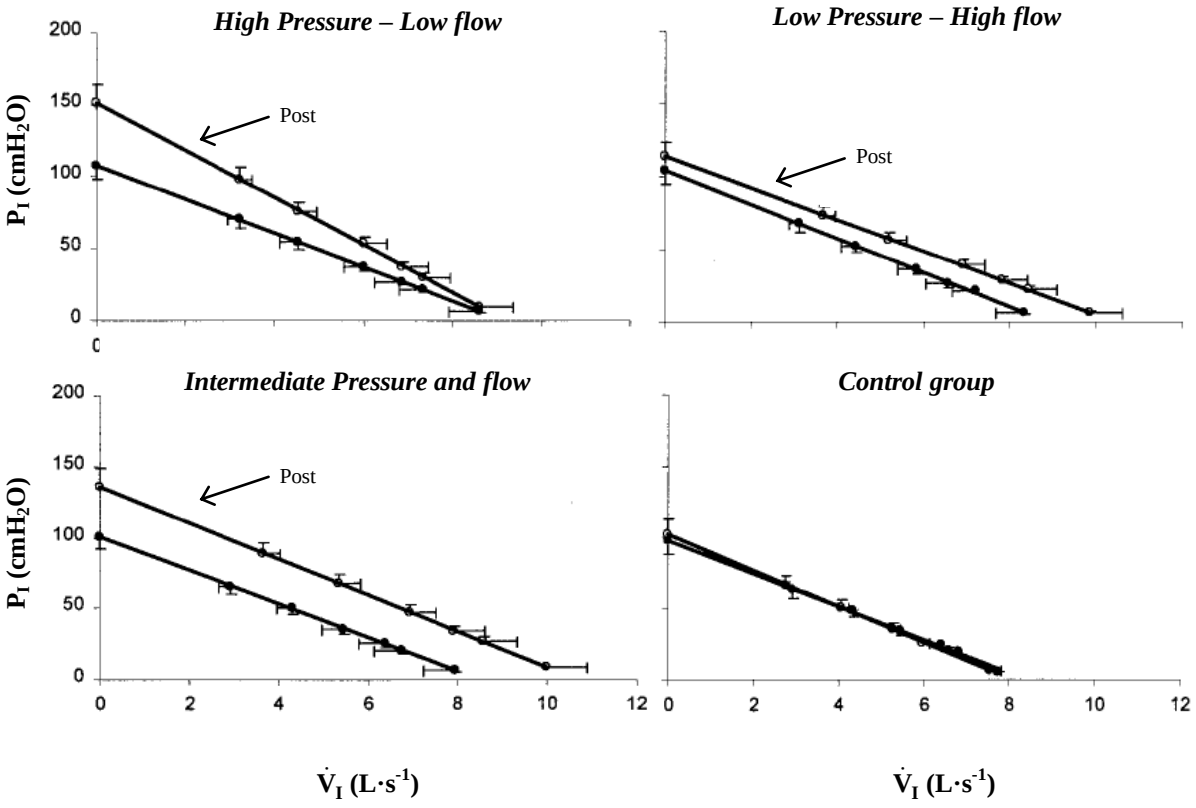

Figure 1.22 Inspiratory mouth pressure (P_I) and inspiratory flow rates ($\dot{V}_I$) before (closed circles) and after (open circles) 9 wk pressure threshold IMT (Romer and McConnell 2003).

The respiratory muscles also show clear detraining effects with inactivity following specific RMT. Leith and Bradley (1976) were the first to observe this. 15 wk post-RMT, subjects lost approximately 50% of their initial improvements in both strength and endurance although there was a large amount of variability in their data (measure of variability not provided). Romer and McConnell (2003) reported that following 9 wk detraining inspiratory muscle strength was reduced, but remained above baseline for 18 wk. Using a longer detraining period, Boutellier and Piwko (1992) reported that improvements in respiratory muscle endurance gained following VIH were completely lost after 72 wk of detraining. It is interesting to note however that a reduction in the training frequency of pressure threshold IMT from 2 sessions·day^{-1} 6 days·wk^{-1} to 2 sessions·day^{-1} 2 days·wk^{-1} preserved inspiratory muscle function for 18 wk (Romer and McConnell 2003). Recently, Leddy et al (2007) reported that following 4 wk VIH, performing 30 min 2 days·wk^{-1} rather than 30 min·day^{-1} for 5 day·wk^{-1} for 3 months, also maintained improvements in 4 mile run time relative to baseline values. These findings clearly demonstrate the plasticity of the respiratory muscles, but also the sensitivity of them to detraining effects.

1.6.3 VOLUNTARY HYPERPNOEA

Voluntary isocapnic hyperpnoea (VIH) requires the participant to increase $\dot{V}_E$ to a prescribed level for a given period of time. A commercially available device for VIH is shown in Figure 1.23. Typical training intensities are 50 to 85% of the individual MVV with a f_R fixed at 30 to 45 breaths·min^{-1} and a and V_T of 50 to 60% VC (~2.5 to 3.0 L) for 30 min·day^{-1} up to 4 wk (Boutellier et al. 1992; Boutellier and Piwko 1992; McMahon et al. 2002; Morgan et al. 1987; Spengler et al. 1999). The accurate monitoring of breathing pattern is fundamental to VIH in order to maintain the prescribed training intensity. VIH causes a rapid reduction in the partial pressure of CO_2 in the arterial blood which can lead to dizziness and fainting. To avoid this, additional CO_2 is added to the inspiratory

breathing circuit using either laboratory based equipment with precise monitoring of end-tidal and / or blood PCO_2, or by using a re-breathing bag. The re-breathing bag, typically fixed at 50% VC, inflates during expiration (the remainder of the expirate is released into the atmosphere). In the subsequent inspiration, the inspirate is comprised of both atmospheric and CO_2-enriched air from the re-breathing bag (Kohl et al. 1997; McMahon et al. 2002). This method is reported to maintain isocapnia during VIH at 38 ± 4 mmHg (Leddy et al. 2007).

Figure 1.23 Example of a commercially available voluntary isocapnic hyperpnoea respiratory muscle training device (www.spirotiger.com; accessed 13.02.2009).

VIH can be used to improve respiratory muscle endurance (rather than strength) by targeting both the inspiratory and expiratory muscles. Following VIH, considerable improvements in ventilatory endurance have been observed. Boutellier and Piwko (1992) observed an increase in breathing endurance following 4 wk VIH training in sedentary individuals from 4.2 min to 15.3 min and from 6.1 min to 40 min (cut off time) in healthy trained individuals (Boutellier et al. 1992). Similar observations were observed in moderately trained healthy males (pre: 9.8 min, post: 36.7 min; Verges et al. 2007b) and in well trained competitive runners (pre: 14.7 min, post: 36.0 min; Leddy et al. 2007).

Although VIH can produce quite remarkable improvements in respiratory muscle endurance and is extremely high in ecological validity, training sessions are time consuming and often require supervision and specialised equipment. Furthermore, evaluating the changes in respiratory muscle endurance post-VIH requires prolonged exhaustive volitional hyperpnoea trials. McConnell and Romer (2004a) also suggest that due to the repeated high flow rates required by VIH, chronic drying of the airways may cause bronchoconstriction in some participants.

1.6.4 FLOW RESISTIVE LOADING

Flow resistive loading (FRL) is a strength training method that specifically targets the inspiratory muscles. During FRL participants inspire through a variable size aperture which provides a resistance. During inspiration, the pressure generated by the inspiratory muscles (i.e. training resistance) is dependent upon the inspiratory flow rate, therefore, monitoring breathing pattern is essential to regulate training load, i.e. it is easy to cheat. Training sessions require subjects to inspire repeatedly against a load of 80 to 100% MIP until the target pressure can no longer be maintained, typically for 3 day·wk^{-1} for 4 to 10 wk (Chatham et al. 1999; Enright et al. 2006; Gething et al. 2004a, b; Hanel & Secher 1991). Some researchers have used a device known as the 'TIRE' system (Test of Incremental Respiratory Endurance) where an inspiratory pressure (based on a percentage of MIP) is attained for six repetitions with 60 s recovery duration between efforts. The recovery time between breaths is subsequently reduced to increase the training intensity (Enright et al. 2006; Gething et al. 2004a, b).

FRL has been shown to improve both inspiratory muscle strength and endurance. Improvements in MIP of 18 to 41% have been observed following 4 to 6 wk FRL training (Chatham et al. 1999; Enright et al. 2006; Gething et al. 2004a, b; Hanel and Secher 1991). Using ultrasonography, Enright et al. (2006) observed a 12% increase in diaphragm thickness at FRC following 9 wks FRL 3 days·wk at 80% MIP (pre: 4.1 mm, post 4.6

mm). An increase in diaphragm thickness was also reported by (Gething et al. 2004b) following 10 wks FRL although the authors fail to report specific data. In addition to improvements in inspiratory muscle strength and endurance, following FRL, increases in VC and TLC have been observed from 4.1 to 4.4 L and 5.7 to 6.1 L, respectively. These improvements were not due to increases in absolute lung volume, but rather the increased ability of the upper chest wall and neck inspiratory muscles to expand the thorax at greater lung volumes (Enright et al. 2006). Although improvements are noted with FRL, the reliance upon monitoring breathing pattern and perhaps PCO_2 render this technique somewhat limited to a laboratory environment where specialist personnel and equipment are readily available. Furthermore the ecological validity of such techniques is questionable.

1.6.5 PRESSURE THRESHOLD LOADING

Pressure threshold IMT requires a negative pressure generated at the mouth which opens a valve. The valve may be a spring loaded solenoid valve (see Figure 1.24), resisted by a weighted plunger or provided by constant negative pressure. Once the threshold pressure is overcome, the valve opens and inspiratory flow begins. When the pressure can no longer be sustained, the valve closes, and passive expiration ensues. A commercially available device for pressure threshold IMT is shown in Figure 1.24. With pressure threshold IMT, each inspiratory manoeuvre is initiated from residual volume and V_T is maximised. A rapid full inspiration followed by a protracted expiration minimises changes in PCO_2, and negates the use of additional CO_2 or a re-breathing bag. Pressure threshold IMT is also near flow-independent (Caine and McConnell 2000), thus monitoring of breathing pattern throughout a training session is not required. A typical IMT training regimen is 30 breaths at ~50% MIP (although 70 to 80% MIP have also been used; Huang et al. 2002 ; Wells et al. 2004 ; Williams et al. 2002), twice daily for 4 to 6 wks. Training durations greater than this appear to have minor effects on inspiratory muscle strength

(Romer and McConnell 2003). Throughout pressure threshold IMT the valve opening pressure is periodically increased to accommodate for the rapid temporal improvement in MIP.

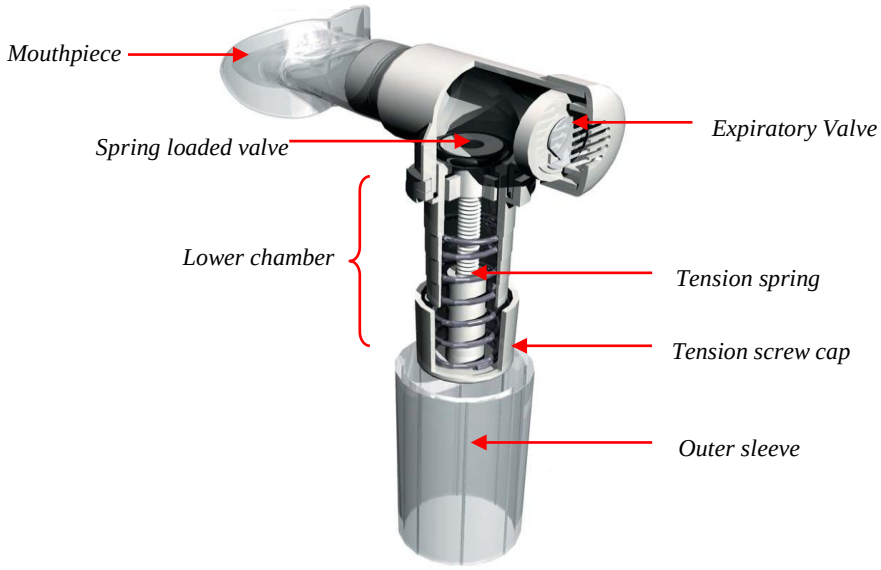

Figure 1.24 Example of a commercially available pressure threshold inspiratory muscle training device (www.powerbreathe.co.uk; accessed 13.02.2009)

Pressure threshold IMT improves MIP by 17 to 55% (Leith and Bradley 1976; Romer et al. 2002a, b, c; McConnell and Sharpe 2005; Volianitis et al. 2001; Tong et al. 2008). The large range in the relative improvements in inspiratory muscle strength may be due, in part, to the baseline inspiratory muscle strength. For example, Johnson et al. (2007) report a 17% improvement in MIP following 6 wk IMT, yet the baseline MIP of the subjects was 137% of the predicted value. Thus in a system with an elevated baseline strength, the scale of physiological adaptation available may be reduced (Åstrand et al. 2003).

Improvements in inspiratory muscle endurance are also noted after IMT. Inbar et al. (2000) used incremental pressure threshold loading in which the resistance was progressively increased every 2 min and observed an increase in the final pressure sustained from 121 to 154 cmH_2O. In addition, pressure threshold IMT improves both the force and velocity (pressure:flow) characteristics of the inspiratory muscles (see Figure 1.22). IMT was shown to increase the maximal inspiratory flow rate (17%), the optimal

pressure development (25%), optimal inspiratory flow rate (17%) and the maximal rate of pressure development (18%). Since MIP also increased (28%), in combination with the increase in the maximal inspiratory flow rate, maximal inspiratory muscle power increased 49% (Romer et al. 2002a; Romer and McConnell 2003). Additionally, a significant 8 to 12% increase in diaphragm thickness has been observed post-IMT (Downey et al. 2007).

Pressure threshold IMT is portable, cheap and easy to use. It is less time consuming than other modes of RMT, with each session usually lasting ~5 min. The pressure generated (and therefore training resistance) is near independent of inspiratory flow rate and with the use of the tensioning load adjustment provided by the screw cap, a high resolution of training loads can be achieved. Other modes of RMT (VIH and FRL) are constrained to velocity axis and pressure axis of the pressure-flow relationship, respectively, whereas both are targeted and improved with pressure threshold IMT (McConnell and Romer 2004a, Romer and McConnell 2003; Romer et al. 2002a).

1.6.6 RESPIRATORY MUSCLE TRAINING AND PERFORMANCE

Numerous studies have investigated the effects of RMT upon whole-body exercise performance. Interpreting the data is confounded by the many different performance tests and modes of training which have been used, inappropriate sample sizes and the lack of appropriate controls. It is clear with the exception of two studies (Edwards and Cooke 2004; Enright et al. 2006) that maximal incremental and constant power intense exercise to volitional tolerance above 90% of either $\dot{V}O_2max$ or $\dot{W}max$ are unaffected by RMT (Fairbarn et al. 1991; Hanel and Secher 1991; Hart et al. 2001; Inbar et al. 2000; Morgan et al. 1987; Riganas et al. 2008; Sonnetti et al. 2001; Wells et al. 2005). At such high intensities, metabolic perturbations within the locomotor muscles outweigh any beneficial effect which the trained respiratory musculature could offer. Furthermore, RMT is unlikely to affect any one component that determine maximal O_2 uptake (where: $\dot{V}O_2max = \dot{Q}max \times a\text{-}vO_2diff$).

Performance was improved during sub-maximal constant power exercise at 70 to 85% $\dot{V}O_2$max / $\dot{W}$max by up to 50% (Boutellier and Piwko 1992; Boutellier et al. 1992; Gething et al. 2004b; Johnson et al. 2007; Leddy et al. 2007; Markov et al. 2001; McMahon et al. 2002; Spengler et al. 1999; Stuessi et al. 2001; Verges et al. 2007b). However, other studies have failed to observe such changes (Kohl et al. 1997; Spengler et al. 1996; Williams et al. 2002). Despite a lack of performance improvements, Spengler et al. (1996) observed ~2 mmol·L^{-1} reduction in $[lac^-]_B$ during exercise and Williams et al. (2002) observed a relationship between the changes in MIP following IMT and the reduction in the perceptions of breathlessness during the last min of the exercise trial which approached significance (no control group; r = -0.650, P = 0.057). Thus it appears that sub-maximal constant power performance is improved with RMT.

Although high in internal validity (i.e. the power output is identical pre and post-RMT), the ecological validity of constant power sub-maximal trials has been questioned since athletic competition does not require a sustained power output, but rather a self-selected oscillating velocity above and below the maximal sustainable intensity. Notwithstanding this, recent evidence suggests that the sensitivity (i.e. the change in performance divided by the error of measurement [SD]) of constant power tests is similar to that of time-trial performance tests. Constant power performance is however constrained by the hyperbolic power-time relationship where small reductions in the ability to produce force results in remarkable reductions in the time to the limit of tolerance (Amann et al. 2008a). Despite this, the greater variance observed in constant power exercise (coefficient of variation: 17 to 40%; Jeukendrup et al. 1996) relative to a time trial (coefficient of variation: 17 to 40%; Jeukendrup et al. 1996) may be deemed negligible since the potential improvements in performance far exceed that observed during time-trial exercise. This results in a sensitivity of constant power exercise tests similar to that of a time-trial. However despite this, Amann et al. (2008a) declare that when investigating the *true* effects of an intervention on "real-life" endurance performance, a time-trial type performance test

is preferable. The sub-conscious selection of a given power-output in such tests avoids working at an intensity which exceeds the lactate threshold and thus the premature draining of the metabolic reserve (Fukuba and Whipp 1999). Above the lactate threshold, performance time is reduced as fatigue develops in proportion to the exercise intensity (Walsh 2000). Furthermore, the athlete can appraise the systemic metabolic environment throughout exercise permitting them to choose an exercise intensity which they feel appropriate to complete the task in the quickest time possible (Amann et al. 2008b; McConnell and Romer 2004a).

Volianitis et al. (2001) were the first to examine the effects of IMT upon rowing time trial performance. Subjects completed either 11 wk of pressure threshold IMT or placebo IMT. The time to complete 5 km and the distance covered in 6 min were improved following IMT by 3.1% (-36 s; $P<0.05$) and 3.5% (+52 m; $P<0.05$), respectively, but remained unchanged following the sham training (5 km: 0.9%, 6 min trial: 1.6%). Improvements have also been reported in 20 and 40 km simulated cycling time-trial on an electromagnetically braked cycle ergometer (3.8 and 4.6%; Romer et al. 2002c) and during a 25 km cycling time-trial where the subjects own racing bicycle was mounted on an air-braked ergometry system (2.7%; Johnson et al. 2007). Leddy et al. (2007) observed a significant 4% (1.2 min) improvement in 4 mile run time above that of a placebo control group and most recently, Edwards et al. (2008) observed a significant 4.3% improvement in 5 km running time trial performance above a placebo (2.2%) following IMT. These findings have also been confirmed and extended by Griffiths and McConnell (2007). They observed a significant 2.7% (~15 m, ~20 W) improvement in a 6 min all out rowing ergometer test following 4 wk IMT but no change in simulated rowing performance following 4 wk of expiratory muscle training (EMT). Furthermore, a subsequent 6 wk period of combined IMT and EMT failed to further change any performance measures. It should be noted that studies investigating EMT and exercise tolerance have been conducted in healthy active subjects (e.g. Griffiths and McConnell 2007). In contrast EMT

appears increase expiratory muscle strength and improve exercise tolerance in clinical populations, such as those with COPD (Weiner et al. 2003). Significant improvements in recovery duration between repeated sprint exercise (Romer et al. 2002b) and distance covered in the Yo-Yo intermittent recovery test (Tong et al. 2008) have also been reported following IMT. Collectively these findings illustrate an ergogenic effect of IMT on whole-body performance but question the use of EMT to further improve performance.

Despite these improvements, Hanel and Secher (1991) observed no differences between groups in the distance covered in a 12 min running test following FRL (8%) and sham-FRL (6%). During IMT, the training regimen required 1 subject from each group to train at the same time, however with sham-IMT the resistance was minimal. Thus it is possible that the sham group were not completely blinded to the outcomes of the study and thus did not participate in a true placebo group. Sonetti et al. (2001) also reported no differences in 8 km cycling time-trial performance following RMT. The time-trial duration was significantly improved by 1.8% but was not different to the placebo control group. In this study, the IMT regimen was a combination of both pressure threshold IMT (~40 breaths once daily) and VIH training (30 min as hard as possible once daily). As a consequence, only modest changes in MIP were observed following the intervention: MIP increased by 8% and 4% in the RMT and placebo groups, respectively. Changes in the maximal sustainable MVV following the intervention were not different between groups although a large coefficient of variation was noted (163%). Therefore, these findings suggest that either the RMT group did not have a sufficient training stimulus throughout the intervention and / or the placebo group also observed a small yet important training stimulus. However, it is known that concurrent strength and endurance training is disadvantageous as strength adaptations are inhibited compared to performing strength training alone. This is likely due to the marked difference in fibre type adaptations that occur with each method and the conflicting intra-cellular signals produced (Leveritt et al. 1999).

In summary, early studies appeared to be limited by the lack of appropriate subject numbers and control / placebo groups. It also seems likely that the nature of the outcome criterion tests selected to assess performance have been inappropriate with open-ended high intensity constant power tests employed which are high in variation. Furthermore, the escalating metabolic acidosis and discomfort associated with intense exercise and the abrupt termination of maximal exercise would overwhelm any potential effects of RMT. More recent, well controlled studies that have employed placebo-controlled methodology have shown significant improvements in both cycling and running time-trial exercise performance as well as intermittent exercise performance. Specific RMT appears to provide an improvement in time-trial type exercise from 1.6 to 4.6%. Romer et al. (2002c) suggest that since the improvements in performance observed following IMT exceeds about half of the natural variance in human performance, the IMT-mediated improvements in exercise performance are likely to present a meaningful ergogenic effect which most importantly would make a difference to an athletes chance of athletic success.

1.6.7 POTENTIAL MECHANISMS

The mechanisms that underpin the improvements in performance following RMT may be (although not exclusively) due to interactions between the respiratory muscles and the brain. Research indicates that improvements in performance are not due to cardiovascular adaptations. Markov et al. (2001) reported improvements in cycling endurance which were not accompanied by changes in stroke volume (SV). Following 15 wk RMT, SV measured using the CO_2 re-breathing technique remained unchanged from baseline during exercise at 60% $\dot{V}O_2$max (pre: 94 ml, post: 93 ml) compared to 15 wk whole-body endurance training (pre: 89 ml, post: 104 ml). Stuessi et al. (2001) also observed no changes in PaO_2, $PaCO_2$ and SaO_2 measured from the radial artery during constant power cycling exercise at 70% $\dot{V}O_2$max. However, in this study, total [Hb] was not measured thus the changes in [CaO_2] which may be affected independent of SaO_2 and

P_aO_2 were not reported. These findings are supported by Edwards and Cooke (2004) who report that the $\dot{V}O_2$ kinetics during the transition from low to moderate intensity exercise (80% of the ventilatory threshold) are unchanged following IMT. Collectively these findings suggest that chronic RMT does not affect the cardiovascular system. This is probably due to the moderate cardiovascular demand placed on the whole-body during RMT. RMT also fails to affect the lactate threshold (Spengler et al. 1999) and the maximal sustainable power output (McConnell and Sharpe 2005; Johnson et al. 2007). A small increase in anaerobic work capacity (which reflects a constant yet finite energy store that is utilised above the maximal sustainable power output) has been observed following IMT but this increase is not great enough to account for the performance improvements. Romer et al. (2002c) suggests that the improvements in exercise tolerance and performance may be due to a number of mechanisms including an RMT-mediated effect upon respiratory muscle fatigue and subsequent locomotor muscle perfusion, changes in breathing mechanics and / or favourable changes in acid-base balance which may affect the perception of both respiratory and locomotor discomfort.

1.6.7.1 ATTENUATED RESPIRATORY MUSCLE FATIGUE

Unloading the respiratory muscles during intense exercise above 85% $\dot{V}O_2$max using PAV attenuates both diaphragm and limb muscle fatigue (Babcock et al. 2002; Romer et al. 2006a) improves exercise tolerance and attenuates the perception of both leg and breathing discomfort (Harms et al. 2000). Dempsey et al. (2006b) state that a reduction in diaphragm fatigue would improve leg blood flow and as a consequence leg exertion is reduced and exercise performance is improved. Whether specific RMT may have similar effects on performance is unknown as the physiological effect of unloading the respiratory muscles is vastly different to the effects of RMT (Wetter and Dempsey 2000). For example, the performance improvement observed when the respiratory muscle are

unloaded by >50% is much smaller (~14%; Harms et al. 2000) than the improvements observed at similar exercise intensities following RMT (>50%).

Post-exercise reductions in MIP, illustrative of inspiratory muscle fatigue are attenuated following IMT during intense rowing exercise (Griffiths and McConnell 2007; Volianitis et al. 2001), cycling time trials (Romer et al. 2002a) and intense constant power exercise in normoxia and hypoxia (Downey et al. 2007). However volitional measures of respiratory muscle fatigue fail to discriminate between a reduction in inspiratory muscle pressure generation and poor coordination / sub-maximal effort. Using magnetic stimulation techniques, Verges et al. (2007b) reported that 4 to 5 wk of RMT attenuated the reduction in twitch *P*di following intense exercise to volitional tolerance at 85% $\dot{W}$ max but only in subjects who demonstrated a reduction in twitch *P*di greater than 10% (absolute reduction twitch *P*di pre: -17 ± 6%, post -9 ± 10%). Interestingly, the reduction in diaphragm fatigue was not correlated with improved exercise performance

An RMT-mediated reduction in inspiratory muscle fatigue may improve performance by attenuating the sympathetic-mediated respiratory muscle metaboreflex. This would improve limb blood flow (and O_2 delivery) and reduce locomotor muscle fatigue; although this has yet to be investigated. There appear to be three lines of evidence that support this hypothesis. Firstly, genuine physiological adaptations within the respiratory muscles are observed following IMT. Muscle biopsy analysis of the human external intercostals following 5 wk pressure threshold IMT has been shown to increase the proportion (38%) and size (21%) of type I and type II muscle fibres (Ramírez-Sarmiento et al. 2002). Animal studies also support this with an increase in diaphragm mitochondrial cytochrome-c oxidase activity observed following 3 wks chronic FRL (Akiyama et al. 1994, 1996). The increase in respiratory muscle oxidative capacity may reduce the reliance on / delay the recruitment of less fatigue resistant type II muscle fibres. An IMT-mediated increase in the maximal force generating capacity reduces the absolute force (Kellerman et al. 2000) generated and detected (Redline et al. 1991) for a given

ventilation. This may reduce inhibitory feedback from the inspiratory muscles to the sensory areas of the brain and attenuate the sympathetic-mediated efferent response.

Secondly, Witt et al. (2007) reported that the sympathetic response to fatiguing diaphragm work during resistive loading at 60% MIP is attenuated post-IMT. Pre-intervention heart rate (HR) and mean arterial pressure increased significantly from 62 ± 3 beats·min^{-1} and 84 ± 1 mmHg at rest to 83 ± 4 beats·min^{-1} and 99 ± 3 mmHg at task failure prior to the intervention (P<0.05). Following 5 wk IMT, when performing the breathing task at the same absolute intensity, the increase in HR and mean arterial pressure from rest was attenuated to 74 ± 2 beats·min^{-1} and 89 mmHg (P<0.05), respectively; no changes were observed in a placebo control group.

Finally, McConnell and Lomax (2006) reported that prior fatigue of the inspiratory muscles using resistive breathing at 60% MIP reduced subsequent isolated plantar flexion performance relative to a control trial. The reduction in performance in the control trial was similar to when a pressure cuff was fastened around the limb which reduced limb blood flow from 7.75 ± 1.70 to 6.86 ± 1.24 ml·min·mmHg^{-1}. Following IMT, despite completing a similar total volume of inspiratory muscle work prior to plantar flexion exercise the time to the limit of tolerance was significantly improved. The improvement in plantar flexion exercise tolerance was attributed to an IMT-mediated improvement in limb blood flow following fatiguing inspiratory muscle work. In support of this, Chiappa et al. (2008a) reported that after 4 wk pressure threshold IMT, calf blood flow during fatiguing inspiratory muscle work was increased, as was forearm blood flow during intense handgrip exercise (repeated 10 s MVC, 20 s rest) following a fatiguing inspiratory breathing challenge in patients with chronic heart failure.

These indirect measures suggest a possible role for RMT in attenuating the respiratory muscle metaboreflex during intense exercise with fatiguing diaphragm work. However, many exercise competitions such as time-trial performance occur at exercise intensities lower than the threshold for diaphragm fatigue and the respiratory muscle

metaboreflex. For example during a 20 to 40 km cycling time-trial, the average power output is ~75% $\dot{W}$ max (Romer et al. 2002c) and the athlete is required to constantly up or down-regulate power output or running velocity in order to effectively pace the bout (Edwards et al. 2008). Consequently, other mechanisms are likely to underpin the improvements in performance observed at sub-maximal exercise intensities. These mechanisms may involve an RMT-mediated change in breathing efficiency and / or systemic $[lac^-]_B$ and their effect upon acid-base balance and the sensations of both respiratory and locomotor discomfort.

1.6.7.2 IMPROVED BREATHING MECHANICS

There are many studies which have reported a reduction in the sensation of breathlessness and changes in exercising breathing pattern following RMT (Downey et al. 2007; Romer et al. 2002b,c Verges et al. 2007b; Volianitits et al. 2001). Verges et al. (2007b) comment that the hyperpnoea response to exercise is extremely plastic after RMT. An RMT-mediated reduction in $\dot{V}_E$ may reduce the work of breathing and possibly the competition for $\dot{Q}$ and O_2 with other metabolically active tissues. However, interpretation of the data is difficult; Romer et al. (2002c) observed an increase in $\dot{V}_E$ caused by an increase in V_T and EILV during a cycling time-trial following RMT. In studies where the same absolute workload is performed prior to and following RMT, others have reported an increase in $\dot{V}_E$ due to an increased f_R without changes in V_T (Holm et al. 2004; Spengler et al. 1999), a decrease in $\dot{V}_E$ due to a lower f_R (Leddy et al. 2007; Suzuki et al. 1995) or a lower V_T (Gething et al. 2004b). Despite the disparity in the breathing response to exercise following RMT, significant relationships are reported between the reduction in $\dot{V}_E$ post RMT and improved exercise tolerance (Kohl et al. 1997; Verges et al. 2007b; Boutellier et al. 1992). Whether an RMT-mediated decrease in $\dot{V}_E$ would reduce whole-body $\dot{V}O_2$ is

unknown since reducing the work of breathing normally performed during intense exercise by >50% using PAV only reduces whole body $\dot{V}O_2$ by ~6.9% (Harms et al. 2000).

Alternatively, performance may be improved following IMT due to a reduction in effort sensations. This is supported most recently by Edwards et al. (2008). In this study, participants were divided into an experimental or a placebo control group and both groups completed 4 wk whole-body cardiovascular training. The experimental group also performed, in addition to the whole-body training, specific IMT. Following the 4 wk intervention, 5000 m running performance was significantly improved in the IMT group only (4.3%, ~50 m). During the 5000 m trial following the intervention, there were no differences in $[lac^-]_B$ and HR at the end of the test. However, RPE was attenuated throughout the training intervention, reaching significance in the 4th wk. Relative to the placebo group, there no changes in the ventilatory threshold or $\dot{V}O_2max$ following the intervention period; this suggested that the performance gain in the IMT group was likely ascribed to the reductions in the perception of effort, which the placebo group did not experience. During exercise, an increase in respiratory drive increases $\dot{V}_E$ which is detected by the sensory brain centres and intensifies the perception of breathing discomfort (El-manshawi et al. 1986). However, following IMT, Huang et al. (2003) observed that the pressure generated during the first 0.1 s of inspiration with the airway briefly occluded (i.e. mouth occlusion pressure after 0.1 s), which provides a measure of respiratory muscle motor drive, decreased by 21.9 ± 5.2%. Kellerman et al. (2000) also reported a reduction in motor output to the respiratory muscles and the magnitude estimation of the inspiratory load with varying inspiratory resistive loads following 4 wk IMT. The authors attribute their findings to an attenuated discharge of respiratory muscle mechanoreceptors. In agreement with this, Sinoway et al. (1996) suggest that group III mechanoreceptors are desensitised due to the repeated high inspiratory loads tolerated throughout IMT. Therefore, it appears that a change in the exercise breathing pattern and / or attenuated sensory feedback between the respiratory muscles to the brain which may influence

subsequent breathing mechanics provides a possible explanation for the overall performance enhancement (Edwards et al. 2008).

1.6.7.3 REDUCED SYSTEMIC BLOOD LACTATE CONCENTRATION

A common and surprising observation following RMT is a reduction in systemic $[lac^-]_B$. This reduction has been observed during maximal incremental cycling (Spengler et al. 1999; Volianitis et al. 2001), 6 min maximal rowing (Griffiths and McConnell 2007), constant power exercise to the limit of tolerance (Leddy et al. 2008; Kohl et al. 1997; Spengler et al. 1999; Boutellier and Piwko 1992; Verges et al. 2007b), exercise at the maximal lactate steady state (McConnell and Sharpe 2005) and during repeated sprint exercise (Romer et al. 2002a; Tong et al. 2008). The reductions observed are often similar to those which occurs following whole-body exercise training (>2 $mmol \cdot L^{-1}$; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999). Interestingly, Verges et al. (2007b) reported a rise in systemic $[lac^-]_B$ following exhaustive volitional hyperpnoea at 70% MVV which was attenuated following VIH training. Despite the attenuated rise in $[lac^-]_B$ throughout hyperpnoea, no changes in the exercising $[lac^-]_B$ were observed. It is therefore unknown whether the changes in exercise $[lac^-]_B$ observed during whole body exercise are due to the respiratory muscles the locomotor muscles and / or other metabolically active tissues. Spengler et al. (1999) suggest that the reductions in $[lac^-]_B$ following RMT may be due to either a decrease in the net lactate efflux by the respiratory muscles or an increase in respiratory muscle lactate consumption although this is yet to be rigorously investigated. Despite this, data from whole body training studies suggests an important role for this small muscle group in attenuating systemic $[lac^-]_B$.

During intense exercise, a reduction in circulating metabolites may attenuate the discharge of chemosensitive afferent fibres located within the diaphragm which are known to trigger the respiratory muscle metaboreflex (Rodman et al. 2003). However, significant reductions in $[lac^-]_B$ are observed even at sub-maximal exercise intensities. Here the lower

$[lac^-]_B$ may be more important in the attenuation of the perceptions of limb discomfort. Romer et al. (2002a) observed a significant correlation between the change in systemic $[lac^-]_B$ and total recovery time taken between repeated sprints as well as the reduction in RPE. Thus favourable change in acid-base balance may be associated with the reduction in the intensity of peripheral effort sensations (Romer et al. 2002a).

Throughout repeated bouts of respiratory muscle training, sensory afferents within the respiratory muscle may become desensitised. Repeated exposures to high metabolite concentrations which may occur during IMT would reduce the afferent-mediated efferent response to a given change in metabolite concentration during subsequent exercise (Sinoway et al. 1992, 1993). In support of this, Sinoway et al. (1992) observed that the discharge of sympathetic afferents was lower in the trained forearm relative to the untrained control. These findings were confirmed in a second experiment where exercise was performed with a pressure cuff around the arm and afferent discharge for a given concentration of lactate and / or pH was lower in trained persons. These findings have since been confirmed in anaesthetised cats. In this study, repeated exposures to high concentrations of lactate reduced the firing frequency of chemosensitive afferent fibres when the hind limbs were electrically stimulated (Sinoway et al. 1993). Therefore, the chronic conditioning of mechanoreceptors and chemoreceptors over the period of RMT may reduce afferent feedback to the brain, attenuating the perceptions of breathing discomfort.

In summary, there are many factors which appear to contribute to an improved whole-body performance following RMT. Given the link between diaphragm fatigue, the respiratory muscle metaboreflex and exercise tolerance, much work has focused on whether RMT attenuates the reflex reduction in limb blood flow. However, given that athletic performance occurs at sub-maximal often steady state exercise intensities where the respiratory muscle metaboreflex does not occur, other mechanism have been explored. Of these mechanisms, one which has received very little attention is the role of lactate.

Lactate is consistently lower during whole body exercise following IMT, yet the possible role this metabolite has in performance improvement following RMT remains elusive. It is also unknown whether the respiratory muscles are capable of influencing systemic lactate kinetics at all. Consequently, the following sections address the functional role of lactate and the possible role the trained and untrained respiratory muscle have in affecting systemic lactate turnover.

1.7 LACTATE: A BRIEF INTRODUCTION

Historically, lactate (commonly referred to as lactic acid) was thought to be a dead end metabolite produced during intense, anaerobic exercise. It was proposed that lactic acid disassociates into the acid salt lactate and a free proton (H^+). This process was called lactic acidosis and was synonymous with metabolic acidosis. The accumulation of intracellular [H^+] would cause fatigue by reducing pH, interfering with the contractile structures of the sarcomere and / or by poisoning the myofibril. This concept, was based on the pioneering work of Hill and Meyerhoff in 1922 (cited in Robergs et al. 2004) who suggested that lactic acid was produced due to muscle hypoxia at the onset of exercise. This theory was later substantiated by a significant negative linear relationship between lactic acid and pH during intense exercise leading to a unanimous, incorrect conclusion that lactic acid caused peripheral muscle fatigue (r = - 0.912; Margaria et al. 1933; Sahlin et al. 1976). However, contemporary physiologists persist that this hypothesis has no biochemical support and is fundamentally flawed (Robergs et al. 2004).

1.7.1 CURRENT CONCEPTS IN LACTATE BIOCHEMISTRY

Current opinion accepts that lactate rather than lactic acid is produced as a direct consequence of anaerobic glycolysis. Furthermore, lactate is produced under fully aerobic conditions (predominantly within the erythrocyte which are void of mitochondria) as it serves as an important carbon source for oxidation and gluconeogenesis. For example, in

the post-absorptive state, approximately 25-50% of carbohydrates passes through the lactate pool and following-exercise, up to 70% of lactate is oxidised (Brooks 1986).

During sub-maximal exercise below the anaerobic threshold, pyruvate (the end product of glycolysis) is formed following a series of ten reactions (see Figure 1.25, reactions ① to ⑩) and enters the mitochondria. Electrons produced during glycolysis and the tricarboxylate cycle (or Krebs cycle; named after Hans Krebs in 1937) enter the electron transport chain (ETC), where under such conditions mitochondrial respiration is sufficient to resynthesise ATP. In the cytoplasm, nicotinamide adenine-dinucleotide (NAD^+) is reduced to $NADH+H^+$ via the glyceraldehyde 3-phosphate dehydrogenase reaction (Figure 1.25: ⑥). Since $NADH+H^+$ can not cross the mitochondrial membrane, it is oxidised and active mitochondrial membrane electron carrier proteins transport the H^+ in to the mitochondria. The delivery of electrons to inter-mitochondrial NAD^+ and FAD by the active carriers allows chanelling to the ETC for subsequent donation to molecular O_2. These shuttles are known as the malate asparate shuttle and / or the glycerol phosphate shuttle. Thus the oxidation of $NADH+H^+$ to NAD^+ maintains the glycolytic rate by preserving the flow of NAD^+ through the glyceraldehyde 3-phosphate dehydrogenase reaction and thus the flow of metabolites to subsequent oxidative phosphorylation (Mougios 2006).

However, with increasing exercise intensities, cytosolic ATP production contributes increasingly to the total [ATP] and the rate of $NADH+H^+$ formation in the cytosol exceeds the rate of NAD^+ regeneration by oxidative phosphorylation. Note: glycolysis yields 2 ATP per glucose molecule and glycogenolyis yields 3ATP per glucose-1-phosphate. The disparity is accounted for by the spending of 1ATP during glycolysis in reaction ① as shown in Figure 1.25. With an increase in the glycolytic rate, lactate dehydrogenase (reaction 12) catalyses the conversion of pyruvate to lactate, therefore, pyruvate is the oxidant of $NADH+H^+$.

Thus the production of lactate restores cytosolic [NAD^+], maintains the glyceraldehyde 3-phosphate dehydrogenase reaction (cytosolic redox potential), but most importantly, facilitates ATP production. It has been suggested that the formation of lactate and NAD^+ from pyruvate requires 2 electrons and 2 H^+, thus, the formation of lactate has an alkalinising effect on the muscle cell by attenuating cellular proton accumulation and retarding the development of a metabolic acidosis (Robergs et al. 2004). Whether this reduces whole-body [H^+] is equivocal given the large volume of readily available H_2O and thus [H^+] and OH^- which may offset such effects. Lactate is therefore an important marker of cytosolic redox potential and anaerobic metabolism (Mougios 2006).

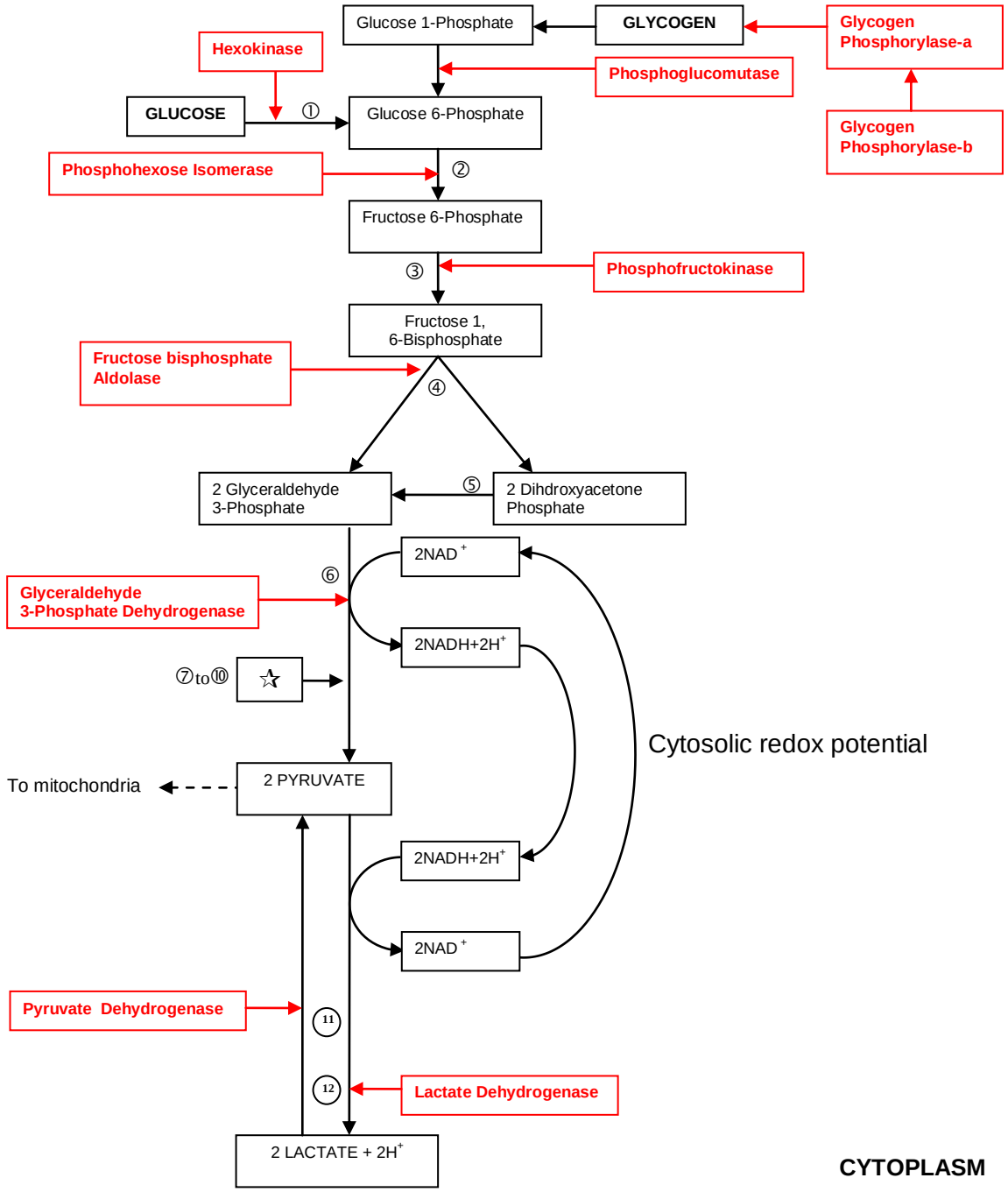

Figure 1.25 Simplified schematic of glycolysis and lactate production. Red font, enzyme; red arrow, site of enzyme activity; H^+, hydrogen ion; NAD^+, Nicatinamide adenine denuclotide; ATP, adenosine triphosphate. ☆, Reactions not shown (⑦ to ⑩ above) include (product [enzyme]) 1,3-Bisphosphoglycerate [Phophoglycerate Kinase], 3-Phosphoglycerate [Phosphoglycerate mutase], 2-Phosphoglycerate [Enolase] and finally the conversion of Phosphoenolpyruvate to Pyruvate [Pyruvate kinase].

Traditionally, lactate was thought to be transported from the cytosol into the blood via passive diffusion and into the mitochondria through the PDH reaction. However significant evidence now supports the lactate shuttle hypothesis which describes both the intracellular and extracellular transport of lactate over both sarcolemmal and mitochondrial membranes (Brooks 1986, Brooks 1991). The active transport of lactate occurs via two

predominant monocarboxylate transporters (MCT), MCT1 and MCT4 with specific functions for lactate influx and efflux, respectively. The presence of the MCT proteins facilitates the efflux of lactate from the glycolytic muscle fibres (type II) and influx to the oxidative muscle fibres (type I). There is ~40% lower [MCT1] in glycolytic fibres compared to their type I counterparts (Juel 2001). MCT mediated lactate transport occurs with a 1:1 coupling between lactate and H^+, therefore, the active movement of lactate may have profound effects on the maintenance of intramuscular acid-base balance.

Much debate remains as to the effects of lactate *per-se* on intramuscular pH. Many researchers maintain that lactate production contributes to reductions in pH as the buffering capacity of the cell is breached (Stringer and Wasserman 2005). Others suggest an integrated physicochemical systems approach to understanding the role of lactate in acid-base balance (Stewart 1983), which appears to be favoured at present (Gladden 2008). Using the Stewart approach, mechanisms accounting for disturbances in acid-base balance during and following exercise can be quantified. Within a given compartment (e.g. muscle, plasma, erythrocyte) the dependent variables which are $[H^+]$ and $[HCO_3^-]$ are determined by the net effect of the strong ion difference ($[SID] = [Na^+] + [K^+] - [Cl^-] + [lac^-]$), PCO_2 and the total concentration of weak acids ($[A_{tot}^-]$), principally albumin. According to this model, assuming minimal change in other strong ions, the $[lac^-]$ of a given compartment may *indirectly* affect $[H^+]$ by causing a *direct* change in [SID] (Lindinger 1995; Kowalchuck and Scheuermann 1995).

Debate exists whether co-transport of $[H^+]$ and lactate across a given membrane affects the compartment pH at all, due to the large expanse of water which can act as a reservoir for free H^+ and OH^- (Putman et al. 2003). Indeed lactate efflux during moderate intensity cycling exercise is up to four times greater than H^+ efflux. These authors suggest that the movement of strong ions across a given membrane affects the destination pH giving an apparent, false H^+ efflux. Regardless of the mechanism(s), the direct effect of a decrease in pH on skeletal muscle fatigue at human physiological temperatures has been

questioned (Westerblad et al. 1997). Notwithstanding this, given that large reductions in $[lac^-]_B$ are observed during whole-body exercise following RMT, if the respiratory muscles are capable of increasing and / or decreasing the compartment $[lac^-]$, this small muscle mass may have important effects on intramuscular and plasma acid-base balance.

1.7.2 RESPIRATORY MUSCLE LACTATE KINETICS: ANIMAL STUDIES

Due to the small muscle mass and high oxidative capacity, the notion that the respiratory muscles may contribute to systemic lactate kinetics by either net lactate production and / or consumption has often been disregarded (Wetter and Dempsey 2000). However, the fibre type distribution of the respiratory muscles is similar to locomotor muscles (see Figure 1.12). Therefore, conceptually it is likely that this muscle group can influence whole-body lactate kinetics. To investigate this hypothesis, research has often favoured animal models as a surrogate for humans owing to the ease in which direct physiological responses can be measured through arterial and venous blood sampling as well as access to diaphragm tissue post exercise.

Ciufo et al. (2001) observed an increase in phrenic venous $[lac^-]_B$ from 3.1 $mmol \cdot L^{-1}$ at rest to 6.4 mmol·L following 30 min of intense flow resistive loading of 32,000 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ to the point of respiratory arrest in anesthetised rats (n = 77;). Arterial $[lac^-]_B$ was unchanged from rest, as was the soleus $[lac^-]_B$ (control), however, diaphragm [glycogen] was reduced from 15.8 to 8.4 mmol·g. These data suggest that the diaphragm may be a net producer of lactate; however, the external validity of such high resistive loads is questionable. In contrast to these data, Bazzy et al. (1989) reported no change in phrenic vein $[lac^-]_B$ following a less intensive resistive load of 150 $cmH_2O \cdot L^{-1} \cdot s^{-1}$ in sheep. The load was induced by a cuffed tracheostomy tube which increased Pdi to 74.7 cmH_2O. However, during resistive loading there was a marked respiratory acidosis increasing PCO_2 from 36.4 mmHg at rest to 70 mmHg at the cessation of the trial. This large increase in PCO_2 may attenuate $[lac^-]_B$ due to the intramuscular inhibition of PFK

(Graham et al. 1986). Both of these studies have little application to exercise as the methods used to increase the work of breathing have been extreme and in the study of Ciufo et al. (2001) maintained until death.

Improving significantly on the ecological validity of previous work, Fregosi and Dempsey (1989) measured intramuscular metabolites during whole-body exercise in wistar rats (n = 120). Animals completed a number of trials on a motorised treadmill including: 10 min sub-maximal exercise, 10 min high-intensity exercise and maximal exercise to exhaustion (~ 4.1 ± 0.3 min). A third group of rats also exercised sub-maximally to exhaustion in hypoxia (F_IO_2: 0.12). During sub-maximal normoxic exercise, there was no change in [lac^-] in any tissues, however, when the intensity exceeded 92% $\dot{V}O_2$max (high-intensity and maximal trials), [lac^-] increased by 234 ± 12 and 214 ± 9% in the diaphragm and intercostals, respectively, and 466 ± 21 and 462 ± 8% in the plantaris and arterial blood, respectively. That diaphragm and intercostal $[lac^-]_M$ was less than arterial suggests that these tissues favoured net lactate uptake. Following the sub-maximal and high intensity prolonged exercise trials, diaphragm and plantaris muscle [glycogen] were unchanged and significantly reduced, respectively, and [glucose 6-phosphate] was increased (267%) and unchanged, respectively. Interestingly, only during exercise to exhaustion in hypoxia was there evidence of glycogen utilisation in the diaphragm, which was also observed in the intercostals and plantaris. In the diaphragm, intercostals and plantaris muscle [glycogen] was 58, 44 and 20% of hypoxic control values. Figure 1.26A and B, shows $[lac^-]_M$ and $[glycogen]_M$ during sub-maximal and maximal exercise. Figure 1.26C shows that increases in $[lac^-]_M$ in the plantaris were related to the decrease in [glycogen] in both conditions (r = 0.67, P<0.01), however, this was only true during exercise in hypoxia for the diaphragm (r = 0.92, P<0.01).

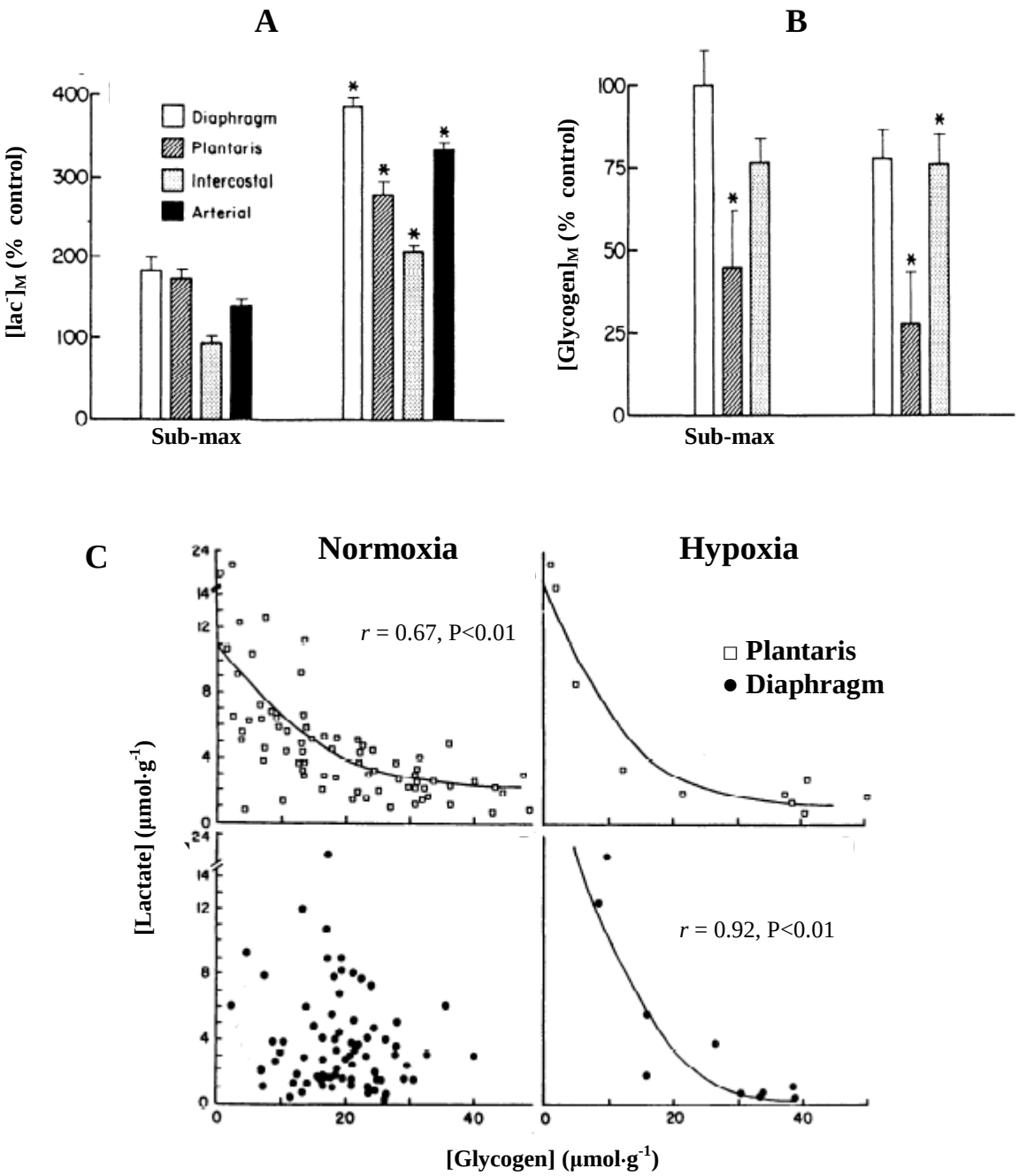

Figure 1.26 Changes in muscle metabolite concentrations. (A) lactate and (B) glycogen concentration following prolonged exhaustive exercise at 68% $\dot{V}O_2$ max (left bars in each figure, 1.32 km·h^{-1} on a 1% gradient) and 84% $\dot{V}O_2$ max (right bars in each figure, 1.44 km·h^{-1} on a 10% gradient). (C) Changes in lactate and glycogen concentrations following exhaustive exercise in normoxia and hypoxia (Fregosi and Dempsey 1989).

The findings of Fregosi and Dempsey suggests that only during prolonged exercise to exhaustion in hypoxia is endogenous glycogen utilised within the rat diaphragm which may result in an increased diaphragm $[lac^-]_M$; although it is important to note however that the end-exercise [glycogen] was still ~50% baseline. Since [glucose 6-phosphate] was increased and muscle glycogen preserved in all trials in normoxia suggests that

endogenous glycogen stores were utilised secondary to blood borne substrates such as liver-derived blood glucose and free fatty acids. Glycogen breakdown is facilitated by the enzyme phosphorylase which in turns is converted to glucose 1-phosphate and via the enzyme phosphoglucomutase forms [glucose 6-phosphate] in the cytoplasm (see Figure 1.25). Secondary to an increased β-oxidation, the increase in [glucose 6-phosphate] attenuates the conversion of the enzyme glycogen phosphorylase-b to its more active form glycogen phosphorylase-a by allosteric regulation. This regulation decreases the formation of glucose 1-phosphate and the net glycolytic flux. The increase in [glucose 6-phosphate] also facilitates endogenous glycogenesis. This latter allosteric regulation does not occur in hypoxia as an increase in catecholamine concentration speeds glycogen breakdown independent of allosteric effects (Watt et al. 2001).

Interestingly, an earlier study which was methodologically similar to that of the study by Fregosi and Demspey, Ianuzzo et al (1987) employed one sub-maximal exercise trial to exhaustion in normoxia and reported somewhat contradictory findings. In contrast to Fregosi and Demspey the authors reported marked glycogen depletion in the albino rat diaphragm (43% of control), intercostals (43%), plantaris (76%) and also the heart (39%). Accounting for these differences in findings is difficult since both employ near-identical exercise protocols and killing / sampling techniques. Interestingly, baseline [glycogen] of the diaphragm, intercostals and plantaris in the study by Ianuzzo and colleagues was 10 to 15 $\mu mol \cdot gm^{-1}$ wet weight greater despite a similar mean animal body mass. The disparity may be explained by sex differences in glycogen utilisation that are sometimes observed during exercise (although neither study report the sex of their animals; Ivey and Gaesser, 1987) and / or strain differences in post-exercise glycogen metabolism that may exist between species (Albino vs. Wistar). More likely, the difference in findings are explained by a combination of tissue sampling delay which was prolonged by Ianuzzo et al. (1.32 min vs. 45 s), sample size (n = 14 vs. n = 90) and exercise time to exhaustion: 48.30 ± 11.45 min vs. 38.0 ± 3.0 min. Longer exercise duration is known to exacerbate glycogen

depletion (Ivey and Gaesser, 1987). What is apparent from these studies is that the diaphragm, either (I) engages in net lactate consumption with the intention of consuming lactate as a respiratory fuel and / or (II) the rat diaphragm, as with other skeletal muscles becomes depleted of glycogen during prolonged exercise in normoxia and hypoxia and may be capable of net lactate production. Given the methodological disparity between these studies and the use of rats, it is difficult to extend these findings to exercising humans.

In a series of excellent studies, Manohar and Colleagues quantified the metabolic demands of the pony diaphragm during short duration maximal (Manohar et al. 1988; Manohar and Hassan, 1990) and prolonged sub-maximal exercise (Manohar and Hassan, 1991). Manohar et al. (1988) studied the changes in diaphragm metabolism during incremental exercise comprising three, 4 min intervals at 16, 24 and 32 $km \cdot h^{-1}$ on a motorised treadmill. Throughout exercise, blood was sampled from the abdominal aorta and the phrenic vein; in the pony the phrenic vein represents the primary site of costal hemi-diaphragm drainage. With increasing exercise intensities despite a marked increase in O_2 extraction and decrease in pH, PCO_2 and $[HCO_3^-]$, there were no differences in arterial-phrenic venous $[lac^-]_B$. However, during heavy and maximal exercise, there was a non-significant reduction in phrenic venous $[lac^-]_B$ of 0.46 and 1.02 $mmol \cdot L^{-1}$, respectively.

In a subsequent study (Manohar and Hassan 1990) ponies exercised at 32 $km \cdot h^{-1}$ to exhaustion on a motorised treadmill (7% grade) and diaphragm metabolism was measured as above (i.e. phrenic arterial-venous $[lac^-]$ difference). Exercise was terminated within approximately 4 min. At the cessation of exercise, arterial $[lac^-]_B$ increased, as did [ammonia], furthermore there was a reduction in pH and PCO_2 due to a marked hyperventilation. In contrast, phrenic $[lac^-]_B$ and [ammonia] were not different to arterial values. Similar to the previous study, there was a non-significant decrease in phrenic venous $[lac^-]_B$ during the final 3 min of exercise ~1.5 $mmol \cdot L^{-1}$ (Figure 1.27). The non-significant yet quite large reduction in phrenic venous $[lac^-]_B$ suggests a possible role, in

agreement with the data of Fregosi and Dempsey, for diaphragm net lactate consumption. Similar results were also noted (Manohar and Hassan 1991) where ponies exercised sub-maximally for 30 min at 24 $km \cdot h^{-1}$ on a 7% grade. At the cessation of exercise, there were no differences in arterial and phrenic venous $[lac^-]_B$, although, at min 5, 10 and 15 inclusive, venous lactate was 1 $mmol \cdot L^{-1}$ lower than the corresponding arterial value (non-significant).

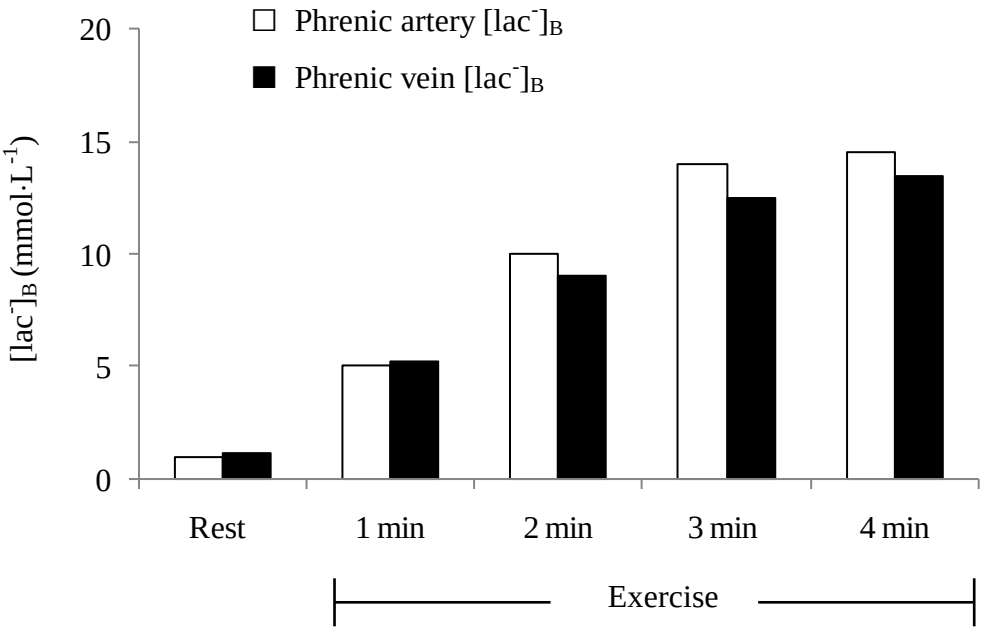

Figure 1.27 Arterial and phrenic-venous $[lac^-]_B$ at rest, and during 4 min of maximal exercise to exhaustion (Manohar and Hassan 1990).

Previous studies that have reported large changes in $[lac^-]_B$ in the animal diaphragm following resistive breathing are questionable since the loads imposed on the inspiratory muscles were exceptionally high. Breathing challenges that employ resistive loading vs. exercise hyperpnoea also differ in the pattern of respiratory muscle recruitment as the protocols occupy different regions of the force-velocity and length-tension relationships. Data from exercising quadrupeds suggest a minimal role of the diaphragm in net lactate production but a more likely role for net lactate consumption (Fregosi and Dempsey 1989). However, the breathing mechanics, and therefore the metabolic characteristics of the quadruped are markedly different to the exercising human. For example, a 1:1 ratio exists

between the breathing and stride frequency in the exercising quadruped (Padilla et al. 2004). Additionally, the axial displacement of the abdominal viscera during exercise (Cobb et al. 1994) facilitates passive diaphragm shortening by functioning as a mechanical piston (Bramble and Carrier 1983). Therefore, with f_R limited by stride frequency and the partial unloading of the diaphragm during the breath cycle, application of these findings to exercising humans is difficult.

1.7.3 RESPIRATORY MUSCLE LACTATE KINETICS: HUMAN STUDIES

Lactate production by the human respiratory muscles has been investigated for almost 50 years. Direct measurement and evaluation of respiratory muscle metabolism in humans is inherently restricted by the anatomical location and proximity of this muscle group to essential organs. Furthermore, given the unique interaction between the primary inspiratory, expiratory and accessory muscles during hyperpnoea, identifying the specific location(s) of lactate production and exchange becomes problematic. This is particularly important since lactate production, release, consumption and transport occur simultaneously within the myofibril (Brooks 1986). Consequently the contribution of the respiratory muscles to lactate kinetics is typically based upon the interpretation of arterialised venous blood lactate measurements. However, caution is warranted when interpreting $[lac^-]_B$ as an estimate of respiratory muscle metabolism. Firstly, $[lac^-]_B$ reflects both the rate of lactate release in to the systemic circulation *and* the rate of lactate removal by metabolically active tissue and organs and secondly, at rest, metabolically active tissues including the brain, liver and the heart as well as other non-skeletal muscles are capable of simultaneously removing, releasing or consuming lactate as a respiratory fuel (Brooks, 1986).

The first study to investigate the contribution of the respiratory muscles to $[lac^-]_B$ in humans was conducted by Eldridge (1966). Given the mistaken biochemistry and fate of lactate (See section 1.14.1), Eldridge suggested that following a breathing challenge whilst

at rest, an increase in arterial $[lac^-]_B$ would directly reflect fatigue of the respiratory muscles. Eldridge (1966) studied eleven subjects of which two were described as "cardiac or pulmonary disease patients". Subjects completed a number of breathing challenges with a spontaneous breathing pattern, these included hypoxia (F_IO_2: 0.15), 1290 ml dead space-induced hyperventilation and flow resistive loading (21 cmH_2O). In these trials $\dot{V}_E$ was 7.9, 28.3 and 21.6 $L \cdot min^{-1}$, respectively. $[lac^-]_B$ was unchanged following each individual breathing challenge, although, combining all three in a subsequent trial elevated $[lac^-]_B$ from 0.61 $mmol \cdot L^{-1}$ at rest to 1.02 $mmol \cdot L^{-1}$. However, given that the methods used to increase the work of breathing in this study do not reflect exercise, the findings of this study are very limited.

In recent years, volitional isocapnic hyperpnoea under resting conditions or imposed upon exercise hyperpnoea has been employed. Reproducing the ventilatory requirements of exercise hyperpnoea whilst all other muscles are otherwise at rest or working at sub-maximal exercise intensities provides a unique model to assess the metabolic response of the respiratory muscles to exercise hyperpnoea. Despite the ecological validity of such experimental methods, Klas and Dempsey (1989) report that even when matching the maximal exercise flow:volume loop, EELV and breathing pattern during volitional hyperpnoea at rest, *P*ga exceeds that observed during exercise. Despite this, Coast et al. (1993) state that respiratory muscle $\dot{V}O_2$ during exercise and volitional hyperpnoea is not different when the absolute $\dot{V}_E$ is between 30 to 130 $L \cdot min^{-1}$ and the breathing pattern achieved suring whole-body exercise, which includes the f_R, V_T and T_I/T_{tot} are mimicked accurately. Therefore, when the breathing pattern is rigorously controlled, isocapnic volitional hyperpnoea can provide an appropriate method to investigate the work of breathing and its metabolic consequences.

Freedman et al. (1983) imposed sustained isocapnic MVV whilst semi-recumbent with f_R fixed at 60 breaths·min^{-1} ($\dot{V}_E$: 108.6 L·min^{-1}; 68% MVV) and reported an absolute increase in $[lac^-]_B$ of 1.1 mmol·L^{-1} following 10 min (range 0.0 to 2.7 mmol·L^{-1}). In another study (Martin et al. 1984) subjects were seated and completed three 5 min bouts of isocapnic volitional hyperpnoea, at 85, 100 and 115% $\dot{V}_E$ max with a relative increase in f_R and a fixed V_T. This breathing pattern was chosen to represent the breathing mechanics of maximal exercise. These relative intensities corresponded to a $\dot{V}_E$ of 117, 138 and 159 L·min^{-1}, respectively. Following hyperpnoea $[lac^-]_B$ was significantly increased when $\dot{V}_E$ was greater than 138 L·min^{-1} (72% MVV; resting $[lac^-]_B$: 0.88 mmol·L^{-1}, post-hyperpnoea at 138 L·min^{-1}: 1.36 mmol·L^{-1}; post hyperpnoea at 159 L·min^{-1}: 1.70 mmol·L^{-1}). Interestingly, the greatest change in $[lac^-]_B$ was positively correlated with the $\dot{V}_E$ from subjects that utilised the largest percentage of their MVV (r = 0.76, P < 0.05). In agreement with this, Verges et al. (2007b) observed a significant increase in $[lac^-]_B$ to 1.7 ± 0.8 mmol·L^{-1} (resting $[lac^-]_B$ not reported) following volitional hyperpnoea to volitional tolerance at 69 ± 7% MVV_{15}. Therefore it appears that a threshold ventilation exists (~70% MVV), below which minimal changes in $[lac^-]_B$ are observed. This is supported by Spengler et al. (2000) who demonstrated no change in $[lac^-]_B$ following 41 ± 9 min volitional hyperpnoea at 51 and 62% MVV_{20} (resting $[lac^-]_B$: 1.6 ± 0.5 mmol·L^{-1}, post-51% MVV: 1.4 ± 0.3 mmol·L^{-1}; post-62% MVV: 1.9 ± 0.9 mmol·L^{-1}). It is clear that when the work of breathing increases above a certain level, the respiratory muscles are capable of net lactate production and release.

A large drawback of assessing the metabolic contribution of the respiratory muscles to whole-body lactate kinetics during volitional hyperpnoea at rest is that there is additional capacity to counter lactate appearance by other metabolically active tissues such as the liver, heart, brain and non-active skeletal muscle (Brooks 1986, 2000). To circumvent these issues, an exercising model has been used (Edwards and Clode 1979;

Engelen et al. 1995; Johnson et al. 2006). Edwards and Clode (1979) examined the effects of spontaneous voluntary hyperventilation on $[lac^-]_B$ whilst exercising at 98 W for 6 min on a cycle ergometer (n = 7 smokers [classified as smoking no more than 5 cigarettes per day]). $\dot{V}_E$ increased from 46.9 L·min^{-1} during exercise to 80.6 L·min^{-1} with volitional hyperpnoea and $[lac^-]_B$ increased from 3.4 to 4.5 mmol·L^{-1}. However, during volitional hyperpnoea, PCO_2 decreased approximately 12 mmHg from 43.5 mmHg at rest to 31.5 mmHg at the end of the trial. The efficacy of these results are questionable since respiratory alkalosis is known to increase $[lac^-]_B$ due to a pH-mediated inhibition of PDH (LeBlanc et al. 2002). Interestingly, when the experiment was repeated maintaining isocapnia, $[lac^-]_B$ did not change from baseline (n = 1; Edwards and Clode 1979). Engelen et al. (1995) also reported no change in $[lac^-]_B$ when spontaneous maximal breathing was imposed upon 10 min constant power cycling exercise above the lactate threshold (94 W; n = 5 healthy subjects). However, the subjects in this study were untrained ($\dot{V}O_2$max: 2.32 L·min^{-1}) and the absolute $\dot{V}_E$ attained during hyperpnoea was 80 L·min^{-1} (45% MVV). Consequently, it is not surprising that changes in $[lac^-]_B$ were minimal.

Most recently, maximal isocapnic volitional hyperpnoea was performed whilst exercising at the MLSS (Johnson et al. 2006). Unlike at rest or during exercise above the lactate threshold, the MLSS represents a relative constant power exercise intensity where the rate of lactate appearance in to the blood is matched by an equal rate of lactate removal from the blood and therefore $[lac^-]_B$ remains constant over time (Figure 1.28, ●). Johnson and colleagues reported a significant 25% (1.0 mmol·L^{-1}; Figure 1.28, ○) increase in $[lac^-]_B$ when maximal spontaneous breathing was imposed from min 20 to 28 of a 30 min constant power cycling trial (mean MLSS power: 207 W). During volitional hyperpnoea, subjects were instructed to attain their maximal spontaneous $\dot{V}_E$ (168.3 L·min^{-1}) which was not different to $\dot{V}_E$max. There was a 6% increase in PCO_2 during the volitional hyperpnoea trial, however, this would only serve to reduce the $[lac^-]_B$ therefore, these findings may

have underestimated the true change in $[lac^-]_B$. Since the MLSS represents an exercise intensity where the capacity for lactate removal is abolished and there is little capacity to counter further lactate appearance, this study suggests that the respiratory muscles are the source of at least part of the increase in $[lac^-]_B$ observed during intense endurance exercise.

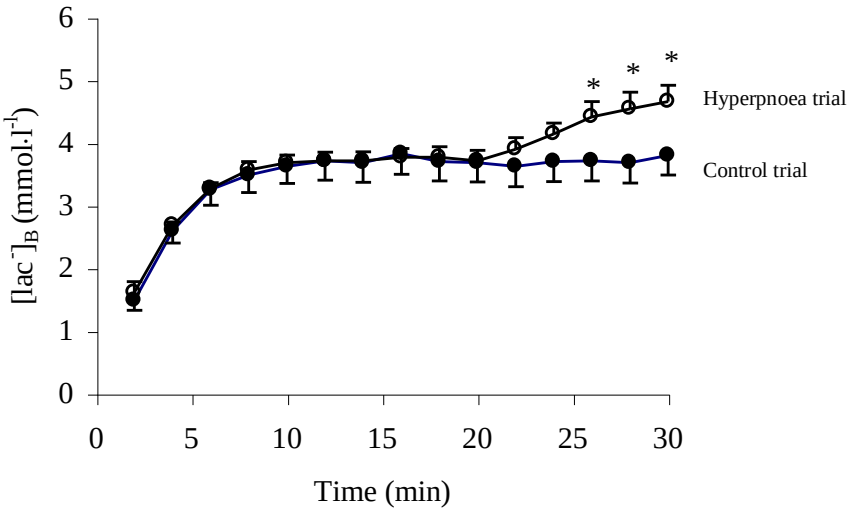

Figure 1.28 Changes in blood lactate concentration ($[lac^-]_B$) and blood acid-base balance during exercise at MLSS power. Control trial (•); experimental trial (o; Johnson et al. 2006).

The precise origin(s) of this increase is unknown, i.e. primary inspiratory, expiratory, or accessory muscles although Babcock et al. (1995) suggest that the diaphragm *per-se* is an unlikely source (Babcock et al. 1995). In this study, subjects performed an endurance exercise test to exhaustion at 85 to 90% $\dot{V}O_2$ max (mean time to volitional tolerance: 13.2 min). On a subsequent trial, participants mimicked at rest and for the same duration the V_T, f_R, T_I/T_{tot} $\int Pdi$ from the final third of the endurance exercise test. Immediately following isocapnic hyperpnoea, there were no changes in evoked *P*di (1, 10, 20 Hz) and minimal changes in $[lac^-]_B$ (mean peak $[lac^-]_B$ 1.1 mmol·L^{-1}). It should be noted however that the exercise test in this study was running, therefore, during the volitional hyperpnoea mimic trial, the work of breathing may have been underestimated as the propulsive forces of locomotion on the thoracic compartment create a cyclical mechanical loading which the respiratory muscles must appose (Bramble and Carrier 1983). In this study by Babcock et al. (1995) the target $\int Pdi$ was calculated as the average of the final

third of the maximal exercise test, i.e. the final 4.4 min. During intense exercise a time and intensity-dependent alveolar hyperventilation occurs which reflects either a respiratory compensation for a metabolic acidosis and / or diaphragm fatigue (Johnson et al. 1993; Sheel, 2002). This hyperventilation is characterised by an increase in $\int Poe/\int Pdi$ (Johnson et al. 1993) and a reduction in the contribution of the diaphragm to the total respiratory muscle power output (Babcock et al. 1998). Thus the changes in $[lac^-]_B$ observed during volitional hyperpnoea in previous studies may be due to the increased recruitment of the less efficient accessory muscles. It is also attractive to speculate that those subjects who achieve a $\dot{V}_E$ that comprises a greater percentage of their MVV will have a greater contribution from the accessory respiratory muscles to increases in systemic $[lac^-]_B$.

To date the majority of studies have investigated the contribution of the respiratory muscles to increases in systemic $[lac^-]_B$. However, in line with the findings of Fregosi and Demspey, recent studies have assessed the ability of the respiratory muscles to consume lactate. This notion is supported by and founded upon studies that report a significant reduction in $[lac^-]_B$ during whole-body exercise performed at the same absolute exercise intensity following specific RMT (e.g. McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al, 1999). Perret and Müeller (2007) investigated the effects of low intensity volitional hyperpnoea ($\dot{V}_E$ 61.6 ± 9.3 $L \cdot min^{-1}$, 30 ± 1% MVV) and a passive recovery (no exercise) on lactate clearance following maximal arm cranking exercise in wheelchair athletes. The decay in $[lac^-]_B$ following exercise was not different between trials (Figure 1.29A). However, since a critical $\dot{V}_E$ (~70% of MVV) is required to cause an increase in $[lac^-]_B$ during volitional hyperpnoea, a relative breathing intensity following exercise closer to this may have a greater impact on $[lac^-]_B$ recovery kinetics as is observed with an active recovery following whole-body exercise (Dodd et al. 1984).

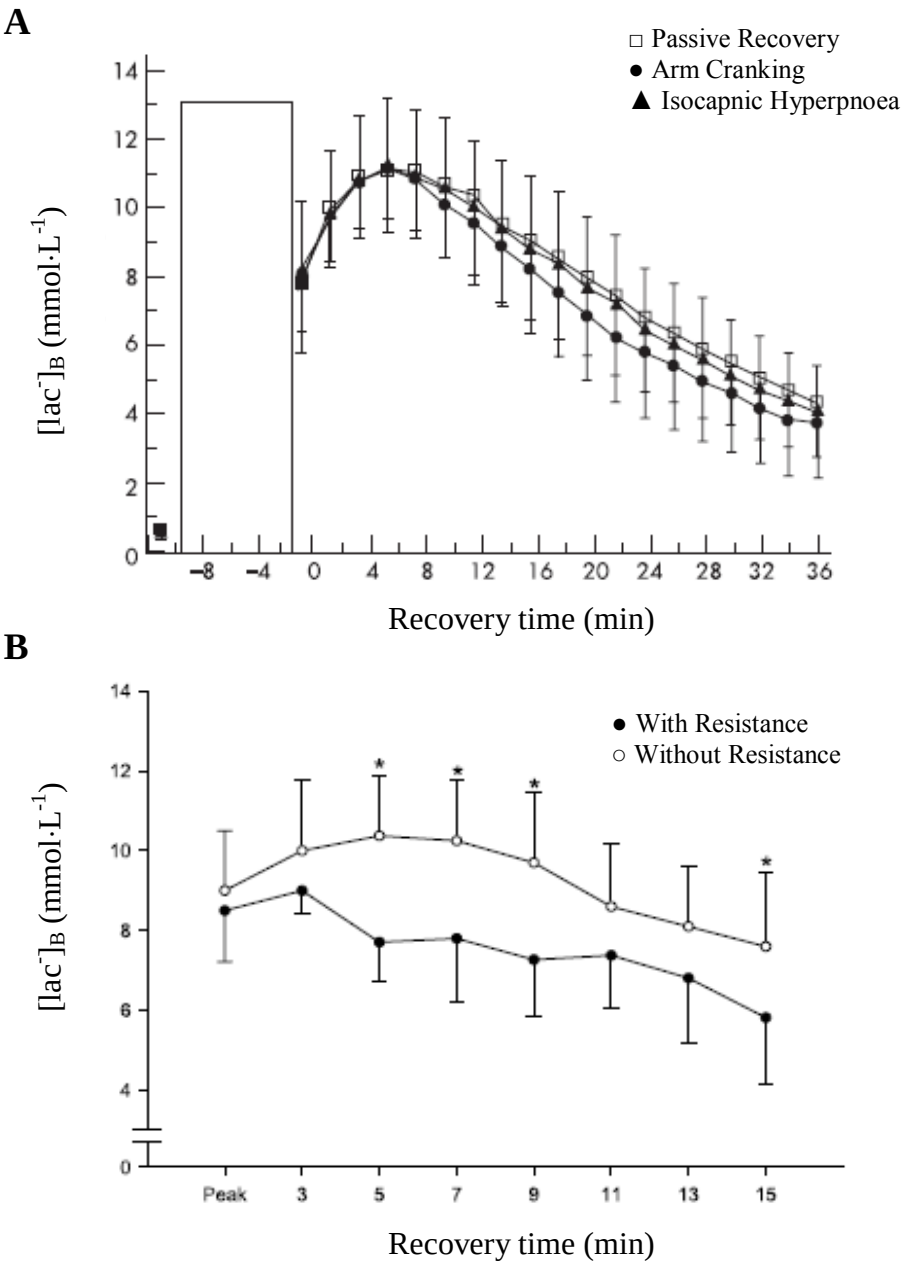

Figure 1.29 Blood lactate following maximal exercise. (A) low intensity volitional hyperpnoea performed following exercise, $\dot{V}_E$ 61.6 ± 9.3 L·min^{-1} (Perret and Müeller 2007); (B) 15cmH_2O inspiratory pressure threshold loading performed following exercise (Chiappa et al. 2008b).

In contrast to these findings, Chiappa et al. (2008b) reported a significant reduction (~2.5 mmol·L^{-1}) in $[lac^-]_B$ following as little as 5 min of recovery when pressure threshold inspiratory loading was performed during recovery from maximal incremental exercise (15 cmH_2O; ~15% MIP; Figure 1.29B, ●). Chiappa et al. (2008b) attributed this reduction to an increased respiratory muscle blood flow during loaded breathing which presented a favourable environment for lactate exchange by increasing the extra- to intra-cellular respiratory muscle lactate gradient. Similar to previous findings, it is not clear as to the

specific site of lactate clearance. Regardless of the site of removal, the findings of Chiappa and colleagues suggest that the inspiratory muscles may possess a large, previously underestimated capacity to affect lactate clearance. However, whether following IMT the ability of the inspiratory muscles to reduce net lactate production and / or increase net lactate clearance during and following whole-body exercise remains unknown.

1.8 GENERAL SUMMARY

The respiratory muscles are a precisely co-ordinated muscle group which power pulmonary ventilation. During intense exercise pulmonary ventilation can exceed 200 $L \cdot min^{-1}$ and the respiratory muscles may demand up to 15% of the total whole-body $\dot{Q}$ and $\dot{V}O_2$. As a consequence of the work of breathing, respiratory muscle fatigue develops which can affect the distribution of systemic $\dot{Q}$ and accelerate locomotor muscle fatigue. Specific training techniques have been developed to increase the strength and endurance of the respiratory muscles independent to whole body training. Furthermore, rigorously designed placebo controlled studies illustrated that RMT can improve exercise tolerance. The mechanism(s) which may underpin this ergogenic effect are multifaceted, and probably involve a unique interaction between respiratory muscle plasticity, systemic metabolites and the supraspinal sensory / motor centres. A surprising observation following RMT is a reduction in $[lac^-]_B$ during whole-body exercise. Amazingly, these reductions are often similar to those observed following whole-body training. However, it remains unknown whether this small muscle group which comprises a modest 3% of total body mass can affect systemic metabolites at all. An RMT-mediated reduction in blood lactate concentration may provide, in part, a possible explanation for the impressive improvements in whole body exercise tolerance due to favourable changes in acid-base balance and / or the intensity of perceived breathing / locomotor discomfort. Therefore, the contribution of the respiratory muscles to systemic $[lac^-]_B$ during and following intense

endurance exercise both prior to and following RMT remains poorly understood and certainly deserves further investigation.

1.9 RESEARCH AIMS

Previous research suggested (c.f. McConnell and Sharpe 2005; Spengler et al. 1999) that the reductions in $[lac^-]_B$ observed during whole-body exercise following RMT may be due to systemically relevant changes in respiratory muscle lactate release and / or clearance. This hypothesis, however, has yet to be rigorously investigated. As a consequence of such a poor understanding of the potential contribution of the trained and untrained respiratory muscles to changes in systemic lactate kinetics, the principal aim of this thesis was to investigate the physiological consequences of the work of breathing and of specific IMT. This thesis aims to provide evidence to support or negate the hypotheses that i) the respiratory muscles contribute to systemic lactate turnover and ii) subsequent to their specific conditioning, attenuate $[lac^-]_B$ during dynamic whole-body exercise and throughout recovery.

In order to test the hypotheses outlined above, 3 primary studies were designed. ***Firstly***, under resting conditions, intense isocapnic volitional hyperpnoea was performed both prior to and following specific IMT. The breathing pattern adopted was matched precisely to that achieved during near-maximal exercise performed using an electromagnetically braked cycle ergometer. Using this approach, an increase in systemic $[lac^-]_B$ from resting concentrations can be attributed, almost exclusively, to respiratory muscle work (e.g. see Martin et al. 1982). Following IMT, it is anticipated that the same absolute breathing challenge as pre-intervention would cause a smaller rise (i.e. a reduction) in the $[lac^-]_B$, illustrating the ability of the inspiratory muscles to engage in lactate exchange / clearance.

There are a number of limitations to this first study. In particular, when performing volitional hyperpnoea under resting conditions adjacent muscle fibres, inactive muscles and organs can engage in lactate exchange: this is known as the lactate shuttle hypothesis (Brooks 1986; see section 1.7.1). Thus, the ***Second*** study of this thesis was designed to minimise the confounding influence of the lactate shuttle by performing isocapnic volitional hyperpnoea whilst cycling at the maximal lactate steady state (MLSS). The MLSS represents the highest power output at which the rate of lactate efflux to and removal from the systemic circulation is equal. Whilst exercising at the MLSS minimal capacity exists for other tissues and organs to influence $[lac^-]_B$. Therefore, following IMT a reduction in the (expected) increase in $[lac^-]_B$ (~1 $mmol \cdot L^{-1}$; Johnson et al. 2006) observed with intense isocpanic hyperpnoea can be attributed with far more confidence to the trained inspiratory muscles.

The ***Third*** study of this thesis was designed to investigate whether the potential reductions observed in $[lac^-]_B$ following IMT during volitional hyperpnoea such as those expected in studies one and two and during whole body exercise such as those observed in previous research (McConnell and Sharpe 2005) are due to an increase in respiratory muscle lactate exchange and / or clearance. To achieve this, the third study investigated the effects of IMT upon the recovery of $[lac^-]_B$ following maximal exercise. The methodology of this study was based largely upon that of recent, pertinent research (Chiappa et al. 2008b, 2009). In these recent studies, $[lac^-]_B$ was lower when a low intensity (15 cmH_2O) inspiratory muscle pressure threshold resistance was attatched to the breathing circuit immediately following maximal cycle ergometry exercise. Given that the $[lac^-]_B$ is reduced with inspiratory muscle loading, whether this may be further enhanced by specific training is an attractive avenue to explore. Unlike the studies by Chiappa et al. (2008b, 2009), in this thesis, individual lactate recovery curves prior to and following the intervention were fitted to a bi-exponential time function using non-linear regression. Modelling lactate recovery in this way provides empirical evidence based upon the systemic $[lac^-]_B$ which

describes i) the rate of appearance of lactate into the arterialised blood (lactate exchange) and ii) lactate clearance (Freund and Zouloumian 1981). An increase in the lactate clearance velocity constant with inspiratory muscle loading and following IMT would provide novel evidence for the ability of the trained inspiratory muscle to engage in lactate turnover. It is anticipated that the findings of these experimental studies will confirm the hypotheses that the respiratory muscles are capable of engaging in lactate exchange and net lactate clearance. Furthermore, they will also confirm the notion that the reductions in $[lac^{-}]_B$ following IMT during whole-body exercise are due, in part, to the trained inspiratory muscles, and in particular, greater lactate clearance.

Finally, as an adjunct to the main focus of the thesis, a subsequent aim was to investigate the potential determinants of inspiratory muscle strength (measured by MIP) both prior to and following IMT. A previous study (Johnson et al. 2007) reported that the baseline MIP (i.e. prior to IMT) and the change in MIP following IMT are correlated. In addition, Hershenson et al. (1988) reported that global inspiratory muscle strength may not be limited by the strength of the diaphragm but rather the relative strengths of the chest wall inspiratory muscles. These intruiging findings warrant further exploration as a large variability exists in between-subject baseline MIP and the IMT-induced changes in MIP in healthy, active persons. Therefore, the aims of this final study were threefold: i) to identify whether the disparity in baseline MIP between-subjects could be explained by the physical characteristics of the participant; ii) to re-affirm the relationship between baseline MIP and the IMT-induced changes in MIP with a larger number of subjects and greater variance in the baseline inspiratory muscle strength; and iii) to establish whether the between-subject differences in MIP and the between-subject increase in MIP following IMT are determined by the strength of the chest wall inspiratory muscles relative to the strength of the diaphragm, and their respective change in strength following IMT.

CHAPTER 2

GENERAL METHODS

2.1 PARTICIPANT PREPARATION

Prior to all research studies, subjects were provided with information packs containing a full description of the aims, potential risks and benefits of the research. Following this, subjects provided their written informed consent, completed a health screen questionnaire and were familiarised with all testing protocols and equipment. The day preceding and the day of a research trial, subjects were instructed not to engage in any strenuous exercise. Each subject completed a 24 h diet record prior to their first trial and this was repeated prior to subsequent tests. Subjects arrived at the laboratory 2 h post-prandial during which they were instructed to consume only water having abstained from alcohol and caffeine in the 24 h prior to testing. Prior to each study, obstructive and restrictive pulmonary disease was assessed using dynamic spirometry (see section 2.5). Any individual with an FEV_1/FVC (see section 2.5) lower than 80% was excluded from participating in any research trials. Prior to all studies, optimal participant numbers were calculated assuming an effect size of 0.30 to 0.50, a statistical power of 0.80 with a-priori α set at $P<0.05$ (Cohen 1988; Dallal 1990). The calculated sample size was then adjusted to concur with previous research (Field 2008).

2.2 LODE EXCALIBUR SPORT CYCLE ERGOMETER

All exercise trials were performed on an externally calibrated electromagnetically braked cycle ergometer (Exalibur Sport, Lode, Groningen, The Netherlands). The ergometer was set in the hyperbolic mode in which power output was constant and independent of pedal cadence. The handlebars and saddle height and their respective horizontal displacements were adjusted for each individual and these configurations were recorded to the nearest mm and replicated for subsequent trials. The handle bars of the ergometer were modified with time trial bars (Aeroforce EA70 Clip on bar, Easton, CA, USA) and when appropriate, subjects were instructed to use their own cycling pedals and cleated cycling shoes. Pedal cadence was displayed at all times on a digital screen mounted

on the handlebars. The power output of the cycle ergometer was controlled by an external PC running specific software on which exercise protocols were programmed to the nearest 1 W and 1 s (Lode ergometry manager, version 5.18.20, Lode, Groningen, The Netherlands).

2.3 INSPIRATORY MUSCLE STRENGTH

A hand-held mouth pressure meter (MicroR.P.M., Micro Medical, Buckinghamshire, UK) measured the maximal inspiratory mouth pressure (MIP) during a quasi-static contraction (Müeller manoeuvre) as an index of global inspiratory muscle strength. The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure during inspiratory efforts and minimise the contribution of the buccal muscles to inspiratory pressure development (Black and Hyatt 1969). Efforts were performed in an upright standing posture, were initiated from residual volume, and sustained for at least 2 s. Inspiratory efforts were separated by 30 s and repeated until serial measures were within 10% or 10 cmH_2O of one another with the highest value recorded for analysis (McConnell 2007); MIP was compared to normal values provided by published reference equations (Wilson et al. 1984).

2.4 CALIBRATION OF THE MOUTH PRESSURE METER

The mouth pressure meter was calibrated using a capacitive sensor prior to commencement of the research programme. The capacitive sensor had a pressure capacity $\geq$1360 cmH_2O (1000 torr; Pirani strain gauge, MKS Barathon, MKS Instruments, MA, USA) and was maintained at a constant temperature (55 ºC) by a digital signal conditioner (MKS Barathon, Type 270B, MKS Instruments, MA, USA). A two-point calibration was performed on the strain gauge daily: firstly the strain gauge pressure was set to zero and secondly to the atmospheric pressure provided by the local meterological station and confirmed with a laboratory based wall-mounted mercury manometer. The assembly for

calibration of the mouth pressure meter is shown in Figure 2.1; the mouth pressure meter was connected via flexible tubing to the strain gauge and an electronic syringe pump fitted with a 60 ml syringe (KD Scientific, KDS 210C, Holliston, MA, USA) using a three-way stopcock. The pump opened the syringe at a constant rate of 70.57 $ml \cdot min^{-1}$. Displacement of the syringe to multiple limits was repeated 12 times generating a range of pressures from 6 to 298 cmH_2O.

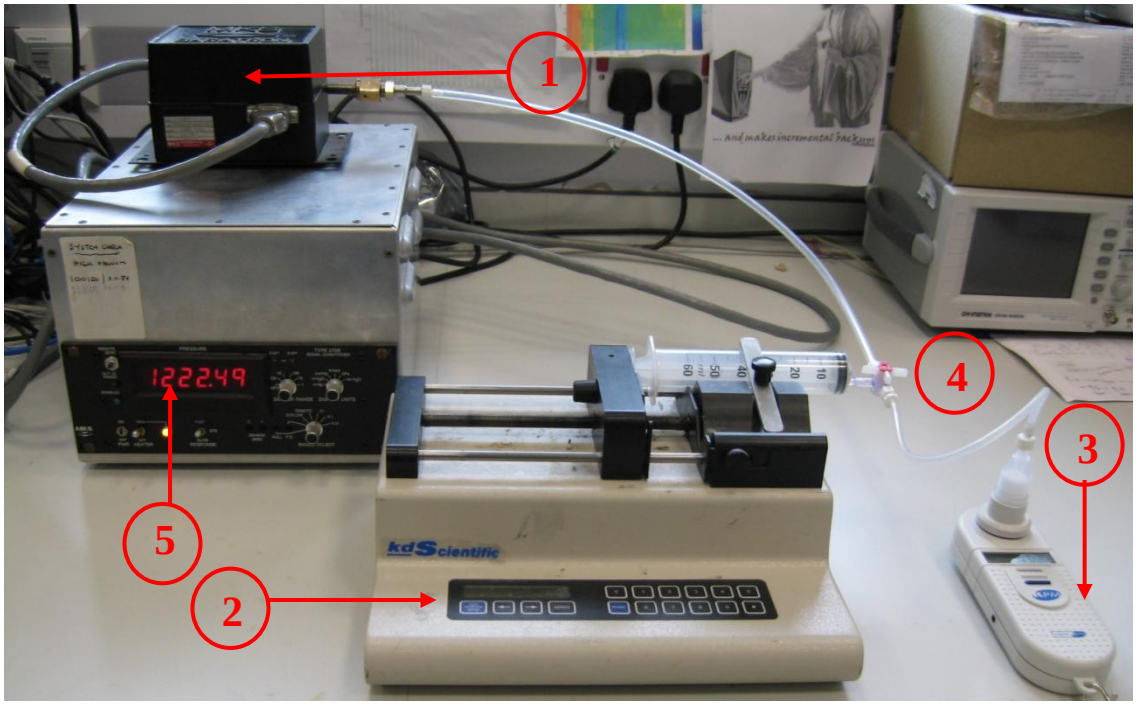

Figure 2.1. Assembly for calibration of the mouth pressure meter. 1) capacitive strain gauge, 2) syringe pump, 3) mouth pressure meter, 4) three-way stopcock and 5) pressure display.

Figure 2.2A shows the relationship between pressure recordings from the mouth pressure meter (P_{MOUTH}) and the calibrated strain gauge (P_{STRAIN}). The relationship between the two was excellent as shown by the correlation coefficient. Figure 2.2B illustrates a Bland and Altman plot (Bland and Altman 1986) of the difference in pressure recordings between P_{MOUTH} and P_{STRAIN} against the mean of both. Also shown in Figure 2.2B are the mean bias, and 95% confidence intervals. Given the narrow limits of agreement, it can clearly be seen that the mouth pressure meter provides a valid measure of pressure.

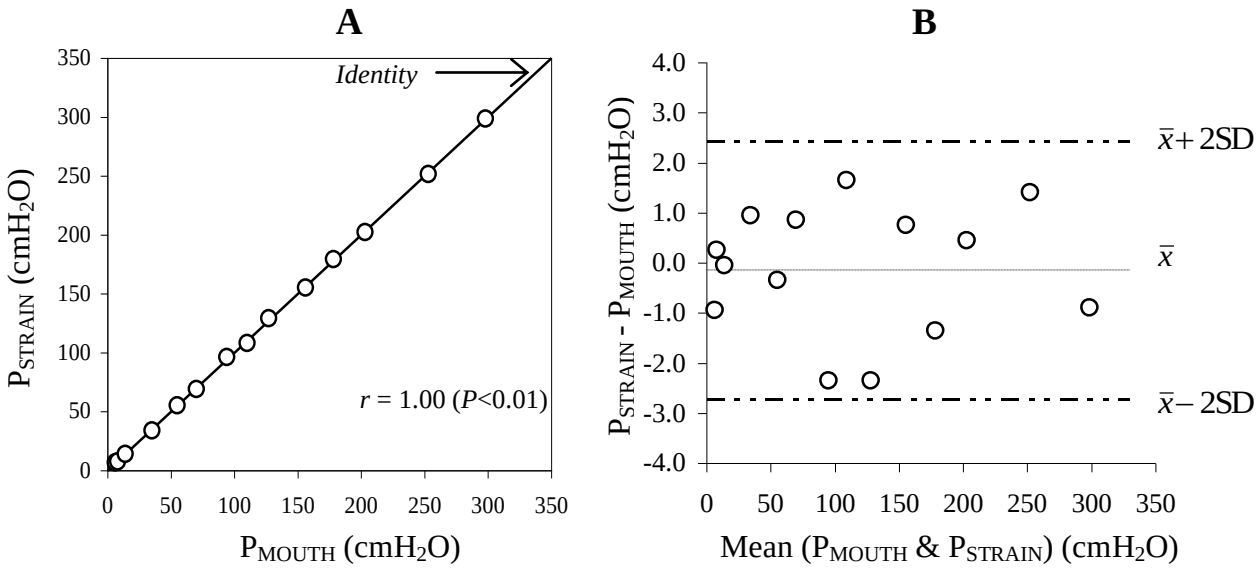

Figure 2.2. A) Strain gauge pressure (P_{STRAIN}) against mouth pressure meter pressure (P_{MOUTH}); B) Difference in pressure between P_{STRAIN} and P_{MOUTH} against the mean (P_{MOUTH} & P_{STRAIN}). 95% confidence intervals for the mean bias, upper limits of agreement and lower limits of agreement were -0.90 to 0.61 cmH_2O, 1.12 to 3.74 cmH_2O and -4.03 to -1.41 cmH_2O, respectively. $\bar{x}$, mean.

2.5 PULMONARY FUNCTION: DYNAMIC SPIROMETRY

Forced lung volumes and flows were assessed in accordance with published guidelines (American Thoracic Society: Quanjer et al. 1993; British Association of Sport and Exercise Sciences: McConnell et al. 2007). Tests were performed using a hand-held pneumotachograph (Pneumotrac, Vitalograph, Buckingham, UK) calibrated using a 3 L syringe. Subjects performed manoeuvres wearing a nose clip and standing upright. A minimum of 3 and maximum of 8 flow-volume loops were performed to determine the forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), peak expiratory (PEF) and peak inspiratory flow rates (PIF) until the within and between-manoeuvre criteria were satisfied, i.e. two largest recordings of FVC and FEV_1 were within 100 ml of each other (Miller et al. 2005). A 10 s maximal voluntary ventilation (MVV_{10}) was performed to determine the maximal breathing capacity. Efforts were separated by 1 min and continued until repeat measurements were within 10% or 20 $L \cdot min^{-1}$ of each other (McConnell 2007). The highest value recorded for the flow-volume loop and MVV_{10} were used for subsequent analysis (Quanjer et al. 1993). All spirometry data were compared to

normal reference values published previously (FVC, FEV_1, PEF, FEV_1/FVC, Quanjer et al. 1993; MVV_{10}, Cotes 1993).

2.6 ZAN 600 USB CPX BREATH BY BREATH ANALYSER

Pulmonary gas exchange and ventilation were measured at the mouth using an online breath by breath gas analysis system operated by an integrated PC (ZAN 600 USB CPX, Nspire Health, Oberthulba, Germany). Pulmonary gases were continuously sampled and analysed using fast response selective analysers at a flow rate of 0.66 $L \cdot s^{-1}$ (O_2: amperometric solid state electrolyte sensor; CO_2: infrared spectroscopy). The expired gases were time aligned with expired airflow using specific software (GPI version 3.0, Nspire Health, Oberthulba, Germany) to provide breath by breath pulmonary gas exchange measurements expressed at STPD. Expired volumes of air were measured using a pneumotachograph (Type II flow sensor, Nspire Health, Oberthulba, Germany) with a low dead space (<40 ml) and expressed at BTPS. The additional resistance provided by the flow pneumotachograph according to the manufacturer's guidelines was 1.0, 1.7, 2.4 and 2.9 cmH_2O for flow rates of 6.2, 8.4, 10.0 and 11.2 $L \cdot s^{-1}$, respectively. The flow pneumotachograph and gas analysers were calibrated prior to all trials using a 3 L syringe and gases of known concentrations (BOC Gases, Guilford, UK), respectively. The pneumotachograph was attached to a facemask (Vmask™ model 7400, Hans Rudolph, KS, USA) with a low dead space (97 ml) and secured around the participants face using elastic quick release mesh head gear. During trials where F_ICO_2 was increased (Chapters 3 and 4) or inspiratory resistance was required (Chapter 5), a two-way non-rebreathing valve (model 2730, Hans Rudolph, Missouri, USA) was connected distal to the pneumotachograph. According to the manufacturer's guidelines, the two way non-rebreathing valve provided inspiratory and expiratory resistances of 7.0, 12.2 and 20.2 cmH_2O and 10.8, 16.3 and 22.2 cmH_2O, respectively, for inspiratory and expiratory flow rates of 5.0, 6.7 and 8.3 $L \cdot s^{-1}$, respectively.

2.7 AGREEMENT BETWEEN ZAN 600 USB CPX AND A 'GOLD STANDARD'

The validity and reliability of the on-line breath by breath expired gas analysis system was assessed against the closed circuit Douglas bag technique since the latter is considered the gold standard of expired gas measurements (Bassett et al. 2001). Expired air was collected into non-permeable bags (200 L capacity) and the concentration of gases and volumes of air were determined. Exercise was performed on an electromagnetically braked cycle ergometer (see section 2.2) at constant power outputs equivalent to 100, 130, 160, 190 and 220 W. Following 4 min of exercise, a 60 s expired air sample was taken. The breath by breath flow pneumotachograph was connected in series with a two-way non-re-breathing valve (model 2730, Hans Rudolph, Missouri, USA) with the Douglas bag corrugated tubing attached proximally to the expiratory port and distally to the Douglas bag. This permitted simultaneous measurement of expired air samples using both methods; this was repeated at each exercise power output.

Douglas bag expired air samples were analysed for the concentrations of CO_2 and O_2 using infrared and paramagnetic analysers, respectively (Servomex Series 1400, Crowborough, UK), calibrated immediately prior to analysis with gases of known concentration (BOC Gases, BOC, Guilford, UK). Expired gas volumes were determined using a dry gas meter calibrated with a known volume of air (Harvard Ltd., Edenbridge, UK); $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were subsequently corrected from ATPS to BTPS and STPD, respectively. Figure 2.3 shows the relationship between $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$, each data series represents a specific date of measurement. Figure 2.3 clearly shows an excellent relationship between both measures of pulmonary gas exchange and ventilation as indicated by the significant correlation coefficients.

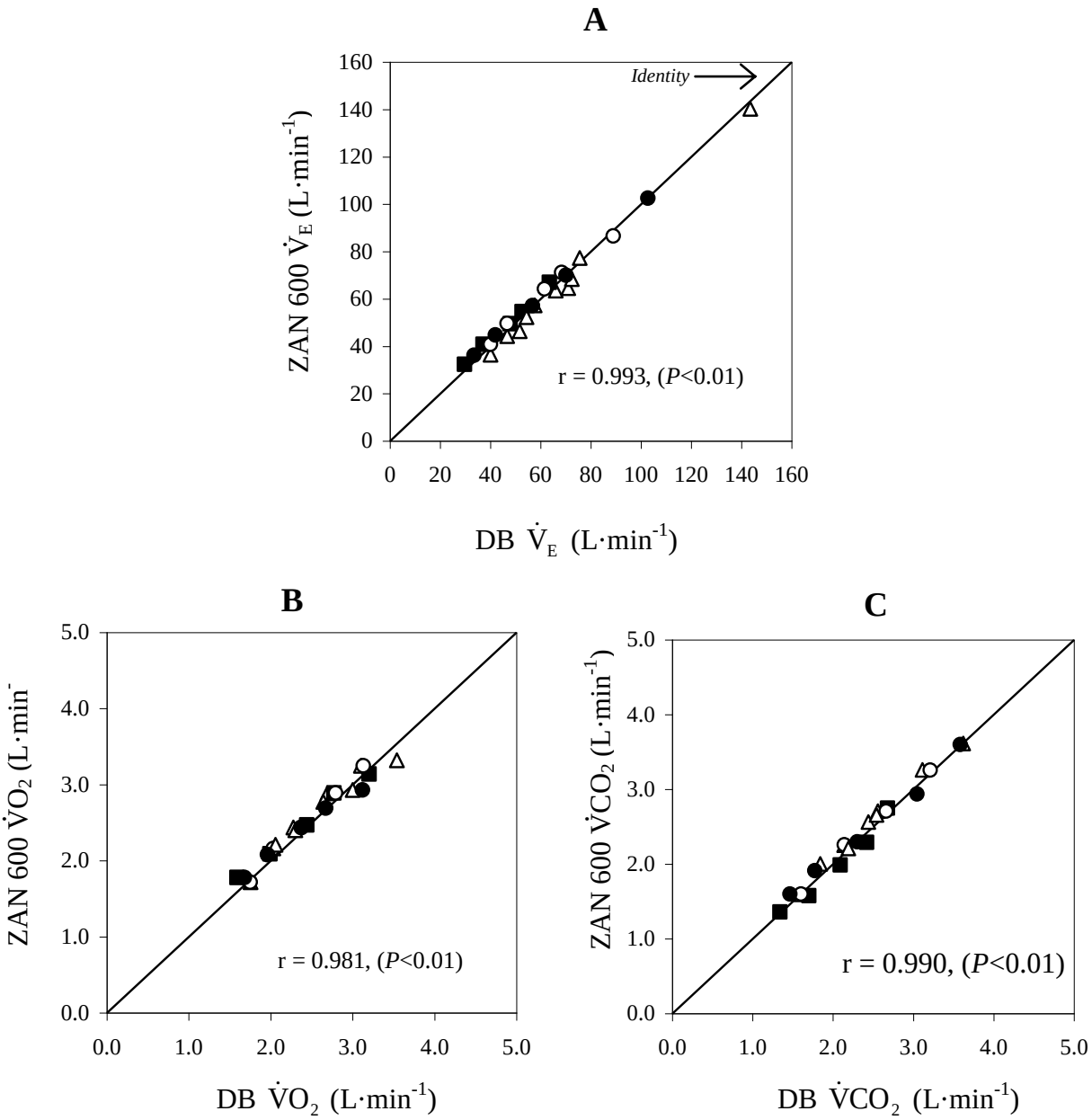

Figure 2.3. Relationships between ZAN 600 USB CPX (ZAN 600) and Douglas bags (DB). A) minute ventilation ($\dot{V}_E$) , B) oxygen consumption ($\dot{V}O_2$), C) carbon dioxide production ($\dot{V}CO_2$). Each data series represent a specific date of measurement: ▲, 02.04.2006; ■, 09.06.2006; ○, 03.03.2007; △, 24.07.07; ●, 11.04.08; each data point within a series reflects a given constant power output.

To minimise the influence of between subject variance on the relationship between the ZAN 600 and Douglas bag method and to observe the relationship between the measurement error and the true value, Bland and Altman plots were constructed. Figures 2.4, 2.5 and 2.6 show the difference between measurements made by the ZAN 600 USB CPX and Douglas bags plotted against the mean of both for $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$,

respectively. The Figures also show the bias of the mean difference, and the 95 % confidence intervals for the lower and upper limits of agreement. Figures 2.4, 2.5 and 2.6 clearly show good agreement for each measurement. These results indicate that the ZAN 600 USB CPX is a valid and reliable breath by breath expired gas analysis system during exercise.

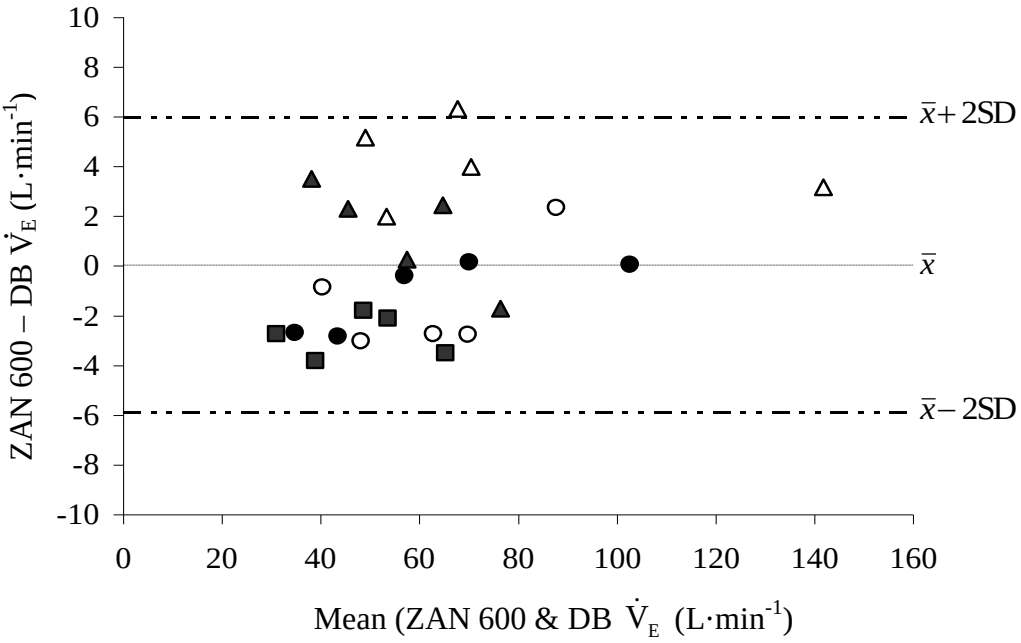

Figure 2.4. Difference in minute ventilation ($\dot{V}_E$) against mean for ZAN 600 USB CPX (ZAN 600) and Douglas bag (DB) methods. 95% confidence intervals for the mean bias, upper limits of agreement and lower limits of agreement were -1.19 to 1.25 L·min^{-1}, 3.84 to 8.07 L·min^{-1} and -8.02 to -3.78 L·min^{-1}, respectively. $\bar{x}$, mean. Each data series represent a specific date of measurement: ▲, 02.04.2006; ■, 09.06.2006; ○, 03.03.2007; △, 24.07.07; ●, 11.04.08; each data point within a series represents a given constant power output.

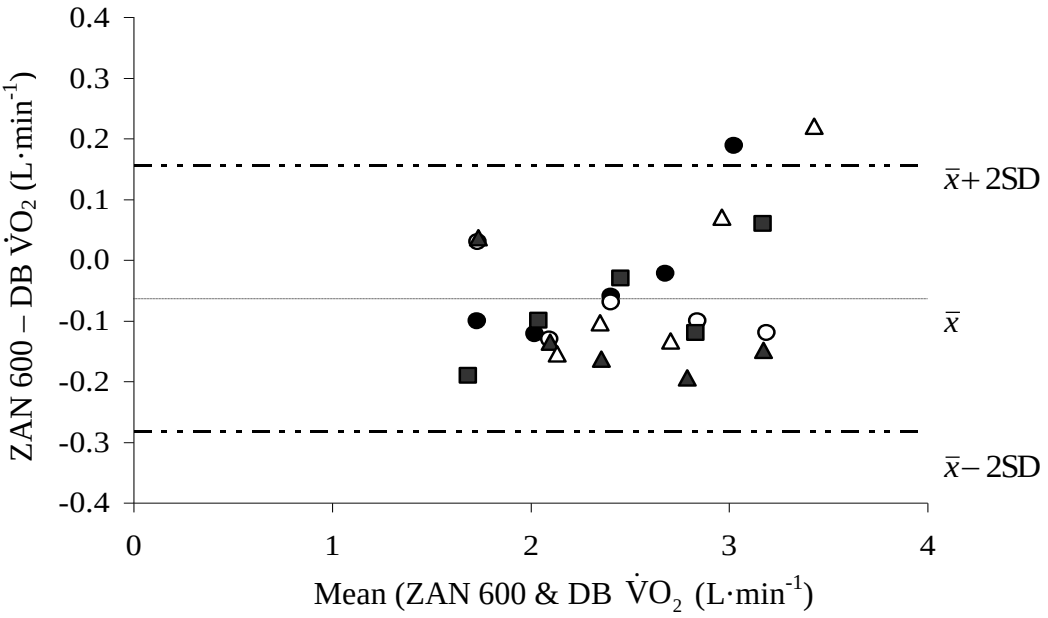

Figure 2.5. Difference in pulmonary oxygen consumption ($\dot{V}O_2$) against mean for ZAN 600 USB CPX (ZAN 600) and Douglas bag (DB) methods. 95 % confidence intervals for the mean bias, upper limits of agreement and lower limits of agreement were -0.11 to -0.02 L·min^{-1}, 0.08 to 0.23 L·min^{-1} and -0.36 to -0.20 L·min^{-1}, respectively. $\bar{x}$, mean. Each data series represent a specific date of measurement: ▲, 02.04.2006; ■, 09.06.2006; ○, 03.03.2007; △, 24.07.07; ●, 11.04.08; each data point within a series represents a given constant power output.

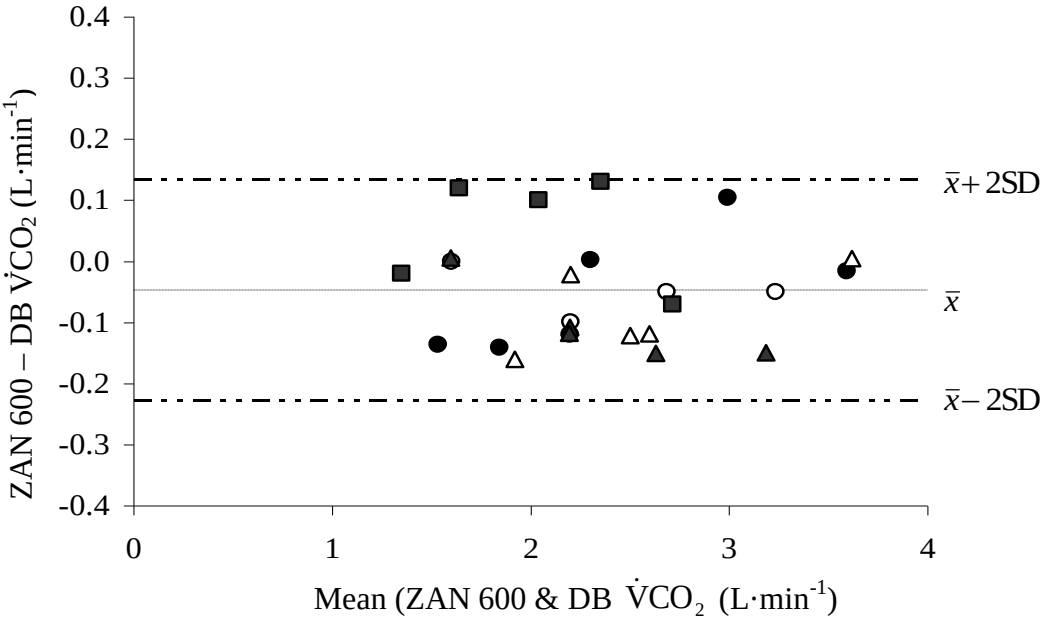

Figure 2.6. Difference in pulmonary carbon dioxide production ($\dot{V}CO_2$) against mean for ZAN 600 USB CPX (ZAN 600) and Douglas bag (DB) methods. 95 % confidence intervals for the mean bias, upper limits of agreement and lower limits of agreement were -0.08 to -0.01 L·min^{-1}, 0.07 to 0.20 L·min^{-1} and -0.29 to -0.16 L·min^{-1}, respectively. $\bar{x}$, mean. Each data series represent a specific date of measurement: ▲, 02.04.2006; ■, 09.06.2006; ○, 03.03.2007; △, 24.07.07; ●, 11.04.08; each data point within a series represents a given constant power output.

2.8 BLOOD SAMPLING AND ANALYSIS

Arterialised venous blood was sampled from a dorsal hand vein via an indwelling 21-G teflon venous cannula (Surflo-W, Terumo, Leuven, Belgium). The cannula was fitted with a 3-way stopcock valve (Becton Dickinson UK Ltd, Oxford, UK) and secured to the hand using adhesive medical tape. This method of arterialised venous blood sampling has been shown to provide excellent agreement with arterial blood for measures of blood lactate concentration ($[lac^-]_B$), pH and the partial pressure of CO_2 (PCO_2) during both steady state and incremental exercise to volitional tolerance (Forster et al. 1972; McLoughlin et al. 1992). Prior to cannulation, arterialisation was ensured by immersing the hand in water ~40˚C for 10 min and during exercise by warming the hand using a stand mounted infrared lamp (Infraphil HP3614, Philips, UK). Dorsal hand skin temperature was measured throughout using a thermistor (Thermocouple 206-3722, RS Components, Northants, UK) with the distance of the lamp adjusted to ensure a constant skin temperature of ~40ºC. Following cannulation, a 5 ml intra-venous infusion of 0.9% sodium chloride (Mini-Plasco Saline, Braun, Melsungen, Germany) was performed to maintain patency. Immediately prior to all blood sampling, residual fluids were withdrawn from the cannula and stopcock using a 1 ml syringe. On completion of a trial, the cannula was removed and medical gauze (Topper 8, Johnson and Johnson Medical Ltd, Skipton, UK) was applied under firm pressure to the puncture site for a minimum of 10 min to avoid superficial haematoma.

$[lac^-]_B$ and glucose concentrations ($[glucose]_B$) were measured using an automated enzymatic method (Biosen, EKF Diagnostics, Barleben, Denmark). Blood samples were drawn into a 1 ml syringe and transferred in to sodium-heparinised 20 µl end-to-end capillary tubes (Biosen, EKF Diagnostics, Barleben, Denmark). Capillary tubes were then placed into a 1 ml micro test tube filled with a glucose/lactate haemolysing solution (Biosen, EKF Diagnostics, Barleben, Denmark) and shaken vigorously for approximately 10 s. The specific $[lac^-]$ contained within the capillary sample was then determined using

the analyser. Here the sample was converted to pyruvate and hydrogen peroxide via the enzyme lactate oxidase using an amperometric electrochemical sensor chip. The products of the aforementioned reaction produce an electrical current on the working electrode site of the sensor directly proportional to the original lactate concentration (Figure 2.6) achieved by comparison to the reference electrode site of the micro sensor where no electrical current is present.

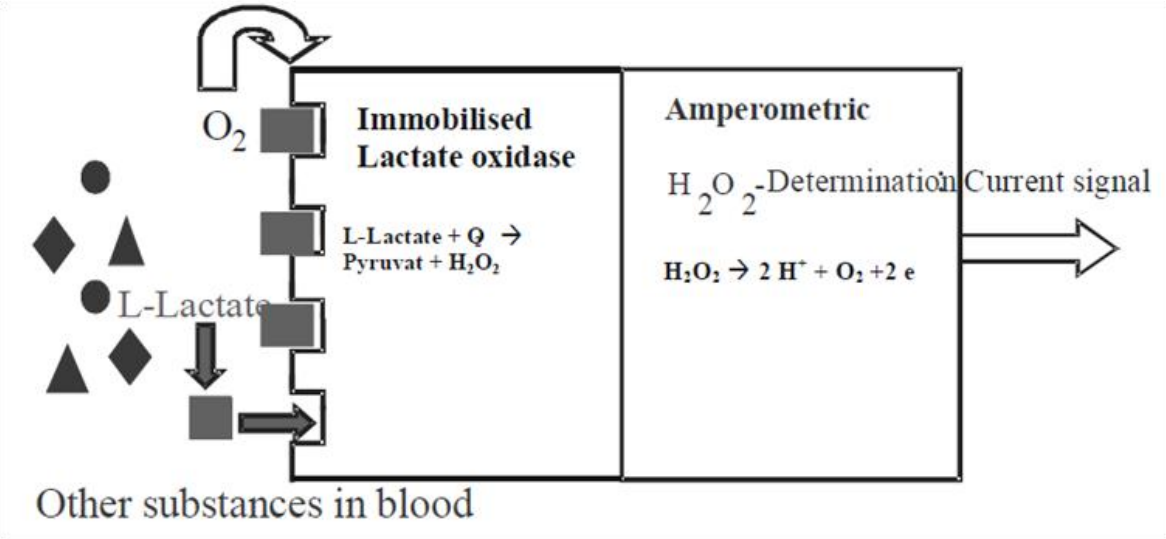

Figure 2.7. Measurement principles of lactate analyser (Biosen, EKF Diagnostics, Barleben, Denmark).

The analyser was calibrated prior to use using a standard solution of known concentration ([lac^-] / [glucose]: 12 $mmol \cdot L^{-1}$). Between day coefficient of variation (CV) in [lac^-] / [glucose] was monitored by the calibration slope of a quality control solution which was <1.5%. The within-sample CV for $[lac^-]_B$ and $[glucose]_B$ was 2.0 ± 0.6 (n = 10 samples) and 1.5 ± 0.4 % (n = 8 samples), respectively. Previous research has confirmed the intra-sample and between-day reliability of this method (Davison et al. 2000), to which the current data agree.

Arterialised venous blood gas measurements including PCO_2 and pH (and thus $[H^+]$) were made using an automated blood gas analyser (ABL 520, Radiometer, Copenhagen, Denmark). After drawing approximately 1.5 ml of blood into a pre-

heparinised syringe (PICO 50, Radiometer, Copenhagen, Denmark), it was rolled vertically between the hands for approximately 10 s and introduced immediately into the analyser. Blood gases were corrected for *in-vivo* changes in core temperature measured using a self-inserted rectal thermistor fixed at 10 cm beyond the anal sphincter (1000 Series Squirrel, Grant Instruments, Cambridge, UK). The Henderson Hasselbalch equation was used to calculate plasma bicarbonate concentration ($[HCO_3^-]$):

$$pH = pK + \log \frac{[HCO_3^-]}{0.03 \times PCO_2}$$

and the Siggaard-Anderson equation was used to calculate base excess of the extracellular fluid (BE_{ECF}; Siggaard-Anderson and Fogh-Anderson, 1995):

$$BE_{ECF} = 0.93 \times \left([HCO_3^-] - 24.40 + 14.83 \times (pH - 7.40)\right)$$

Automatic calibration of the analyser occurred every 8 hr; additionally, during periods of heavy use, quality control solutions stored in air tight glass ampules were injected in to the analyser (QUALICHECK 3+, Radiometer, Copenhagen, Denmark). Four quality control solutions were introduced into the analyser each containing certified values of PCO_2 and pH; the range of values within the quality control solutions for PCO_2 were 20.4 to 24.4, 40.0 to 46.0, 65.4 to 75.4 and 88.3 to 104.3 mmHg and for pH were 6.797 to 6.827, 7.076 to 7.116, 7.376 to 7.416 and 7.565 to 7.605, respectively.

2.9 Effects of storage duration on $[lac^-]_B$ and $[glucose]_B$

Throughout exercise trials approximately 45 min separated blood sampling and analysis. Therefore the effects of 50 min time delay on $[lac^-]_B$ and $[glucose]_B$ was investigated. Five males performed 5 min cycling exercise at a constant power output between 100 and 300 W using an electromagnetically braked cycle ergometer and blood sampling procedures described in sections 2.2 and 2.8, respectively. To achieve maximal $[lac^-]_B$ and $[glucose]_B$, a 5 ml blood sample was drawn approximately 5 min post-exercise and divided in to 11 aliquots of 20 µl. The first aliquot was analysed immediately (time: 0)

with each subsequent blood sample analysed every 5 min thereafter. The effect of storage duration on $[lac^-]_B$ and $[glucose]_B$ is shown in Figure 2.7.

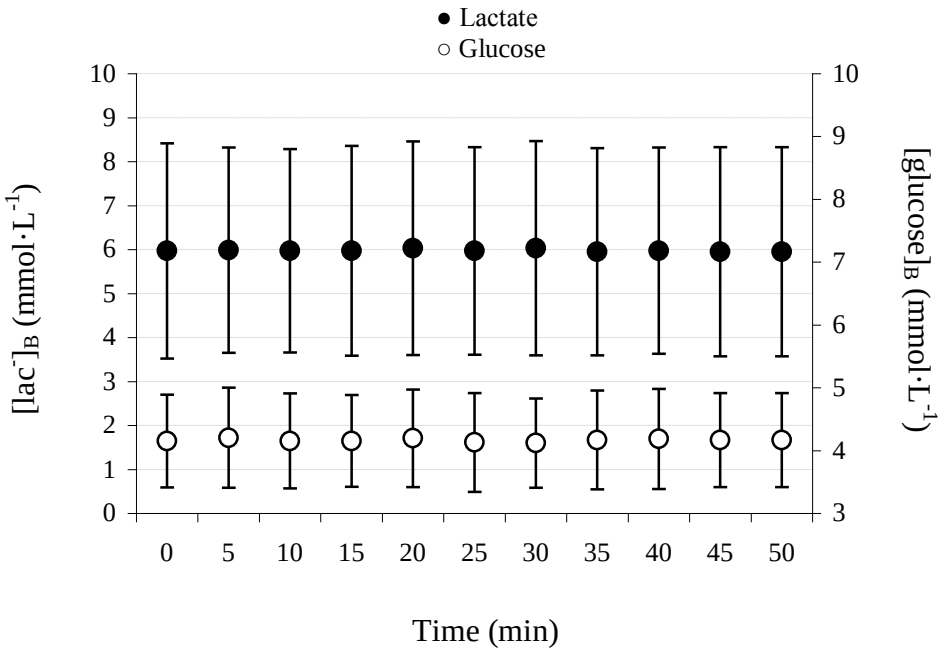

Figure 2.8. Effect of storage duration (time delay prior to analysis) on blood lactate ($[lac^-]_B$; ●) and blood glucose ($[glucose]_B$; ○) concentrations.

A linear regression was fitted to the values of $[lac^-]_B$ and $[glucose]_B$ over time in order to determine if the slope of the relationship was significantly different to zero using the equation:

$$t = \frac{b(SD_x)\left(\sqrt{N-1}\right)}{SEE}$$

where b = is the slope, SD_x = standard deviation (SD) of x where x is time and SEE = the standard error of the estimate (σ_{est}) calculated as:

$$\sigma_{est} = \sqrt{\frac{\sum\left(Y - Y'\right)^2}{N}}$$

where $\left(Y - Y'\right)^2$ is the sum of the squared errors of predictions of Y and N is the number of predictions. The slope of the relationships were not significantly different to zero,

therefore, a time delay of up to 50 min did not influence the measurement of either arterialised venous $[lac^-]_B$ or $[glucose]_B$.

2.10 INSPIRATORY MUSCLE TRAINING

Inspiratory muscle training (IMT) was performed using a commercially available pressure threshold loading device (POWERbreathe®, Gaiam, UK; Figure 1.24). The device has been shown to provide near flow-independent pressure threshold loading over a range of inspiratory flow rates (Caine and McConnell 2000). During inspiration, subjects were required to generate a negative pressure at the mouth sufficient to open a spring loaded valve until the threshold pressure could no longer be sustained. At such time, the valve closed and unloaded expiration was initiated. 30 dynamic inspiratory efforts were performed twice daily for a period of 4 to 6 wk against a pressure-threshold load of ~50% MIP. Throughout the training intervention, subjects were instructed to periodically increase the load to a threshold pressure which would permit them to only just complete 30 breaths. Each inspiratory manoeuvre was initiated from residual volume and subjects strove to maximise V_T. To avoid hypocapnia, subjects were instructed to expire slowly and fully, thus attenuating f_R. This protocol is known to be effective in eliciting an adaptive response (see section 1.15.3). Throughout the intervention period, subjects completed a training diary to record IMT adherence and habitual training (Appendix 1).

2.11 STATISTICAL TREATMENT OF DATA

For all data included in statistical analyses, normal distribution of the sample was used to indirectly confirm normal distribution expected within the sample population (Field 2008). Normality was confirmed by subjective interpretation of frequency distribution histogram plots and interpretation of the empirical skewness and kurtosis. Interpretation of the Kolmogorov-Smirnov statistic was used to objectively interpret whether the distribution of data was significantly different from a comparable normal

distribution with an identical mean ± SD. Data was considered normal when the Kolmogorov-Smirnov α-level was greater than 0.05 (Field 2008).

Homogeneity of variance, i.e. the assumption that the variance of one variable / group was similar at all levels of another variable, was confirmed using Levene's test (Field 2008). Briefly, a one-way ANOVA was performed upon the deviance scores of the variable(s) to reveal the absolute difference between the mean of the group and each individual variance score. A levene's statistic greater than 0.05 confirmed homogeneity of variance.

Througout the thesis, all data are presented as mean ± SD unless stated otherwise. All statistical analyses (including the assessment of normal distribution and homogeneity of variance) were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). The modelling of oxygen uptake / lactate kinetics at the onset of constant power exercise (Chapter 4) and lactate kinetics throughout recovery from maximal exercise (Chapter 5) were performed using the statistical packages GraphPad Prism (Version 5.01, GraphPad software, Inc. CITY, USA) and SYSTAT (Version 12, SYSTAT software Inc., CA, USA), respectively.

2.12 THE LACTATE MINIMUM TEST AND THE MAXIMAL LACTATE STEADY STATE (MLSS)

The lactate minimum test (Johnson et al. 2009; Tetgbur et al. 1993) was used to predict the maximal lactate steady state power output (MLSS). The lactate minimum test consisted of three consecutive phases: phase one: a maximal incremental exercise test to volitional tolerance; phase two: 8 min of constant power cycling at 60 W to maximise $[lac^-]_B$; and phase three: five, 4 min increments in power output at 45, 50, 55, 60 and 65% of the maximal power output ($\dot{W}max$) achieved during phase one. During the maximal incremental test, power output started at 0 W and was increased by 10 W every 15 s to elicit exercise intolerance within approximately 10 min; exercise intolerance was determined when, despite verbal encouragement, cycling cadence could no longer be

maintained above 60 r.p.m. Throughout phase 3, $[lac^-]_B$ was determined in the final seconds of each 4 min increment. Subsequently, a 2nd order polynomial was fitted to the relationship of $[lac^-]_B$ and power output. The asymptote of the curve was calculated through differentiation of the quadratic equation and defined the lactate minimum power output (i.e. estimated MLSS power).

The MLSS represents the highest power output that can be sustained over time where a steady state in $[lac^-]_B$ is observed (i.e. the rate of appearance and disappearance of lactate from the systemic circulation is equal). Therefore, in order to resolve the MLSS power output, a minimum of two, 30 min constant power trials were performed, preceded by a 3 min warm-up at 50% power output. The first trial was performed at the lactate minimum power output. Throughout these tests, $[lac^-]_B$ was determined every 3 min from min 15 to 30 using procedures outlined in section 2.10. MLSS was defined as the highest cycling power output where $[lac^-]_B$ increased no more than 0.5 $mmol \cdot L^{-1}$ from min 15 to 30 (Aunola and Rusko 1992; Bacon and Kern 1999). If $[lac^-]_B$ increased or decreased more than 0.5 $mmol \cdot L^{-1}$ during the first trial, power output during the subsequent trial was decreased or increased by 2.5 %, respectively. This process was repeated until MLSS power output was confirmed.

2.13 EFFECTS OF PEDAL CADENCE ON THE PHYSIOLOGICAL RESPONSES TO EXERCISE AT THE MAXIMAL LACTATE STEADY STATE

2.13.1 INTRODUCTION

In Chapter 4, isocapnic volitional hyperpnoea was imposed upon cycling exercise at the MLSS to determine the contribution of respiratory muscle work to systemic $[lac^-]_B$. Preliminary investigations showed that at the onset of volitional hyperpnoea where $\dot{V}_E$ was increased in a square wave manner from ~80 $L \cdot min^{-1}$ to ~150 $L \cdot min^{-1}$, pedal cadence demonstrated a transient rise by ~10 r.p.m. Previous studies have demonstrated that large (± 50 r.p.m.) increases in pedal cadence have big effects upon the $[lac^-]$ response to

exercise at the MLSS. Whether much smaller increases in pedal cadence (as observed in our preliminary work) *per-se* has any meaningful effect upon physiological responses reflective of the MLSS is unknown. A pedal cadence-mediated alteration in physiological parameters independent of the imposed volitional hyperpnoea may confound the interpretation of physiological responses. Therefore, the aim of this pilot study was to quantify the effects of pedal cadence upon physiological responses to 30 min cycling exercise at the MLSS. The findings of this study provide novel methodological recommendations for the control of pedal cadence during exercise with imposed volitional hyperpnoea used in Chapter 4.

2.13.2 METHODS

2.13.2.1 PARTICIPANTS

Following ethical approval and written informed consent, 11 non-smoking, active males were recruited for the study (Table 2.1). Participants followed pre-exercise instructions outlined in section 2.1. All tests were separated by a minimum of 48 h and performed at a similar time of day and in similar laboratory conditions (temperature: 16.5 ± 1.7°C; relative humidity: 46.2 ± 8.7%).

2.13.2.2 EXPERIMENTAL PROCEDURE

On the first visit, all subjects were familiarised with the testing procedures. In subsequent visits, MLSS cycling power output was determined as described in section 2.12. Following determination of MLSS and the subjects' preferred cadence, three 30 min experimental cycling trials were performed at MLSS on different days and in random order. The first 15 min of each trial was performed at the preferred cycling cadence and for the remaining 15 min subjects maintained this (control) or cycled 15 r.p.m. above (+15r.p.m.) or 15 r.p.m. below (-15r.p.m.) the control. All exercise trials were performed on an electromagnetically braked cycle ergometer (see section 2.2).

Table 2.1 Descriptive characteristics of the subjects.

$n = 11$	
Age (years)	33.1 ± 8.0
Body mass (kg)	81.5 ± 8.2
Height (m)	1.79 ± 6.4
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	4.10 ± 0.61
$\dot{W}$max (W)	387 ± 42
MLSS (W)	214 ± 29
$MLSS_{INTENSITY}$ (MLSS/ $\dot{W}$max ; %)	55 ± 4

Values are expressed as mean ± SD.

2.13.2.3 RESPIRATORY, CARDIOVASCULAR AND PERCEPTUAL MEASUREMENTS

Throughout all experimental trials, respiratory variables and pulmonary gas exchange were measured breath by breath (see section 2.6). Heart rate (HR) was measured every 2 min using telemetry (Polar S610, Polar, Kempele, Finland) and ratings of perceived exertion (RPE) were recorded every 2 min using the Borg 6-20 scale (Borg 1982).

2.13.2.4 BLOOD SAMPLING

Arterialised venous blood samples were drawn every 2 min and analysed immediately for PCO_2, pH, $[lac^-]_B$ and $[glucose]_B$ (see section 2.8); $[HCO_3^-]$ and BE_{ECF} were calculated as in section 2.8.

2.13.2.5 STATISTICAL ANALYSES

Statistical analyses were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). All trials were divided into and averaged over two discrete periods: steady state period (min 10 to 14) and an intervention period (min 16 to 30). Since dependent

variables were not different between trials during the steady-state phase, data are presented from the steady state period of the control trial only. Differences and trial interactions were compared using a factorial ANOVA for repeated measures and Tukey's HSD post-hoc analysis. Interactions were defined for "trial" (control vs. +15r.p.m. vs. -15r.p.m.) and "time" (steady state vs. intervention). Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

2.13.3 RESULTS

During the control trial, subjects preferred cycling cadence was 87 ± 6 r.p.m. Throughout +15r.p.m. and -15r.p.m. cycling cadence increased to 102 ± 6 r.p.m. ($P<0.05$) and decreased to 72 ± 6 r.p.m. ($P<0.05$).

2.13.3.1 PERCEPTUAL RESPONSES

RPE was similar between trials during the steady state period (11.4 ± 0.1). Relative to this, during the intervention period (min 16 to 30) RPE increased to 12.1 ± 1.2, 13.4 ± 2.1 and 12.4 ± 1.6 (all $P<0.05$) in the control, +15r.p.m. and -15r.p.m. trials, respectively. The increase in RPE in +15r.p.m. was greater than the control trial and -15r.p.m ($P<0.05$).

2.13.3.2 VENTILATORY RESPONSES

Group mean $\dot{V}_E$, V_T and f_R are shown in Table 2.2. Breathing pattern was not different between trials during the steady state period, however, $\dot{V}_E$ was significantly increased and decreased from min 16 to 30 in +15r.p.m. and -15r.p.m., respectively ($P<0.001$). In +15r.p.m., this was due predominantly to an increase in f_R ($P<0.001$) with only a small change in V_T. In contrast, the lower $\dot{V}_E$ in -15r.p.m. was primarily due to a reduction in V_T with no change in f_R. Duty cycle was unchanged between and within all

trials. An increase and decrease in cadence resulted in an increase and decrease in $\dot{V}O_2$, $\dot{V}CO_2$ and RER (Table 2.2).

Table 2.2 Pulmonary ventilation and gas exchange responses to 30 min exercise at MLSS during the control, +15r.p.m. and -15r.p.m. trials.

	Control trial		+15r.p.m.	-15r.p.m.
	Steady state (min 10-14)	Intervention (min 16-30)	Intervention (min 16-30)	Intervention (min 16-30)
$\dot{V}_E$ ($L \cdot min^{-1}$)	72.2 ± 12.0	78.3 ± 13.9 *	91.8 ± 18.7 *†	70.8 ± 13.7 †
V_T (L)	2.46 ± 0.69	2.36 ± 0.68 *	2.48 ± 0.83	2.23 ± 0.73 *†
f_R ($breaths \cdot min^{-1}$)	31 ± 7	35 ± 8 *	40 ± 10 *†	34 ± 8 *
T_I/T_{tot}	0.49 ± 0.03	0.49 ± 0.03	0.49 ± 0.02	0.48 ± 0.03
$\dot{V}O_2$ ($L \cdot min^{-1}$)	2.85 ± 0.37	2.88 ± 0.37	3.03 ± 0.53 *	2.66 ± 0.43 †
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	2.76 ± 0.39	2.81 ± 0.41	3.03 ± 0.51 *†	2.56 ± 0.43 †
$\dot{V}_E / \dot{V}O_2$	25.35 ± 2.16	27.12 ± 2.72*	30.37 ± 3.60*†	27.67 ± 2.08*
$\dot{V}_E / \dot{V}CO_2$	26.15 ± 2.08	27.84 ± 2.41*	30.26 ± 2.77*†	26.59 ± 2.30
RER	0.97 ± 0.03	0.97 ± 0.03	1.00 ± 0.05 *†	0.96 ± 0.03

Values are expressed as mean ± SD. * $P<0.05$ vs. steady state. † $P<0.05$ vs. intervention period of control trial.

2.13.3.3 CARDIOVASCULAR RESPONSES

There were no differences in steady state HR between all trials (146.7 ± 8.2 $beats \cdot min^{-1}$). A transient increase in HR was observed in the control trial from min 16 to 30 (155.7 ± 9.4 $beats \cdot min^{-1}$, $P<0.05$), however, this was exceeded during +15r.p.m. (160.3 ± 11.8 $beats \cdot min^{-1}$; $P<0.05$) and attenuated in -15r.p.m. (152.5 ± 12.3 $beats \cdot min^{-1}$; $P<0.05$; interaction effect trial × time, $P<0.05$).

2.13.3.4 BLOOD AND ACID-BASE RESPONSES

Acid-base status was not different between trials during the steady state period. Relative to the control trial, during min 16 to 30 reducing cadence by 15 r.p.m. lead to a reduction in $[H^+]$ and an increase in $[HCO_3^-]$ and BE_{ECF}; PCO_2 remained unchanged. Conversely in +15r.p.m., $[H^+]$ was increased and $[HCO_3^-]$, BE_{ECF} and PCO_2 decreased. The changes observed in BE_{ECF} and $[HCO_3^-]$ in +15r.p.m. and -15r.p.m. exceeded that of the control trial (interaction effect trial × time, $P<0.001$; Table 2.3).

Table 2.3. Acid-Base and blood glucose responses to 30 min exercise at MLSS during the control, +15r.p.m. and -15r.p.m. trials.

	Control trial		+15r.p.m.	-15 r.p.m.
	Steady state (min 10-14)	Intervention (min 16-30)	Intervention (min 16-30)	Intervention (min 16-30)
$[H^+]$ (nmol·L^{-1})	44.5 ± 3.0	43.6 ± 2.9 *	44.4 ± 2.3	43.1 ± 2.3 *
$[HCO_3^-]$ (mmol·L^{-1})	23.1 ± 2.0	22.9 ± 1.8	21.5 ± 2.8 *†	24.1 ± 3.0
BE_{ECF} (mEq·L^{-1})	-1.87 ± 2.01	-1.84 ± 1.95	-3.31 ± 2.69 *†	-0.76 ± 2.71 *
PCO_2 (mmHg)	43.6 ± 3.2	42.0 ± 2.9 *	39.9 ± 4.8 *	43.5 ± 6.5
$[glucose]_B$ (mmol·L^{-1})	4.04 ± 0.37	4.06 ± 0.48	4.05 ± 0.60	3.88 ± 0.48

Values are expressed as mean ± SD. * $P<0.05$ vs. steady state. † $P<0.05$ vs. intervention period of control trial.

The $[glucose]_B$ was not different between or within trials (Table 2.3). During all trials $[lac^-]_B$ reached a steady state following ~10 min (3.91 ± 1.89 mmol·L^{-1}). Following 30 min, $[lac^-]_B$ was increased and decreased in +15r.p.m. and -15r.p.m. to 4.96 ± 2.01 mmol·L^{-1} (+43 ± 38 %) and 3.15 ± 1.75 mmol·L^{-1} (-25 ± 22 %), respectively (interaction effect trial × time, $P<0.001$; Figure 2.9). The rate of change in $[lac^-]_B$ in +15 and -15r.p.m. was 0.09 and -0.03 mmol·L^{-1}·min^{-1}, respectively.

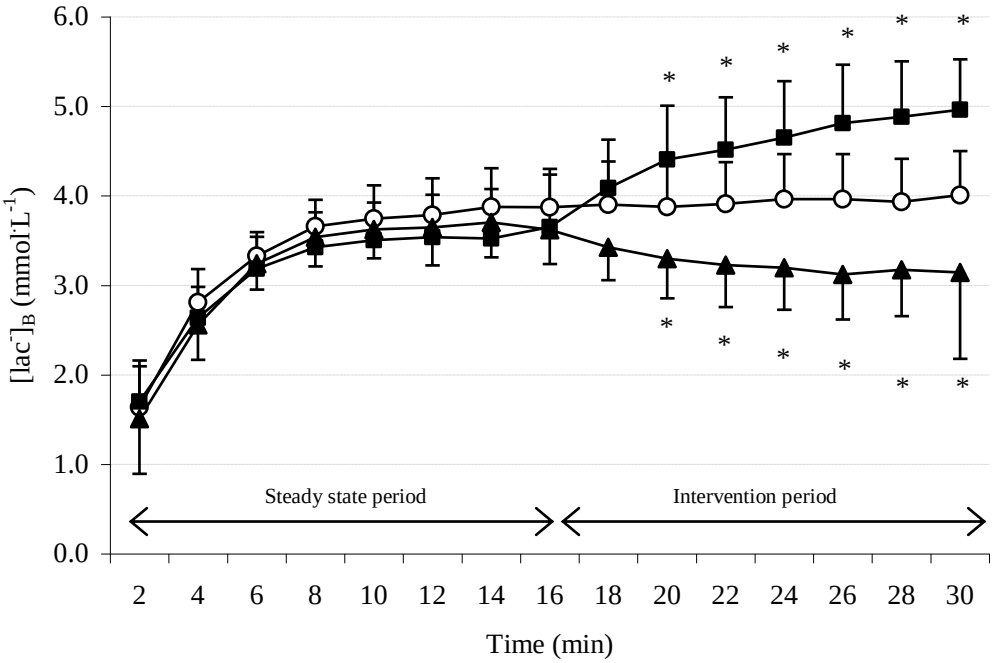

Figure 2.9 Blood lactate concentration ($[lac^-]_B$) during 30 min exercise at MLSS. ○, control trial; ■, +15r.p.m.; ▲, -15r.p.m.. * $P<0.05$ vs. control trial.

2.13.4 DISCUSSION

The aim of this pilot study was to investigate the effects of pedal cadence upon physiological responses to 30 min cycling at MLSS. The between day coefficient of variation in preferred cadence was 0.5 ± 0.3% which was superior to that reported previously (4.4 ± 0.9%; Moussay et al. 2003). The main findings were that increasing and decreasing pedal cadence by 15 r.p.m. significantly altered physiological, acid base and psychophysical responses. Therefore, in subsequent studies, failing to control for changes in pedal cadence during exercise at the MLSS would significantly affect the interpretation of physiological parameters.

At the onset of volitional hyperpnoea imposed upon exercise, preliminary work indicated that pedal cadence increases. This rise in pedal cadence is explained by the neural link between breathing and locomotion (Eldridge et al. 1981) which is termed locomotor-respiratory coupling or the 'entrainment' of breathing (Bramble and Carrier 1983). Recent evidence shows that the supero-lateral primary motor cortex (the cortex area associated with volitional hyperpnoea) is also activated during and following leg exercise

in humans (Haouzi 2006). Therefore, when intense volitional hyperpnoea is imposed upon exercise, the increase in central respiratory motor output appears to also activate the supraspinal locomotor efferent centres causing an increase in motor output to the limbs and thus pedal cadence.

The MLSS is suggested to represent a relative exercise intensity at which a steady state is observed in metabolite concentrations, cardiorespiratory parameters and breathing pattern (Baron et al. 2003). In +15r.p.m. an increase in f_R was observed and V_T remained unchanged. Conversely, in -15r.p.m. f_R remained unchanged and V_T was reduced. The increase in breathing frequency in +15r.p.m. may be due to the entrainment of respiratory and locomotor motor output. This is supported by the significant negative correlation between ΔPCO_2 and $\Delta \dot{V}_E$ (r = -0.57, P<0.05) and the significant increase in the ventilatory equivalents for O_2 and CO_2 (Table 2.2) above that of the control and -15r.p.m. trials. This demonstrates an excessive respiratory motor drive and pulmonary $\dot{V}_E$ for the exercise metabolic demand and would also account for the increase in $\dot{V}CO_2$ and RER.

Alternatively, the greater pedal cadence may have increased the recruitment of less efficient type II muscle secondary to the increased rate of muscle shortening. The greater recruitment of type II muscle fibres with an increase in pedal cadence is supported by the increase in $[lac^-]_B$ (Figure 2.9) and is consistent with the transient on-time of the glycolytic flux (Crowther et al. 2002). This is also supported by the increase in $\dot{V}O_2$ in +15r.p.m. (Ferguson et al. 2001) which is likely due to the greater work performed when pedal cadence is higher and places the type I and type II muscle fibres on a more disadvantageous and optimal position on their efficiency-rate of muscle shortening curve (He et al. 2000). The recruitment of type II muscles fibres would increase glycogenolysis (Beelen et al. 1993) and in support of this notion, Table 2.2 shows an increase in RER from 0.97 in the control trial to 1.00 in +15r.p.m. Therefore, the resultant tachypnoea may reflect a respiratory compensation for the greater utilisation of endogenous carbohydrates stores.

However, the potential for a respiratory alkalosis to increase $[lac^-]_B$ is also well documented (Davies et al. 1986; LeBlanc et al. 2002). In +15r.p.m. significant relationships were observed between $\Delta[lac^-]_B$ and $\Delta \dot{V}_E$ (r = 0.83, P<0.05), $\Delta \dot{V}_E$ and ΔPCO_2 (r = -0.57, P<0.05), and $\Delta[lac^-]_B$ and ΔPCO_2 (r = -0.74, P<0.01; Figure 2.10). The decrease in PCO_2 (-13%) is similar to previous studies although the increase in $[lac^-]_B$ (+43%) is larger (Davies et al. 1986; LeBlanc et al. 2002). The differences between studies is most likely explained by differences in study protocol since previous studies imposed moderate intensity hyperventilation upon steady state exercise to achieve a reduction in PCO_2. Therefore, it is possible that the increase in $[lac^-]_B$ occurred secondary to the reduction in PCO_2. Interestingly, pH remained unchanged in +15r.p.m. despite the increase in $[lac^-]_B$ and decrease in BE_{ECF}, although this was likely due to the 5 mmHg reduction in PCO_2. It would certainly be interesting in future study to investigate the effects of pedal cadence upon responses to exercise at MLSS with a breathing pattern matched to that observed in the control trial thus identifying the respiratory contribution to changes in systemic metabolites and acid-base balance.

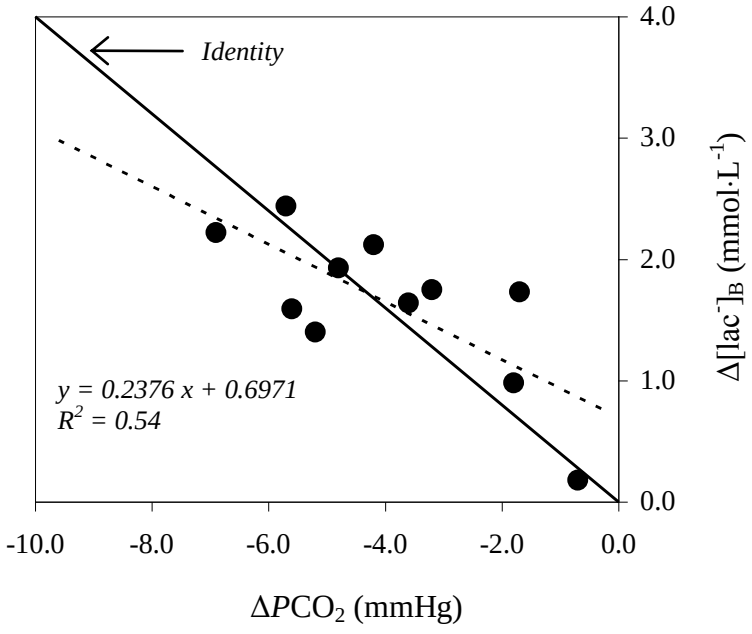

Figure 2.10 Change in $[lac^-]_B$ versus change in PCO_2 in the +15r.p.m. trial. Note: correlation coefficient: r = -0.74, P = 0.01.

The increase in $\dot{V}_E$ in +15r.p.m. was caused almost exclusively by a greater f_R. This would increase the recruitment of the less efficient accessory muscles and therefore the work of breathing. In a previous study, increasing the work of breathing up to 50% via inspiratory resistive loads resulted in a decreased vascular conductance and blood flow in the working limb muscles (Harms et al. 1997) which resulted in a significant increase in limb muscle fatigue (Romer et al. 2006a). In +15r.p.m. the greater ventilatory work may have attenuated limb blood flow secondary to a reflex reduction in limb vascular conductance and thus O_2 delivery which would increase the reliance upon type II muscle fibres. The increase in type II muscle fibre recruitment may increase the glycolytic flux and thus lactate production and release by the locomotor muscles. However, reductions in limb blood flow with inspiratory muscle loading does not affect arterial-venous femoral [lac^-] (Harms et al. 1997). Furthermore, the absolute increase in $\dot{V}_E$ in +15r.p.m. was less than previous studies (Harms et al. 1997; Romer et al. 2006a). With a low $\dot{V}_E$ is unlikely to trigger a sympathetic efferent response which importantly, is known to preserve locomotor perfusion (Wetter et al. 1999). Accordingly, it is unlikely that an increased ventilatory work contributed to the observed increase in $[lac^-]_B$ in +15r.p.m.

2.13.5 CONCLUSIONS

In summary, this pilot study shows the importance of controlling pedal cadence throughout steady state exercise when determining physiological parameters. The pedal cadence-mediated change in these parameters is probably due to type II muscle fibre recruitment and / or the development of a respiratory alkalosis. In chapter 4, performing volitional hyperpnoea upon exercise at the MLSS whilst maintaining a spontaneous (and increasing) pedal cadence has the potential to affect the interpretation of physiological variables due to the coupling between breathing and locomotion.

CHAPTER 3

INSPIRATORY MUSCLE TRAINING REDUCES BLOOD LACTATE CONCENTRATION DURING RESTING ISOCAPNIC VOLITIONAL HYPERPNOEA

3.1 INTRODUCTION

Specific respiratory muscle training (RMT) can be performed using voluntary isocapnic hyperpnoea (VIH), flow-resistive loading, or pressure-threshold loading; with the exception of VIH, these are commonly referred to as inspiratory muscle training (IMT). Ventilatory endurance is enhanced with all three techniques, whereas IMT also increases diaphragm thickness (Downey et al. 2007; Enright et al. 2006) and the maximal strength, shortening velocity and power of the inspiratory muscles (for a full review see McConnell and Romer 2004a). Furthermore, well controlled studies have shown improvements in endurance exercise performance following both IMT (Gething et al. 2004a, b; Griffiths and McConnell 2007; Johnson et al. 2007; Romer et al. 2002a; Volianitis et al. 2001) and VIH (Leddy et al. 2007).

The mechanisms underlying such performance improvements remain speculative but may include altered breathing mechanics, reduced perception of effort (Downey et al. 2007; Gething et al. 2004a, b; Griffiths and McConnell 2007; Romer et al. 2002a; Verges et al. 2007b; Volianitis et al. 2001) and possibly reductions in both diaphragm fatigue (Verges et al. 2007b) and an associated metaboreflex that attenuates limb blood flow (McConnell and Lomax 2006; Witt et al. 2007). The notion that genuine physiological adaptation explains, in part, RMT-mediated improvements in endurance exercise performance is further supported by the frequently observed reduction in blood lactate concentration ($[lac^-]_B$) during whole-body exercise following both IMT (Griffiths and McConnell 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Volianitis et al. 2001) and VIH (Leddy et al. 2007; Spengler et al. 1999). Furthermore, correlations have been reported between reductions in $[lac^-]_B$ and performance improvements following RMT (Romer et al. 2002b; Spengler et al. 1999), with up to 52% of the variation in performance being attributed to the reduced $[lac^-]_B$ (Romer et al. 2002b).

The mechanism(s) by which RMT reduces $[lac^-]_B$ remains equivocal. An RMT-mediated change in minute ventilation ($\dot{V}_E$), which may conceivably alter both the work of

breathing and acid base balance, is an unlikely mechanism since reductions in $[lac^-]_B$ following RMT have been observed irrespective of whether $\dot{V}_E$ is lower (Leddy et al. 2007), unchanged (McConnell and Sharpe 2005; Spengler et al. 1999; Volianitis et al. 2001), or increased (Kohl et al. 1997). The concept that RMT-mediated respiratory muscle adaptations explain, in part, the reductions observed in $[lac^-]_B$ remains contentious: the small size of these muscles and observations that loading and unloading of the respiratory muscles during exercise fails to influence both the systemic (Romer et al. 2006a) and femoral arterial-venous $[lac^-]_B$ (Harms et al. 1997) argue against this premise (Wetter and Dempsey 2000). However, volitional hyperpnoea increases $[lac^-]_B$ both at rest (Martin et al. 1984; Verges et al. 2007b) and when superimposed upon steady state exercise (Johnson et al. 2006) suggesting that the respiratory muscles are capable of net lactate production and release. Furthermore, VIH appears to attenuate such net release during volitional hyperpnoea (Verges et al. 2007b). However, this latter study did not rigorously control isocapnia which is essential for the interpretation of changes in $[lac^-]_B$. Also, the use of a breathing challenge based upon maximum voluntary ventilation (MVV) limits external validity as both the breathing pattern and work of breathing are unreflective of that seen during exercise (Coast et al. 1993). Since many of the muscle adaptations associated with endurance-orientated training (i.e. VIH) are different from those associated with strength-orientated training (i.e. pressure threshold IMT), it also remains uncertain whether IMT would reduce $[lac^-]_B$ during volitional hyperpnoea.

Therefore, to investigate this issue further the present study examined the hypothesis that 6 weeks of IMT would attenuate the increase in $[lac^-]_B$ caused by mimicking, at rest, the breathing pattern observed during high-intensity endurance exercise.

3.2 METHODS

3.2.1 PARTICIPANTS

Following approval from Nottingham Trent University's ethics committee, 22 non-smoking, recreationally active males provided written informed consent to participate in the study. Throughout the study subjects were instructed to adhere to their usual training regimen and followed pre-exercise instructions outlined in section 2.1. Descriptive characteristics of the subjects are presented in Table 3.1.

Table 3.1 Descriptive characteristics of the subjects.

	Control (n = 11)	IMT (n = 11)
Age (years)	28.5 ± 4.1	22.4 ± 4.5 *
Body mass (kg)	75.5 ± 5.6	78.6 ± 9.7
Height (cm)	176.9 ± 7.4	181.6 ± 7.6
FVC (L)	5.32 ± 0.55 (104 ± 8)	5.67 ± 0.92 (106 ± 12)
FEV_1 (L)	4.28 ± 0.62 (99 ± 11)	4.93 ± 0.67 (109 ± 11)
FEV_1/FVC (%)	80.3 ± 7.1 (96 ± 9)	87.7 ± 8.3 (103 ± 9) *
MVV_{10} ($L \cdot min^{-1}$)	176.3 ± 15.0 (102.3±10.9)	173.4 ± 53.7 (122.4±30.3)
MIP (cmH_2O)	163 ± 19 (113 ± 4)	147 ± 27 (119 ± 5)
$\dot{V}O_2 max$ ($L \cdot min^{-1}$)	3.75 ± 0.55	3.77 ± 0.75
$\dot{W}max$ (W)	353 ± 44	362 ± 38

Values are expressed as means ± SD. Values in parentheses represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984). * P<0.05 between groups.

3.2.2 EXPERIMENTAL PROCEDURE

Baseline pulmonary function and MIP were measured during the first laboratory visit. On subsequent visits separated by at least 48 h, subjects performed a maximal incremental cycling test, and two 10 min isocapnic volitional hyperpnoea tests (the first being a familiarisation test). The volitional hyperpnoea tests were performed at the $\dot{V}_E$, tidal volume (V_T), breathing frequency (f_R) and duty cycle (T_I/T_{tot}) associated with 85% maximal exercise $\dot{V}_E$ ($\dot{V}_E$ max). During volitional hyperpnoea tests blood samples were taken every 2 min from 0-10 min, inclusive, and respiratory variables were measured breath by breath and averaged over 2 min intervals. Subjects were subsequently matched for 85% $\dot{V}_E$ max and divided into an IMT group (n = 11) or a control (no IMT) group (n = 11). No more than 1 week following a 6 wk intervention MIP was measured and at least 48 h following this, subjects repeated the volitional hyperpnoea test.

3.2.3 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY MOUTH PRESSURE

Pulmonary function was assessed using a pneumotachograph and a hand-held mouth pressure meter measured MIP as an index of global inspiratory muscle strength according to sections 2.3 and 2.5, respectively.

3.2.4 MAXIMAL EXERCISE TEST

Subjects performed a maximal incremental cycling test on an electromagnetically-braked cycle ergometer (see section 2.2). Cycling began at 0 W and power was subsequently increased by 10 W every 15 s in order to result in exercise intolerance within ~10 min. This rapid incremental protocol was selected to maximise $\dot{V}_E$ at the cessation of the test. The power at which exercise intolerance ensued defined maximal power output ($\dot{W}$ max), and the highest oxygen uptake ($\dot{V}O_2$) and $\dot{V}_E$ recorded in any 30 s period defined $\dot{V}O_2$ max and $\dot{V}_E$ max, respectively.

3.2.5 VOLITIONAL HYPERPNOEA

Volitional hyperpnoea was performed whilst seated on the cycle ergometer in an identical body position to that adopted during the maximal exercise test. Subjects were instructed to increase $\dot{V}_E$ and f_R in a square wave manner to a level commensurate with 85% $\dot{V}_E$max, which during pilot work was shown to represent the maximum square wave response that could be maintained for 10 min. An audio metronome paced f_R and real-time visual feedback of $\dot{V}_E$ was provided throughout the test. The prescribed breathing pattern ($\dot{V}_E$, V_T, f_R and T_I/T_{tot}) during volitional hyperpnoea was identical pre- and post-intervention and was chosen to provide a breathing challenge reflective of the work of breathing associated with exercise hyperpnoea. This methodology is deemed superior to an arbitrary %MVV as it more closely reflects the work of breathing during whole-body exercise: for a given $\dot{V}_E$ greater than approximately 60 L·min^{-1} the work of breathing of exercise hyperpnoea can be overestimated by as much as 25% when a spontaneous breathing pattern is adopted during volitional hyperpnoea (Coast et al. 1993). Isocapnia was maintained during volitional hyperpnoea by adding CO_2 into the inspiratory circuit in order to maintain a resting PCO_2.

3.2.6 INTERVENTION

IMT was performed using an inspiratory pressure-threshold device as described in section 2.10. Subjects completed a training diary to record IMT adherence and habitual training, which the control group also recorded. The control group did not perform sham IMT since the duration of the volitional hyperpnoea test and the breathing pattern employed was identical pre- and post-intervention, thus responses would not be influenced by either motivation or expectation.

3.2.7 BLOOD SAMPLING AND RESPIRATORY MEASUREMENTS

Arterialised venous blood was sampled from a dorsal hand vein via an indwelling cannula and analysed immediately for PCO_2, pH, and $[lac^-]_B$ as outlined in section 2.8. Plasma $[HCO_3^-]$ and BE_{ECF} were calculated as described in section 2.8. Pulmonary ventilation and gas exchange were measured breath by breath (see section 2.6). During volitional hyperpnoea tests, a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) and a 1.5 m length of corrugated tubing was attached distally to the pneumotachograph allowing additional CO_2 to be added to the inspirate.

3.2.8 STATISTICAL ANALYSES

Statistical analyses were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). Within group changes over time during volitional hyperpnoea were determined using one-way ANOVA for repeated measures and Tukey's HSD post-hoc analysis. Within and between group interaction effects were determined using two-way ANOVA for repeated measures. Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

3.3 RESULTS

3.3.1 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY PRESSURE

Baseline pulmonary function and MIP were all within normal limits (Table 3.1). The IMT group demonstrated excellent training compliance (91% adherence) and subjects' habitual training remained unchanged in both IMT and control groups. MIP increased from 147 ± 27 to 189 ± 27 cmH_2O (+31 ± 22%) following IMT ($P<0.01$). No change was observed in the control group (pre: 163 ± 19 vs. post: 166 ± 20 cmH_2O).

3.3.2 RESPIRATORY AND ACID-BASE RESPONSES TO VOLITIONAL HYPERPNOEA

Group mean values for ventilatory and acid base responses to 10 min volitional hyperpnoea pre- and post-intervention are shown in Table 3.2. Before and after the intervention, $\dot{V}_E$, V_T, f_R, T_I/T_{tot}, and measures of acid base balance, were not different between groups and remained unchanged over time during volitional hyperpnoea. The mean $\dot{V}_E$ during volitional hyperpnoea represented 72 ± 8% and 81 ± 19% of MVV_{10} in control and IMT groups, respectively. PCO_2 was maintained at resting levels throughout volitional hyperpnoea prior to and following the intervention and was not different between groups (Figure 3.1).

Table 3.2 Ventilatory and acid-base responses to 10 min volitional hyperpnoea pre- and post-intervention.

	Control		IMT	
	Pre	Post	Pre	Post
$\dot{V}_E$ ($L \cdot min^{-1}$)	127.1 ± 2.3	128.7 ± 2.4	132.9 ± 9.6	136.8 ± 3.2
V_T (L)	2.62 ± 0.04	2.64 ± 0.07	2.60 ± 0.03	2.66 ± 0.06
f_R ($breaths \cdot min^{-1}$)	50 ± 0	50 ± 0	52 ± 0	52 ± 0
T_I/T_{tot}	0.44 ± 0.00	0.44 ± 0.00	0.52 ± 0.00	0.49 ± 0.00
$[H^+]$ ($nmol \cdot L^{-1}$)	40.6 ± 2.9	39.4 ± 2.2	40.2 ± 2.2	40.3 ± 1.0
$[HCO_3^-]$ ($mmol \cdot L^{-1}$)	26.0 ± 0.9	26.9 ± 2.5	26.5 ± 1.4	27.0 ± 1.3
BE_{ECF} ($mEq \cdot L^{-1}$)	1.38 ± 0.91	1.72 ± 2.04	1.52 ± 1.11	2.35 ± 1.23

Values are expressed as means ± SD.

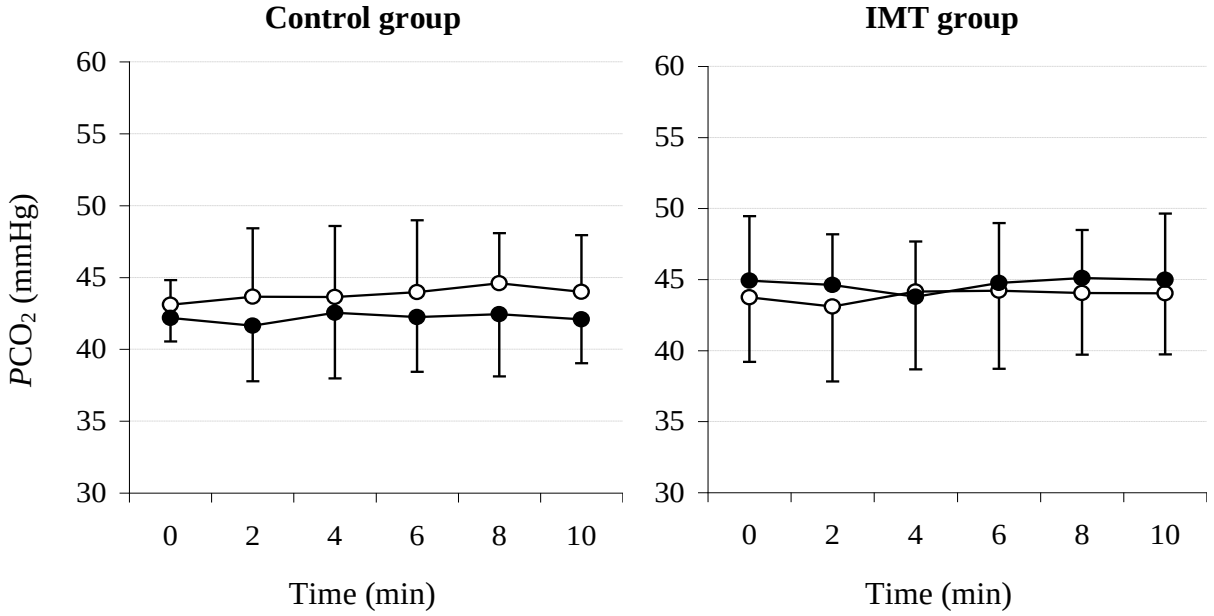

Figure 3.1 Partial pressure of carbon dioxide in arterialised venous blood (PCO_2) during volitional hyperpnoea pre- (○) and post- (●) intervention in control and IMT groups.

3.3.3 LACTATE RESPONSES TO VOLITIONAL HYPERPNOEA

Individual plots demonstrating the change in $[lac^-]_B$ following 10 min of volitional hyperpnoea pre and post-intervention for both groups is shown in Figure 3.2; note the reduction in group mean $[lac^-]_B$ following IMT only (solid black line).

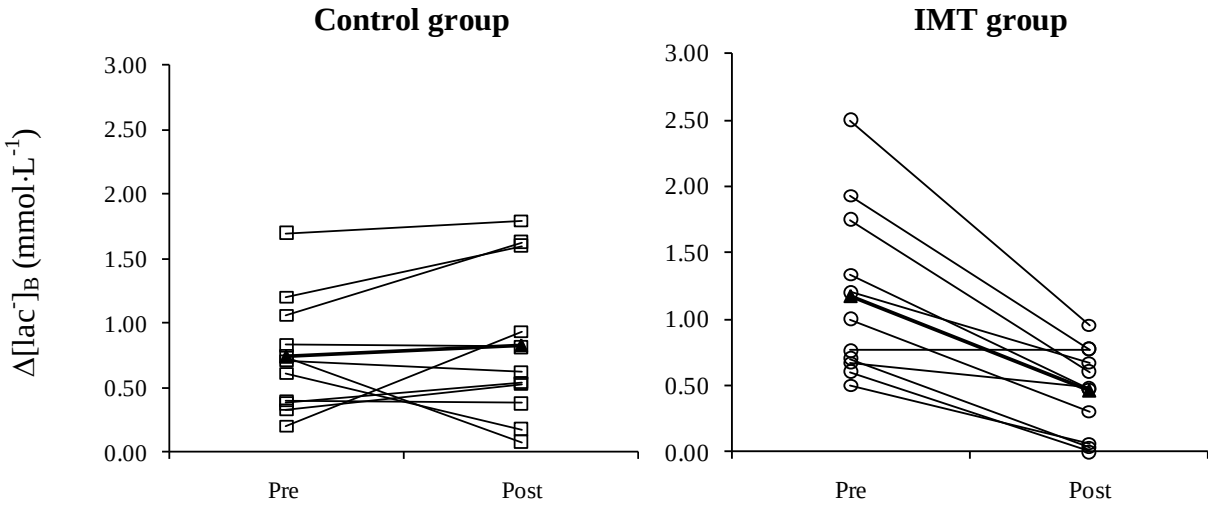

Figure 3.2 Individual changes in blood lactate concentration ($[lac^-]_B$) during 10 min volitional hyperpnoea pre- and post-intervention in control and IMT groups.

Prior to the intervention, significant increases in $[lac^-]_B$ above rest were observed following 10 min of volitional hyperpnoea in IMT and control groups (P<0.05; Figure 3.3) and such changes were not different between groups. Following the intervention the $[lac^-]_B$ response to volitional hyperpnoea was unchanged in the control group. Conversely, $[lac^-]_B$ during volitional hyperpnoea was reduced following IMT, with 17 ± 37% and 25 ± 34% reductions observed at 8 and 10 min, respectively (P<0.05). These reductions exceeded changes observed in the control group (P<0.05).

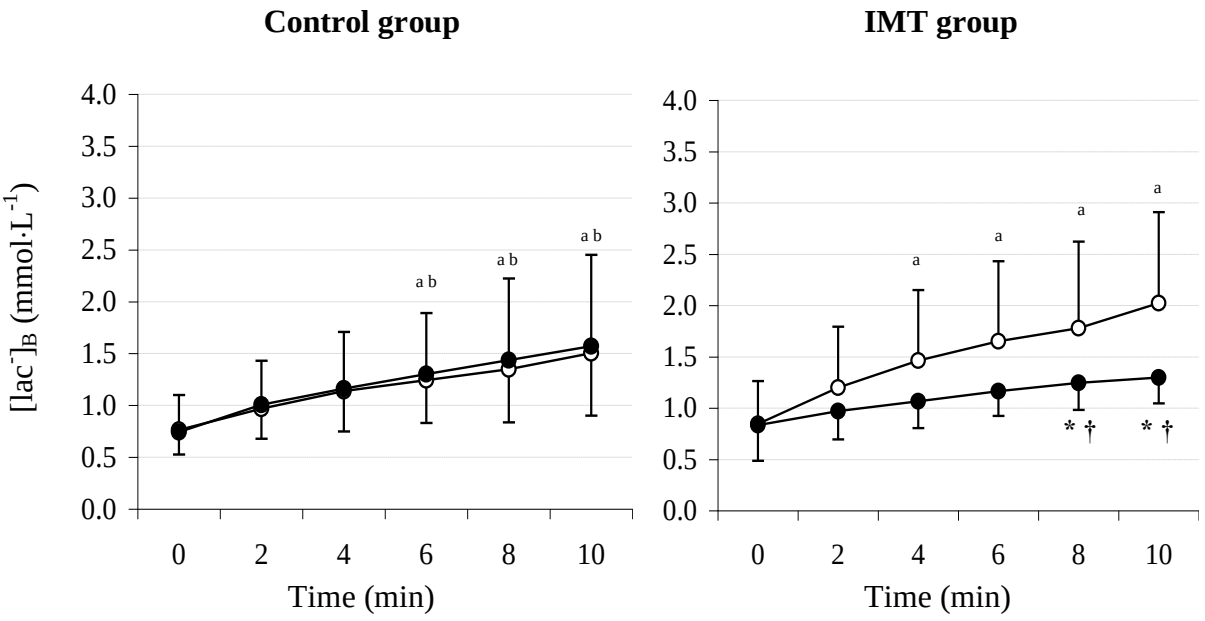

Figure 3.3 Blood lactate concentration ($[lac^-]_B$) during volitional hyperpnoea pre- (○) and post- (●) intervention in control and IMT groups. [a] P<0.01 vs. rest pre-IMT; [b] P<0.01 vs. rest post-IMT; * P<0.05 vs. pre-IMT; [†] P<0.05 interaction effect.

3.3.4 CORRELATIONS AMONGST VARIABLES

Prior to the intervention, increases in $[lac^-]_B$ during volitional hyperpnoea did not correlate with any measure of pulmonary function, MIP, endurance training status ($\dot{V}O_2$ max, $\dot{W}max$) or ventilatory responses to volitional hyperpnoea. Increases in $[lac^-]_B$ during volitional hyperpnoea did not correlate with the absolute $\dot{V}_E$ nor when expressed as %MVV. The attenuated increase in $[lac^-]_B$ during volitional hyperpnoea after IMT was not

correlated with increases in MIP. However, baseline MIP was negatively correlated with relative IMT-induced increases in MIP (r = -0.70, P<0.05; Figure 3.4).

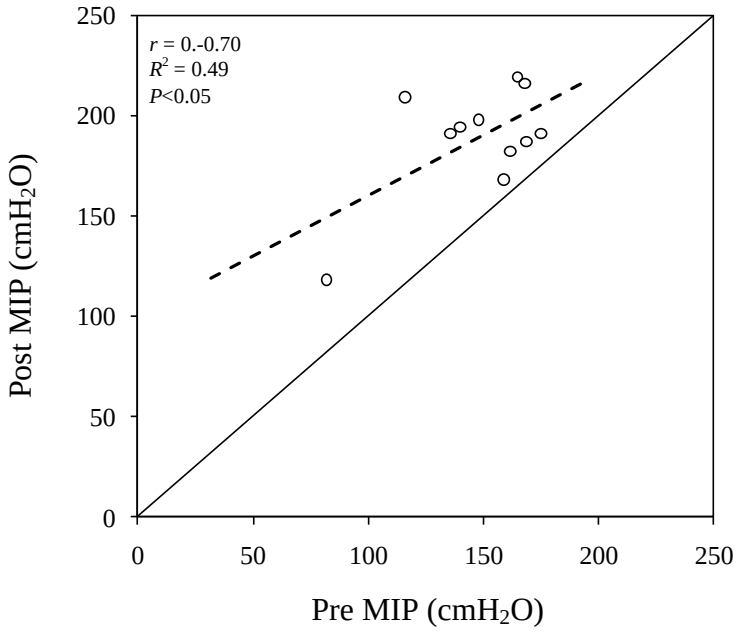

Figure 3.4 Identitiy plot of pre and post-IMT maximal inspiratory pressure (MIP).

3.4 DISCUSSION

The main finding of this study was that 10 min of volitional hyperpnoea approximately doubled resting $[lac^-]_B$, and that 6 wks of pressure-threshold IMT attenuated this increase by 25%. These findings strongly support the notion that the respiratory muscles are capable of increasing $[lac^-]_B$ and are the first to show that this can be attenuated through specific pressure threshold IMT. This observation may help to explain some of the IMT-mediated reductions in $[lac^-]_B$ previously observed during whole-body exercise.

Resting $[lac^-]_B$ was ~0.84 mmol·L^{-1} following 10 min of intense volitional hyperpnoea at 85% $\dot{V}_E$max (130.7 ± 19.7 L·min^{-1}, 77 ± 15% MVV_{10}; n = 22) increased by 0.96 ± 0.58 mmol·L^{-1} (range: 0.20 – 2.50 mmol·L^{-1}; n = 22) during. Comparable increases in $[lac^-]_B$ have been reported whilst breathing at similar (72% MVV, Martin et al. 1984;

70% MVV, Verges et al. 2007b), but not lower (62% MVV, Spengler et al. 2000), relative intensities. Therefore it is apparent that when $\dot{V}_E$ surpasses a certain level the respiratory muscles are capable of net lactate release. However, the potential for respiratory alkalosis to elevate $[lac^-]_B$ is well documented (Davies et al. 1986; LeBlanc et al. 2002). Consequently, we were careful to maintain, with considerable accuracy, resting PCO_2 throughout the 10 min of volitional hyperpnoea (see Figure 3.1). Other measures of acid base status also remained unchanged from rest during volitional hyperpnoea in both groups pre- and post-intervention. We are thus confident that the increase in $[lac^-]_B$ during volitional hyperpnoea was a consequence of increased lactate efflux from the respiratory muscles rather than respiratory alkalosis.

The attenuated increase in $[lac^-]_B$ during volitional hyperpnoea following IMT is similar to that observed in healthy subjects performing an exhaustive respiratory endurance test at ~70% MVV following VIH training, although this reduction did not exceed that of a control group (Verges et al. 2007b). Given the aforementioned importance of maintaining isocapnia it is also unfortunate that end-tidal CO_2 and/or PCO_2 was not controlled during the respiratory endurance test. Furthermore, subjects were prescribed a pre-determined arbitrary breathing pattern which has previously received criticism for failing to accurately represent the work of breathing during exercise hyperpnoea (Coast et al. 1993). Notwithstanding this, VIH- and IMT-mediated reductions in $[lac^-]_B$ observed during volitional hyperpnoea are similar to the reductions often observed during submaximal, whole-body exercise (Griffiths and McConnell 2007; Leddy et al. 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999; Volianitis et al. 2001); however, whether these observations during volitional hyperpnoea and exercise share a common mechanistic explanation is unclear.

RMT-mediated reductions in $[lac^-]_B$ at sustainable and submaximal exercise intensities occur (e.g. see Leddy et al. 2007; McConnell and Sharpe 2005; Spengler et al. 1999; Volianitis et al. 2001) when net lactate production from the respiratory muscles is

probably negligible given the relatively low $\dot{V}_E$ and minimal activation of the less efficient accessory muscles (Martin et al. 1984; Johnson et al. 2006). Hence, under such conditions it seems more likely that reductions in $[lac^-]_B$ result from increased uptake and metabolism of lactate by the trained respiratory muscles (Griffiths and McConnell 2007; Spengler et al. 1999) rather than a decrease in net lactate release. Conversely, during high-intensity exercise where $\dot{V}_E$ relative to MVV approaches/exceeds levels achieved in the breathing challenge of this study (e.g. see Edwards and Cooke 2004; Kohl et al. 1997; Spengler et al. 1999), it is possible that RMT-mediated respiratory muscle adaptation contributes to lowering $[lac^-]_B$ through affecting both lactate clearance by and efflux from the trained respiratory muscles.

The plasticity of the inspiratory muscles has been well documented (McConnell and Romer 2004a; Powers et al. 1997; Ramírez-Sarmiento et al. 2002). It is thus attractive to suggest that changes in inspiratory muscle morphology may explain, in part, the attenuated hyperpnoea-mediated increase in $[lac^-]_B$ following IMT. An approximate 10% increase in diaphragm thickness (Downey et al. 2007; Enright et al. 2006), and a 21% increase in the size of type II muscle fibres in the external intercostal muscles (Ramírez-Sarmiento et al. 2002), has been reported following 6 and 5 weeks of IMT, respectively. Increasing inspiratory muscle fibre cross-sectional area and subsequently strength decreases the relative intensity for a given absolute work load, which may reduce/delay fast twitch fibre recruitment and thus lactate production (Marcinik et al. 1991). A decrease in the relative workload per muscle fibre may also decrease blood flow occlusion, which may influence lactate production and/or clearance (Marcinik et al. 1991).

Increased muscle MCT protein content, which facilitates inter- and intra-cellular lactate shuttling in sarcolemmal and mitochondrial membranes, respectively (Brooks et al. 1999; Dubouchaud et al. 2000), has been reported following endurance (Baker et al. 1998; Burgomaster et al. 2007) and strength (Juel et al. 2004) based training regimens. It is thus possible (cf. McConnell and Sharpe 2005) that similar adaptations would occur in the

respiratory muscles following both IMT (strength-orientated) and VIH (endurance-orientated) training and may explain, in part, the decrease in $[lac^-]_B$ observed during whole-body exercise and volitional hyperpnoea following these dissimilar training stimuli.

Finally, the attenuated $[lac^-]_B$ response to volitional hyperpnoea following IMT (and VIH training) may also reside in a training-induced increase in the oxidative capacity of the inspiratory muscles. In support of this notion, Ramírez-Sarmiento et al. (2002) reported a 38% increase in the proportion of type I muscle fibres in the external intercostals following 5 weeks IMT. Moderate intensity, high repetition strength training, similar to the IMT protocol used in the present study, can increase oxidative enzyme activity (Costill et al. 1979; Sale et al. 1990) thereby reducing net lactate production (Holloszy and Coyle 1984). Since similar oxidative adaptations would be expected to occur following VIH (endurance-orientated) training (Holloszy and Coyle 1984), this also offers an attractive explanation for the decrease in $[lac^-]_B$ observed during whole body exercise (Griffiths and McConnell 2007; Kohl et al. 1997; Leddy et al. 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999; Volianitis et al. 2001) and volitional hyperpnoea (present study; Verges et al. 2007b).

3.5 CONCLUSIONS

In summary, the present study provides novel evidence that increases in $[lac^-]_B$ when mimicking the breathing pattern observed during heavy exercise can be attenuated following IMT. These data suggest that the inspiratory muscles were the source of at least part of this reduction, and provide a possible explanation for at least some of the IMT-mediated reductions in $[lac^-]_B$ previously observed during whole-body exercise. The precise mechanisms that underpin these changes remain unknown, but an IMT-mediated increase in the oxidative and/or lactate transport capacity of the inspiratory muscles is an attractive possibility that merits further investigation.

CHAPTER 4

INSPIRATORY MUSCLE TRAINING REDUCES BLOOD LACTATE DURING VOLITIONAL HYPERPNOEA PERFORMED DURING EXERCISE

4.1 INTRODUCTION

In Chapter 3 it was demonstrated that respiratory muscle work and training influenced the systemic $[lac^-]_B$. This surprising notion contradicts the traditional view that the small size (~0.5% total body mass) and large oxidative capacity of this muscle group precludes any systemically relevant lactate exchange (Wetter and Dempsey 2000). However, supporting evidence comes from three avenues of investigation. Firstly, studies investigating the effects of specific IMT have consistently reported large decreases in $[lac^-]_B$ during whole body exercise (see section 1.17.3). Furthermore, reductions in $[lac^-]_B$ and improved performance appear to be correlated (Romer et al. 2002b; Spengler et al. 1999) with up to 52% of the variation in performance accounted for by the reduced $[lac^-]_B$ (Romer et al. 2002b).

Secondly, and most recently Chiappa et al. (2008b) showed that increasing the work of breathing (via low intensity pressure threshold loading) during recovery from maximal exercise significantly accelerated the rate of lactate clearance. During recovery, $[lac^-]_B$ was up to 20% lower with loaded breathing than without. The authors explain their findings by suggesting that under these conditions the inspiratory muscles are capable of net lactate consumption.

Finally, a third line of evidence comes from investigations in which the work of breathing is increased via volitional hyperpnoea whilst at rest. Such interventions typically result in a twofold increase in resting $[lac^-]_B$ following approximately 10 min (Chapter 3; Johnson et al. 2006; Martin et al. 1984; Verges et al. 2007b). Presumably once a critical level of hyperpnoea is reached (suggested to be around 70% MVV) the respiratory muscles are capable of significant net lactate production. Intriguingly, specific RMT attenuates the hyperpnoea-mediated increase in $[lac^-]_B$ at rest (Chapter 3; Verges et al. 2007b), although such reductions do not occur during constant power high-intensity exercise at 85% $\dot{V}O_2max$ (Verges et al. 2007b). Indeed, it is possible that at such exercise intensities which surpass the lactate threshold, metabolic perturbations within the locomotor muscles

overshadow any changes which may be occurring due to an RMT-mediated increase in respiratory muscle plasticity.

When performing volitional hyperpnoea under resting conditions adjacent muscle fibres, inactive muscles and organs can engage in lactate exchange (Hashimoto and Brooks 2008), furthermore, when exercising at a high-intensity fixed work rate lactate exchange within the locomotor muscles may also mask genuine changes in respiratory muscle lactate kinetics. To circumvent this problem we developed a protocol in which isocapnic volitional hyperpnoea was imposed upon cycling exercise at the MLSS and previously observed a significant increase in $[lac^-]_B$ (~25%; Johnson et al. 2006). The MLSS represents the highest power output at which the rate of lactate efflux to and removal from the systemic circulation is equal and minimal capacity exists for other tissues and organs to influence $[lac^-]_B$. Therefore the observed increase in $[lac^-]_B$ was more fully attributable to the respiratory muscles. It has also been shown that IMT attenuates the lactate response to cycling exercise at the MLSS without modifying the absolute exercise intensity (McConnell and Sharpe 2005).

Therefore the primary aim of the current study was to extend the work of Johnson et al. (2006), McConnell and Sharpe (2005) and those in Chapter 3. We hypothesised that 6 wk pressure threshold IMT would attenuate steady state and the hyperpnoea-mediated increase in $[lac^-]_B$ when cycling at MLSS. It was anticipated that the findings would further reveal the extent to which training the respiratory muscles can affect their capacity for net lactate production and clearance.

4.2 METHODS

4.2.1 PARTICIPANTS

Following approval from Nottingham Trent University's ethics committee 20 healthy non-smoking males provided written informed consent to participate in the study (Table 4.1). Throughout the study subjects were instructed to adhere to their usual training

regimen and followed pre-exercise instructions outlined in section 2.1. Descriptive characteristics of the subjects are presented in Table 4.1. All exercise was performed in similar laboratory conditions (temperature: 17.5 ± 2.6˚C; relative humidity: 47.3 ± 9.7%).

Table 4.1. Descriptive characteristics of the subjects.

	Control group (n = 10)	IMT group (n = 10)
Age (years)	25.9 ± 4.8	36.7 ± 6.1 *
Body mass (kg)	77.8 ± 10.0	84.8 ± 13.9
Height (cm)	180.8 ± 8.4	179.4 ± 7.1
FVC (L)	5.79 ± 1.14 (107 ± 15)	5.41 ± 0.88 (108 ± 11)
FEV_1 (L)	4.47 ± 0.91 (99 ± 15)	4.13 ± 0.65 (100 ± 14)
FEV_1/FVC (%)	77.6 ± 7.1 (92 ± 7)	76.6 ± 6.0 (94 ± 8)
MVV_{10} ($L \cdot min^{-1}$)	187.5 ± 25.2 (100 ± 13)	205.8 ± 38.7 (119 ± 23)
MIP (cmH_2O)	149.3 ± 24.7 (115 ± 5)	163.5 ± 23.4 (104 ± 6)
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	3.82 ± 0.41	4.09 ± 0.66
$\dot{W}$max (W)	366 ± 37	393 ± 47
MLSS (W)	205 ± 29	210 ± 28

Values are expressed as means ± SD. *, P<0.05 between groups. Values in parentheses represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984).

4.2.2 EXPERIMENTAL PROCEDURE

Subjects were initially familiarised with testing procedures and completed pulmonary function and MIP tests. Subsequently, MLSS was estimated using the lactate minimum test and resolved using 30 min constant power tests. Subjects were then matched for 90% $\dot{V}_E$ max and MLSS and divided equally into an IMT or control group. Prior to and following a 6 week intervention (IMT or no IMT) subjects completed in random order a 30 min reference trial at MLSS, and a 30 min experimental trial at MLSS during which

from 20-28 min $\dot{V}_E$ and breathing pattern were volitionally matched to that commensurate with 90% $\dot{V}_E$ max. All exercise trials were performed using an electromagnetically-braked cycle ergometer (see section 2.2).

4.2.3 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY MOUTH PRESSURE

Pulmonary function was assessed using a pneumotachograph and a hand-held mouth pressure meter measured MIP as an index of global inspiratory muscle strength according to sections 2.3 and 2.5, respectively. MIP was reassessed following 2, 4 and 6 wk of the intervention using the same protocol in both groups.

4.2.4 DETERMINATION OF MLSS

MLSS cycling power output was predicted and resolved using the protocols described in section 2.11. Following the intervention MLSS was re-assessed, starting at the pre-intervention MLSS power, using the same criteria.

4.2.5 REFERENCE TRIAL (WITHOUT VOLITIONAL HYPERPNOEA)

Following a 3 min warm-up at 50% MLSS power, subjects cycled for 30 min at MLSS. $[lac^-]_B$ was determined at rest and every 2 min during exercise, whereas blood gases were determined every 4 min from 0-20 min and every 2 min thereafter. Breath by breath respiratory variables were averaged over the final 30 s of every 2 min interval. Heart rate (HR) was measured using short range telemetry (Polar S610, Polar, Kempele, Finland) and arterial oxygen saturation (SpO_2) was estimated using infrared pulse oximetry (Model 8600, Nonin, Minnesota, USA). Ratings of perceived exertion (RPE) and dyspnoea (RPD) were recorded every 2 min using the Borg 6-20 and modified Borg 1-10 scales, respectively (Borg et al. 1982). Pedal cadence was constant throughout.

4.2.6 EXPERIMENTAL TRIAL (WITH VOLITIONAL HYPERPNOEA)

The experimental trial was identical to the reference trial except that from 20-28 min, subjects volitionally increased $\dot{V}_E$ to 90% of the $\dot{V}_E$ max which was measured during the maximal incremental phase (Phase I) of the lactate minimum protocol (see section 2.11). Pilot work showed that the breathing pattern equal to 90% $\dot{V}_E$ max was the highest value that could be maintained for 8 minutes in a square wave fashion. During volitional hyperpnoea tidal volume (V_T), breathing frequency (f_R) and duty cycle (T_I/T_{tot}) were matched to that used to achieve the target $\dot{V}_E$ during maximal exercise. An audio metronome paced f_R and real-time visual feedback of $\dot{V}_E$ and V_T were provided. Following volitional hyperpnoea, subjects returned to a spontaneous breathing pattern for the final 2 minutes of exercise. According to the methodological recommendations reported in section 2.12, pedal cadence was rigorously controlled and matched to that achieved during min 2 to 20 throughout the period of volitional hyperpnoea (see Table 4.2).

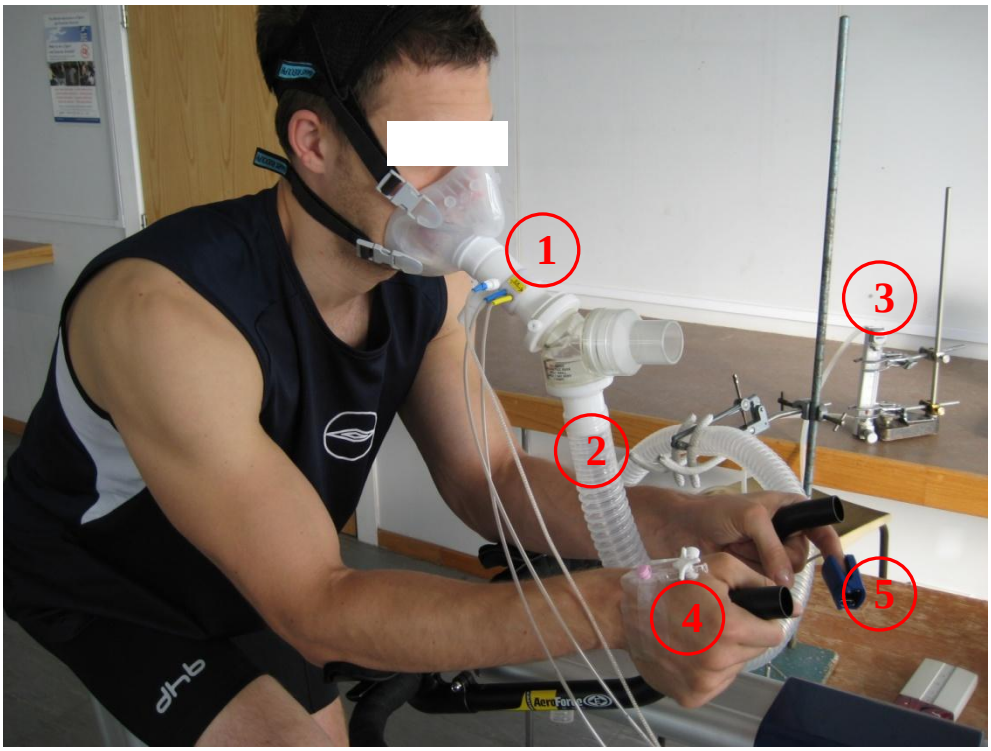

Figure 4.1. Participant set-up for experimental exercise trials requiring isocapnic volitional hyperpnoea. 1) pneumotachograph; 2) 1.5 m length of corrugate tubing attached via the inspiratory port of two-way non-rebreathing valve to the pneumotachograph; 3) flow meter controlling the flow of CO_2 into the inspiratory circuit; 4) cannula inserted into a dorsal hand vein and 5) finger pulse oximeter.

4.2.7 INTERVENTION

IMT was performed using an inspiratory pressure-threshold device as described in section 2.10. Subjects completed a training diary to record IMT adherence and habitual training, which the control group also recorded. The control group continued with their habitual physical training schedule and were not exposed to an intervention. A placebo treatment was not applied to the control group since the study outcome measures could not be influenced by either motivation or expectation. Subjects were informed that they belonged to a control group prior to commencement of the study and to avoid any possible disadvantage were afforded the opportunity to undertake 6 weeks of inspiratory muscle training after completion of the study.

4.2.8 BLOOD SAMPLING AND RESPIRATORY MEASUREMENTS

Arterialised venous blood was sampled from a dorsal hand vein via an indwelling cannula (Figure 4.1, number 4) and analysed immediately for PCO_2, pH, and $[lac^-]_B$ as outlined in section 2.9. Plasma $[HCO_3^-]$ and BE_{ECF} were calculated as described in section 2.8. Throughout experimental trials, pulmonary ventilation and gas exchange were measured breath by breath (see section 2.6). During volitional hyperpnoea tests, a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) and a 1.5m length of corrugated tubing was attached distally to the pneumotachograph (Figure 4.1 numbers 1 and 2) allowing additional CO_2 to be added to the inspirate in order to maintain blood PCO_2 at levels commensurate with steady state exercise.

4.2.9 PULMONARY $\dot{V}O_2$ KINETICS

The effects of IMT upon the pulmonary $\dot{V}O_2$ exercise onset response to constant power heavy exercise was characterised by fitting a single monoexponential model to the exercise $\dot{V}O_2$ data (0 to 20 min only). Breath by breath data were linearly interpolated to provide second by second values following the elimination of outlying breaths (defined as

those ± 4 SD of the previous 5 breaths; Lamarra et al. 1987). For each subject, prior to and following the intervention, two square wave transitions from 50% MLSS to the MLSS power (i.e. the reference and experimental trials) were time aligned and averaged to improve the signal to noise ratio and thereby the underlying features of the $\dot{V}O_2$ response. This provided one set of second by second data for pre- and post-intervention comparisons. The amplitude and time delay of the primary (phase II) $\dot{V}O_2$ response was then modelled using iterative non-linear regression with a 20 s time delay to exclude the cardiodynamic component (phase I):

$$\dot{V}O_2(t) = \dot{V}O_{2,b} + A(1 - e^{-(t-TD)/\tau})$$

where $\dot{V}O_{2,b}$ is the baseline $\dot{V}O_2$ measured during the final minute of the warm-up preceding the onset of exercise; *A* is the amplitude of the exponential curve and defined as the increase in $\dot{V}O_2$ from $\dot{V}O_{2,b}$ to the end of phase II and τ and TD are the time constant and the time delay of the response, respectively. The amplitude of the $\dot{V}O_2$ slow component (phase III) was defined as the difference in $\dot{V}O_2$ between TD (end of phase II) and the average $\dot{V}O_2$ from 19.5 to 20 min. The parameters of the exponential model were calculated using commercially available software (GraphPad Prism Version 5.01, GraphPad software, Inc. CITY, USA).

4.2.10 BLOOD LACTATE KINETICS

The effects of IMT upon the lactate response to constant power heavy exercise was characterised by iterative least mean squares non-linear regression. For each subject, the reference and experimental trials (from 0 to 20 min only; i.e. excluding volitional hyperpnoea) were time aligned and averaged. This provided one set of $[lac^-]_B$ against time data per-subject for pre- and post-intervention comparisons. Data were fitted to the following exponential time function:

$$La(t) = La0 + A\left(1 - e^{\tau \cdot t}\right)$$

Where La(t) (mmol·L^{-1}) is the $[lac^-]_B$ for a given time (t; min); La(0) (mmol·L^{-1}) is the $[lac^-]_B$ at the onset of exercise; A and τ are the amplitude of the exponential curve defined as the increase in $[lac^-]_B$ from La(0) to steady state (where $\Delta[lac^-]_B/\Delta t = 0$) and the time constant of the response, respectively. The parameters of the exponential curve were calculated using commercially available software (GraphPad Prism Version 5.01, GraphPad software, Inc. CITY, USA).

4.2.11 STATISTICAL ANALYSES

Statistical analyses were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). In order to compare the discrete physiological responses between steady state exercise and volitional hyperpnoea, the reference and the experimental trials were averaged and analysed over two time periods: a steady state period from 12 to 20 min (as it takes approximately 12 min to reach a steady state in $[lac^-]_B$) and a volitional hyperpnoea period from 22 to 28 min. When physiological responses to the reference trial only were analysed and compared, data were averaged from 12 to 30 min. This method of analyses is similar to that used previously when volitional hyperpnoea was imposed upon exercise at the MLSS (Johnson et al. 2006). Pre- and post-intervention results and group interactions were assessed using one-way or two-way ANOVA for repeated measures and Tukey's HSD post-hoc analysis. Interactions were defined for "group" (IMT vs. Control), "trial"

(reference vs. experimental) and "time" (min 12 to 20 [steady state period] or min 22 to 28 [volitional hyperpnoea period]). Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

4.3 RESULTS

4.3.1 PULMONARY FUNCTION, MAXIMAL INSPIRATORY PRESSURE AND MLSS

Baseline pulmonary function and MIP were all within normal limits (Table 4.1). Inspiratory muscle training compliance was excellent in the IMT group (96 ± 4%) and inspection of training diaries revealed habitual training remained constant in both groups. MIP was unchanged following the intervention period in the control group (pre vs. post: 149 ± 25 vs. 147 ± 27 cmH_2O). In contrast, MIP increased from 164 ± 23 cmH_2O at baseline to 174 ± 24, 187 ± 23 and 194 ± 21 cmH_2O (+19 %) following 2, 4 and 6 weeks of IMT, respectively ($P<0.01$). MLSS power was unchanged following the intervention period in both groups with the exception of one subject in the control group in whom MLSS power fell by 2.5% (7 W).

4.3.2 RESPIRATORY RESPONSES

In the IMT (see Figure 4.2 and Table 4.2) and control groups, volitional hyperpnoea represented 90.3 ± 9.6% $\dot{V}_E$ max (76 ± 19% MVV) and 91.2 ± 4.9% $\dot{V}_E$ max (81 ± 15% MVV), respectively. $\dot{V}_E$ and breathing pattern during both the reference and experimental trials were not different between groups before or after the intervention period. Therefore the breathing challenge was repeated with considerable accuracy by both groups after the 6 wk intervention period.

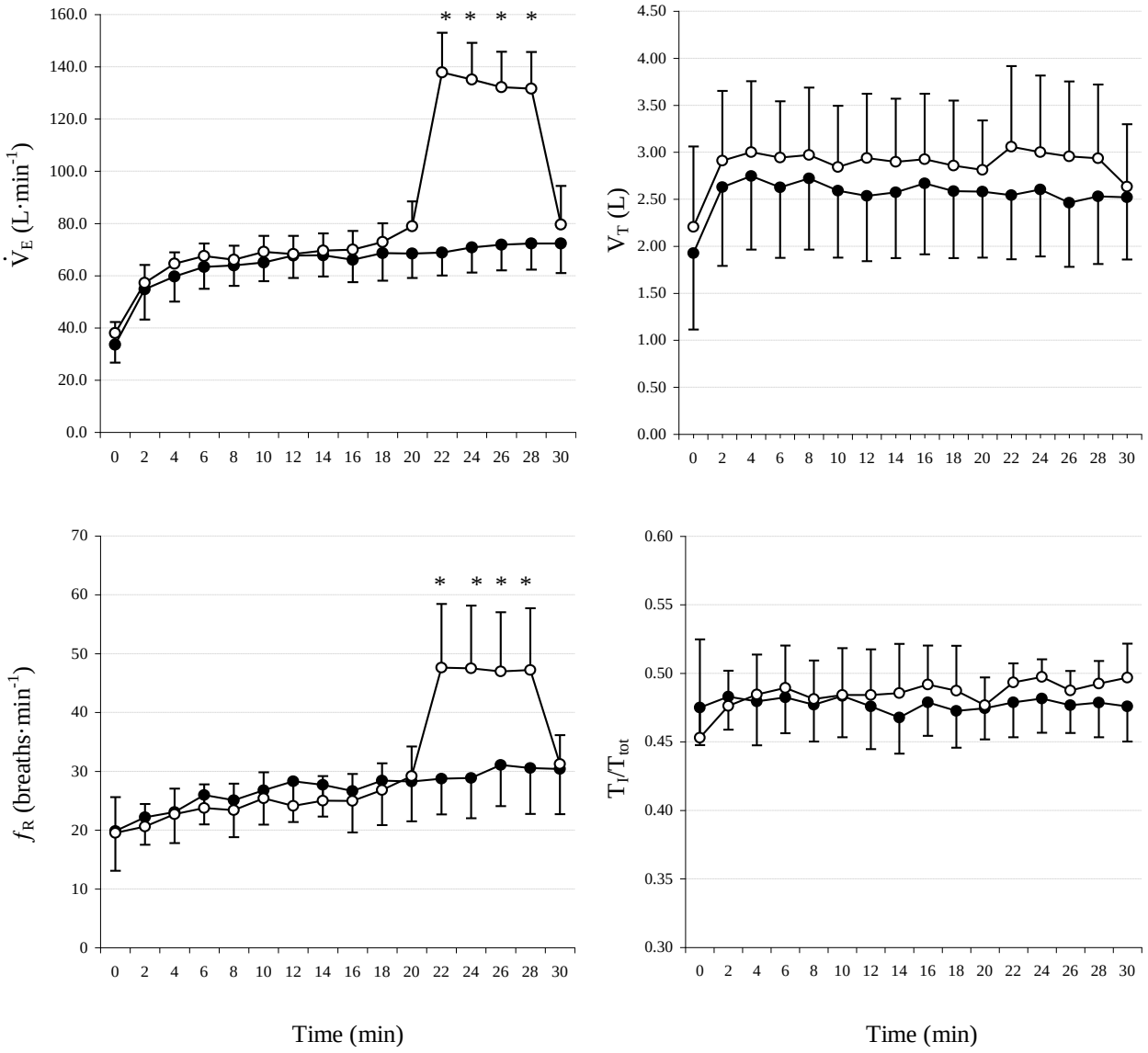

Figure 4.2. Pre-intervention breathing pattern during the reference trial (●) and experimental trial (○) for the IMT group only (n = 10). Data from the IMT group following the intervention and the control group both prior to and following the intervention has been omitted since it was not different to the data illustrated within the figure. Volitional hyperpnoea was imposed from 20 to 28 min. Time 0 reflects the end of a 3 min warm-up at 50% MLSS power. *, P<0.05, hyperpnoea period significantly different from steady state period (12 to 20 min) both prior to and following the intervention.

Following IMT, SpO_2 was slightly lower (-0.6 ± 0.8%; P<0.05) from 22 to 28 min relative to the pre-intervention reference trial. However, $\dot{V}O_2$, $\dot{V}CO_2$ and RER throughout the reference trial and $\dot{V}O_2$ and $\dot{V}CO_2$ throughout the experimental trial were not different between groups and were unchanged following the intervention (Table 4.2).

Table 4.2. Physiological responses to 30 min cycling exercise during the reference and experimental trials prior to and following IMT. Data from the control group both prior to and following the intervention has been omitted since it was not different to pre-IMT.

IMT Group	12 – 20 min	22 – 28 min			
	Pre-IMT	Pre-IMT		Post-IMT	
	Reference trial	Reference trial	Experimental trial (hyperpnoea)	Reference trial	Experimental trial (hyperpnoea)
$[H^+]$ ($nmol \cdot L^{-1}$)	44.2 ± 3.8	43.4 ± 3.1*	46.1 ± 2.8*	42.0 ± 2.8*	45.5 ± 5.5*
$[HCO_3^-]$ ($mmol \cdot L^{-1}$)	23.2 ± 2.7	23.1 ± 2.7	22.8 ± 3.0*	23.4 ± 2.4	23.5 ± 2.8§
BE_{ECF} ($mEq \cdot L^{-1}$)	-1.75 ± 2.87	-1.74 ± 2.91	-2.33 ± 3.25*	-1.28 ± 2.43§	-1.60 ± 3.23§
PCO_2 (mmHg)	42.6 ± 3.1	41.6 ± 3.0*	43.6 ± 3.6	40.9 ± 3.3*	44.2 ± 3.4*
SpO_2 (%)	95.3 ± 1.2	95.3 ± 1.0	95.8 ± 1.2	94.7 ± 1.2†	95.9 ± 1.3
$\dot{V}_E$ ($L \cdot min^{-1}$)	67.3 ± 8.7	71.0 ± 9.4	134.2 ± 13.8*	75.8 ± 9.6	131.1 ± 14.8*
$\dot{V}O_2$ ($L \cdot min^{-1}$)	2.55 ± 0.39	2.55 ± 0.42	3.28 ± 0.43*	2.81 ± 0.37	3.25 ± 0.47*
$\dot{V}CO_2$ ($L \cdot min^{-1}$)	2.46 ± 0.41	2.54 ± 0.43	-	2.78 ± 0.36	-
RER	0.98 ± 0.02	0.99 ± 0.03	-	0.96 ± 0.04	-
HR ($beats \cdot min^{-1}$)	144.6 ± 11.0	152.4 ± 13.6	160.0 ± 14.5	152.7 ± 12.0	156.6 ± 13.9§
RPE	12.1 ± 0.7	12.8 ± 0.9*	13.1 ± 1.4	12.5 ± 2.1*	12.0 ± 1.0
RPD	2.9 ± 0.6	3.1 ± 0.9	5.6 ± 1.5*	3.1 ± 0.6*	4.0 ± 1.4*†§
Cadence (r.p.m.)	91 ± 8	91 ± 8	92 ± 9	91 ± 7	90 ± 9

* $P<0.01$ vs. 12-20 min; † $P<0.05$ vs. pre; § $P<0.05$ trial × time interaction. $\dot{V}CO_2$ and RER data not shown from 22 to 28 min of experimental trial due to technical limitations.

The parameters of the pulmonary $\dot{V}O_2$ exercise onset response to constant power heavy exercise are shown in Table 4.3 and displayed graphically for the IMT group in Figure 4.3. With the exception of τ which was reduced following IMT from 45.2 ± 13.8 s to 31.3 ± 18.4 s ($P<0.05$), no parameter of the phase II or III $\dot{V}O_2$ kinetics were modified following the intervention in both groups.

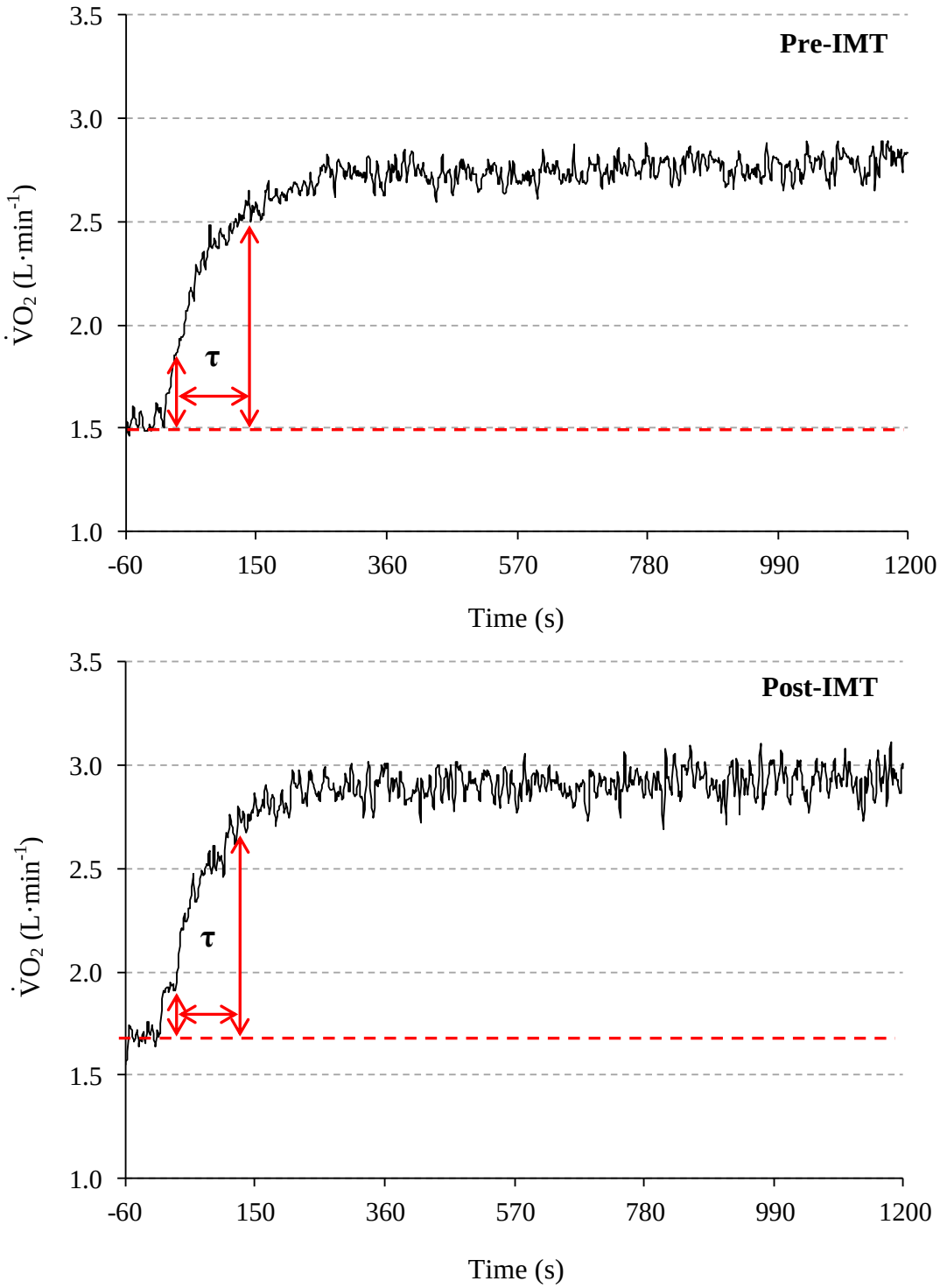

Figure 4.3. Pulmonary $\dot{V}O_2$ exercise onset response to constant power heavy exercise prior to (Upper panel) and following (lower panel) IMT. Note data are displayed as 1 s interpolated average. Time: -60 to 0 reflects a 60 s warm-up at 50% MLSS power output. Power output was increased in a square wave manner to MLSS power at time 0. Dashed red horizontal line, baseline $\dot{V}O_2$ ($\dot{V}O_{2,b}$).

Table 4.3. Pulmonary oxygen uptake kinetics.

	Control		IMT	
	Pre	Post	Pre	Post
Phase II				
TD (s)	15.9 ± 4.8	13.0 ± 10.5	15.7 ± 4.2	16.3 ± 4.4
A ($L \cdot min^{-1}$)	0.94 ± 0.36	1.02 ± 0.19	0.91 ± 0.26	0.86 ± 0.25
τ (s)	56.1 ± 61.0	51.6 ± 18.6	45.2 ± 13.8	31.3 ± 18.4 *
Phase III				
A ($L \cdot min^{-1}$)	1.89 ± 0.32	1.81 ± 0.34	1.83 ± 0.31	1.94 ± 0.30

A, amplitude; τ and TD are the time constant and the time delay of the response, respectively. *Phase III* A, difference in $\dot{V}O_2$ between TD (end of phase II) and the average $\dot{V}O_2$ from 19.5 to 20 min. * $P<0.05$ pre vs. post-intervention: IMT group only.

4.3.3 BLOOD LACTATE RESPONSES TO EXERCISE AND VOLITIONAL HYPERPNOEA

The blood lactate response to the reference and the experimental trials for the IMT group only are shown in Figure 4.4. $[lac^-]_B$ remained unchanged between minutes 12 to 30 during the reference trial in both groups, before and after the intervention; this reflects the successful determination of MLSS in all subjects. Pre-intervention mean $[lac^-]_B$ over 12 to 30 min of the reference trial was 3.74 ± 1.83 $mmol \cdot L^{-1}$ and 3.94 ± 1.57 $mmol \cdot L^{-1}$ in the IMT and Control groups respectively. Prior to the intervention volitional hyperpnoea increased $[lac^-]_B$ with the greatest increases occurring at min 30. Relative to the mean of 12-20 min $[lac^-]_B$ was increased at min 30 by 0.88 ± 0.72 (25 %) and 0.96 ± 0.58 $mmol \cdot L^{-1}$ (27 %) in the control and IMT groups respectively.

In the control group $[lac^-]_B$ during the reference trial and throughout the experimental trial (both 12 to 20 min and 22 to 28 min) were unchanged following the intervention. Conversely, following IMT $[lac^-]_B$ was reduced during the reference trial by 0.65 ± 1.68 $mmol \cdot L^{-1}$ (-8 %) from minutes 12 to 30. A main effect for 'trial' ($P<0.05$) was

observed, although pairwise comparisons revealed no significant differences at individual time points. During the experimental trial $[lac^-]_B$ was also reduced by 0.94 ± 1.77 mmol·L^{-1} (-15 %) from 12-20 min (main effect 'trial', P<0.05) although once more, individual time points were not statistically different. Interestingly, the time constant and the amplitude of the increase in $[lac^-]_B$ from the start of exercise to the steady-state ($\Delta[lac^-]_B / \Delta t = 0$) was significantly reduced by 26 and 17%, respectively (Table 4.4). During volitional hyperpnoea, i.e. 22 to 28 min of the experimental trial, the increase in $[lac^-]_B$ observed prior to the intervention was abolished. At minute 30, relative to pre-intervention, $[lac^-]_B$ was reduced by 1.84 ± 1.28 mmol·L^{-1} (-26 %; group × trial × time interaction; P<0.01; Figure 4.4).

Table 4.4. Blood lactate kinetics at the onset of exercise.

	Control		IMT	
	Pre	Post	Pre	Post
La(0) (mmol·L^{-1})	0.95 ± 0.32	0.86 ± 0.25	0.98 ± 0.10	0.94 ± 0.22
A (mmol·L^{-1})	3.13 ± 1.65	3.66 ± 1.83*	3.07 ± 2.16	2.18 ± 1.33*
τ (s)	3.84 ± 1.11	3.89 ± 1.20	4.09 ± 1.37	2.91 ± 1.31*
$\Delta[lac^-]_B / \Delta t = 0$ (mmol·L^{-1})	4.08 ± 1.75	4.52 ± 1.88*	3.07 ± 2.16	2.18 ± 1.33

* P<0.05 vs. pre intervention.

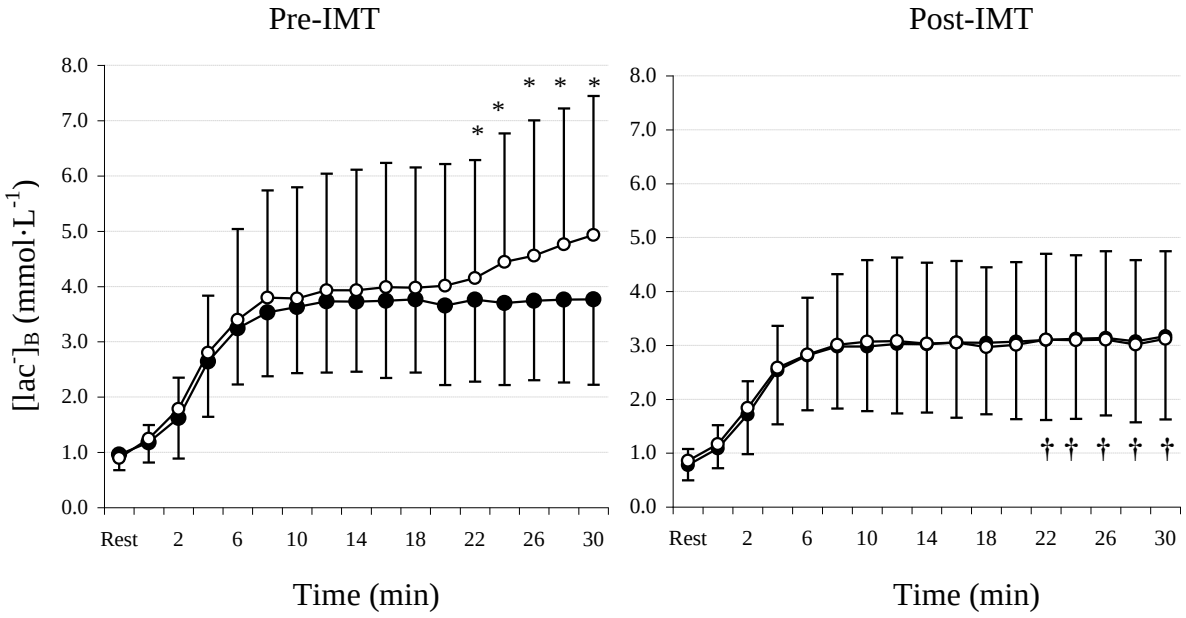

Figure 4.4. Blood lactate concentration ($[lac^-]_B$) during 30 min exercise at MLSS in the reference trial (●) and experimental trial (○) for the IMT group only; pre and post intervention data in the control group were not different from IMT-Pre. Time 0 represents the end of a 3 min warm-up at 50% MLSS power. Volitional hyperpnoea was imposed from min 22 to 28. *, $P<0.05$, experimental trial greater than reference trial; †, $P<0.05$, experimental trial post-IMT lower than experimental trial pre-IMT.

4.3.4 ACID-BASE BALANCE RESPONSES

Whilst cycling at MLSS, $[H^+]$ increased over time: prior to the intervention the mean $[H^+]$ between 22-28 min was higher than that between 12-20 min in the reference and experimental trials in both groups (Table 4.2). Relative to the pre-intervention reference trial, $[H^+]$ was lower from 22 to 28 min during the post-IMT reference trial, but this just failed to reach significance ($P=0.07$). Pre- and post-intervention, volitional hyperpnoea (22 to 28 min of the experimental trial) did not change $[H^+]$ relative to the same time period (22 to 28 min) of the reference trial in either group.

$[HCO_3^-]$ and BE_{ECF} remained constant over time during the pre-intervention reference trial, in both groups. However, relative to 22 to 28 min of the reference trial, in both groups pre-intervention, volitional hyperpnoea caused a reduction in $[HCO_3^-]$ and BE_{ECF}. Post intervention these responses were unchanged in the control group. Following IMT relative to the pre-intervention reference trial, from 22 to 28 min, $[HCO_3^-]$ was unchanged, but BE_{ECF} was higher (trial × time interaction; $P<0.05$). Relative to the pre-

intervention experimental trial, the reductions in [HCO_3^-] and BE_{ECF} observed during volitional hyperpnoea were both attenuated (trial × time interaction; $P<0.05$).

PCO_2 was similar between groups and between trials prior to and following the intervention (Table 4.2). PCO_2 during volitional hyperpnoea and over the equivalent time period of the reference trial were not different, thus isocapnia was maintained successfully.

4.3.5 HEART RATE AND PERCEPTUAL RESPONSES

In both groups there were no differences in HR and RPD between groups during the reference trial either prior to or following the intervention. Prior to the intervention there were also no differences in HR and RPD between groups during min 12 to 20 of the experimental trial. During volitional hyperpnoea (22 to 28 min) in the experimental trial, HR ($P<0.01$) and RPD ($P<0.01$) increased in both groups prior to the intervention and were similar in the control group following the intervention. However, following IMT, HR and RPD during volitional hyperpnoea were both lower than pre-intervention values (trial × time interaction; $P<0.05$, Table 4.2). Following IMT, RPE was lower during 12-20 min of the experimental trial (pre: 13 ± 1 vs. post: 12 ± 1, $P<0.05$) and during 20-28 min of the experimental trial (pre: 13 ± 1 vs. post: 12 ± 1; $P = 0.07$). There were no such trends observed during the reference trial for the IMT group or either trial in the control group.

4.3.6 CORRELATIONS

Pre-intervention increases in $[lac^-]_B$ during volitional hyperpnoea were correlated with the target $\dot{V}_E$ relative to MVV ($r = 0.68$, $P<0.05$). Baseline MIP was negatively correlated with relative IMT-mediated increases in MIP ($r = -0.79$, $P<0.05$). Both absolute ($r = 0.75$, $P<0.05$) and relative ($r = 0.66$, $P<0.05$) increases in MIP following IMT were correlated with the absolute reduction in $[lac^-]_B$ observed at min 30 of the experimental trial. All relationships are displayed in Figure 4.5.

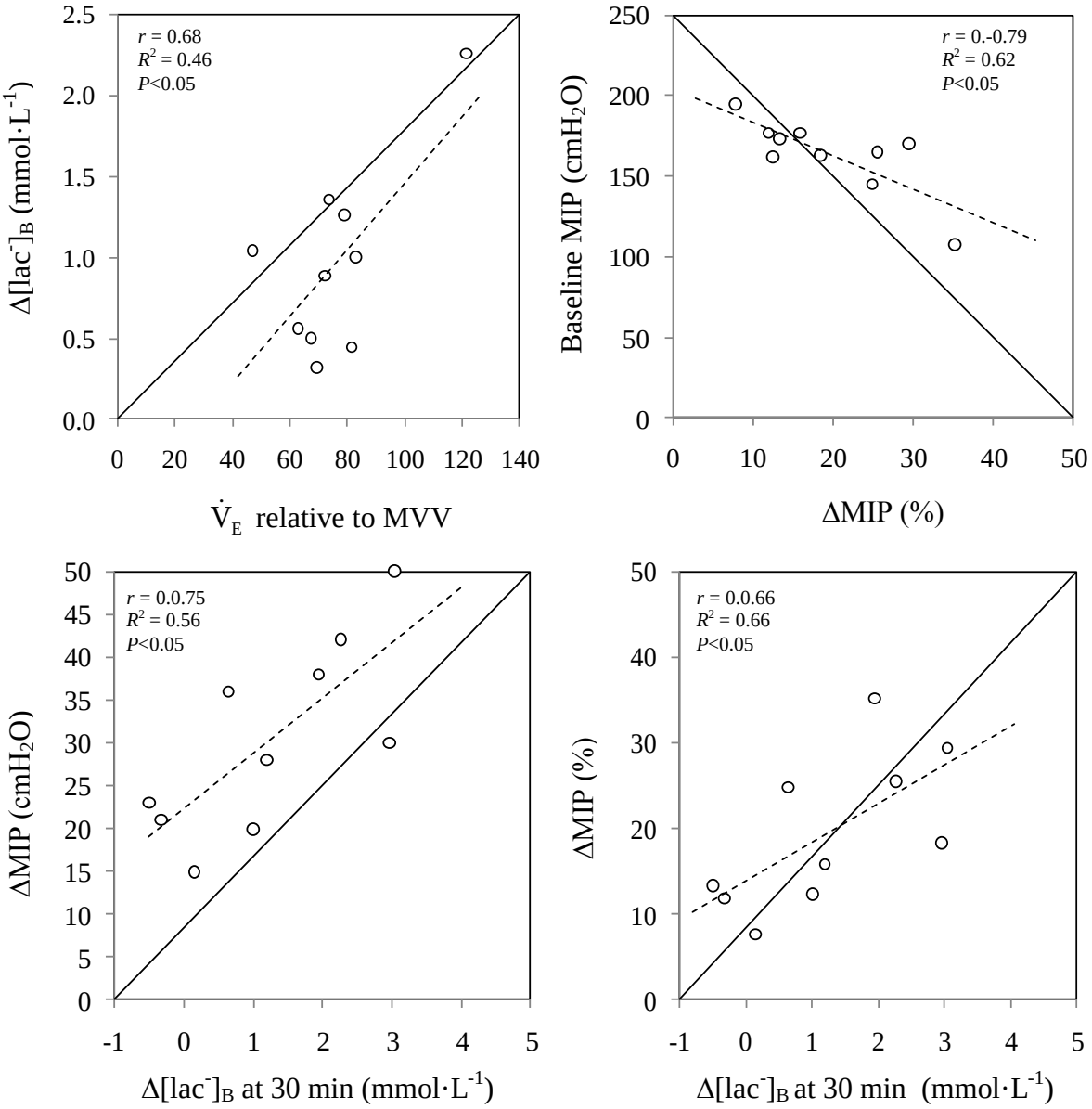

Figure 4.5. Relationship between the absolute reduction in $[lac^-]_B$ and the relative demand of volitional hyperpnoea (Top left), baseline MIP and the relative IMT-mediated increase in MIP (Top right) and finally the absolute (Bottom left) and relative (Bottom right) IMT-induced change in MIP and the reduction in lac at 30 min.

4.4 DISCUSSION

The primary finding of this study was that 6 wk IMT reduced the steady state $[lac^-]_B$ and abolished the increase in $[lac^-]_B$ observed when volitional hyperpnoea was superimposed upon cycling exercise at MLSS. We also show that IMT reduced dyspnoea and HR during volitional hyperpnoea.

The observation that hyperpnoea elevates $[lac^-]_B$ is consistent with the findings of previous studies performed during exercise at the MLSS (Johnson et al. 2006) and those presented in Chapter 3. The approximate 25% increase in $[lac^-]_B$ caused by the relatively

modest (~60 $L \cdot min^{-1}$) increase in $\dot{V}_E$ in this study indicates that the respiratory muscles contribute significantly to systemic lactate evolution. However other studies (Babcock et al. 1995), using much larger increases in $\dot{V}_E$ failed to observe a change in $[lac^-]_B$. This may be because their challenge was performed at rest where, unlike at MLSS, there is spare capacity for lactate clearance by other tissues. An alternative explanation may lie in the nature of the breathing challenge. Similar to Babcock et al. (1995) we mimicked exercise f_R, V_T and $\dot{V}_E$. However, we did not control the work of breathing associated with exercise, i.e. the pressure-volume characteristics per breath. The work of breathing (particularly that associated with expiration) during voluntary hyperpnoea exceeds that of an identical $\dot{V}_E$ during exercise (Klas and Dempsey 1989). Therefore we recognise that the increase in net respiratory muscle lactate production in this study probably exceeds that likely to be observed during exercise. Nonetheless this study's primary finding that the hyperpnoea-mediated increase in $[lac^-]_B$ was completely abolished following inspiratory muscle training is a stark illustration of this muscle groups plasticity and capacity to affect systemic blood chemistry.

Our findings suggest that IMT causes an upward shift of the critical ventilation rate at which net respiratory muscle lactate production occurs (around 70% MVV in the untrained state; Chapter 3; Martin et al. 1984) as following IMT our breathing challenge appears to fall below this critical level. The IMT-induced adaptations that may explain this deserve further elucidation. Hypertrophy of the diaphragm (Downey et al. 2007; Enright et al. 2006) and external intercostals (Ramírez-Sarmiento et al. 2002) may decrease the relative intensity for a given absolute $\dot{V}_E$, thus reducing/delaying the recruitment of less efficient muscle fibres and thus lactate production (Marcinik et al. 1991). The shift in the critical ventilation rate may also be explained by an increased prevalence of inspiratory muscle type I fibres (Ramírez-Sarmiento et al. 2002), and/or increased muscle mitochondria mass (Juel et al. 2004) and oxidative enzyme activity (Costill et al. 1979).

That a primarily strength-based training intervention would cause such alterations in a relatively short (6 wk) time frame seems unlikely at first sight. However, it has been argued that the inspiratory muscles are unusually plastic and that pressure threshold IMT improves the functional capacity of both the pressure (strength) and flow (velocity of shortening) axes of the inspiratory muscle pressure-flow (force-velocity) relationship (McConnell and Romer 2004a).

The IMT-induced decrease in $[lac^-]_B$ whilst cycling at MLSS is similar to that reported by McConnell and Sharpe (2005). We also show that a steady state in $[lac^-]_B$ (defined as $\Delta[lac^-]_B/\Delta t = 0$) was reached more quickly and the time constant of the primary (phase II) $\dot{V}O_2$ response to the onset of exercise at MLSS was reduced after IMT. Notwithstanding these changes and similar to previous work (Johnson et al. 2007; McConnell and Sharpe 2005) we found that MLSS power remained unchanged. Therefore it appears that IMT reduces some of the transient physiological flux associated with mitochondrial inertia (Xu and Rhodes 1999) at the commencement of constant power exercise thereby "re-setting" the $[lac^-]_B$ associated with MLSS. Given the small mass of the respiratory muscles relative to the locomotor muscles this explanation may seem counterintuitive. However, recently published work (Chiappa et al. 2008b) indicates that the respiratory muscles may be large consumers of lactate: the addition of a relatively light inspiratory resistance resulted in accelerated lactate clearance during recovery from maximal exercise. The authors suggest that the rich perfusion and high oxidative capacity of the inspiratory muscles promotes net lactate clearance when respiratory muscle perfusion is increased. Reduced $[lac^-]_B$ during whole-body exercise following IMT could thus be explained by increased lactate clearance by inspiratory muscles secondary to increased expression of sarcolemmal and mitochondrial membrane-bound monocarboxylate transporters (MCT; Juel et al. 2004) and/or increases in oxidative enzyme activity (Costill et al. 1979). Although the marked reductions in $[lac^-]_B$ following IMT seem surprising given the relatively small muscle mass of the inspiratory muscles,

their metabolic capacity is high due to an impressive capillary supply and high aerobic oxidative enzyme activity (Polla et al. 2004).

The 8 min period of volitional hyperpnoea was sufficient to cause a (small) increase in $[H^+]$ and decrease in $[HCO_3^-]$ relative to the same time point of the reference trial; we also observed a significant reduction in BE_{ECF} and increase in $[lac^-]_B$. Collectively these findings demonstrate that mimicking the breathing pattern associated with intense exercise promotes a metabolic acidosis (Johnson et al. 2006). Following IMT, during volitional hyperpnoea $[H^+]$ remained unchanged relative to pre-intervention, yet the reductions in $[HCO_3^-]$ and BE_{ECF} were attenuated; we also observed a significant reduction in $[lac^-]_B$ (see Table 4.2 and Figure 4.4). Thus, despite a similar $[H^+]$ during volitional hyperpnoea pre- and post-intervention, we interpret these data as an IMT-mediated reduction in metabolic acidosis. We explain these conclusions according to the integrated physicochemical systems approach of acid-base balance (Stewart 1983). Using this method, the plasma $[H^+]$ is determined by the strong ion difference ($[SID] = [Na^+] + [K^+] - [Cl^-] + [lac^-]$; see section 1.18.1), PCO_2 and the total concentration of weak acids ($[A_{tot}^-]$). Following IMT, the significant reduction in $[lac^-]_B$ would conceivably increase the [SID] and (assuming negligible changes in $[A_{tot}^-]$) increase $[H^+]$. However, that $[H^+]$ was unchanged during volitional hyperpnoea following IMT is probably accounted for by the small ~3 mmHg increase in PCO_2 which according to this model would counteract the increase in [SID]. The attenuated metabolic acidosis is further supported by the greater BE_{ECF} during volitional hyperpnoea following IMT. Under conditions of metabolic acidosis, this parameter describes the amount of alkali required to return the plasma to a normal pH (7.4) with a constant PCO_2 (40 mmHg; Siggaard-Anderson and Fogh-Anderson 1995) and provides very similar observations to the physicochemical approach (Stewart 1983). Thus based on the attenuated reduction in BE_{ECF} and the likely effects of $[lac^-]_B$ on the [SID], it would appear that following IMT, the hyperpnoea-mediated metabolic acidosis was attenuated. This may have also been the case during the reference trial, since

following IMT from 22 to 28 min $[H^+]$ was lower (P=0.07), $[lac^-]_B$ was significantly lower and the reduction in BE_{ECF} was attenuated (see Table 4.2). However, since we did not quantify changes in acid-base disturbance using the physicochemical approach, the possible effects of IMT upon the mechanisms accounting for changes in plasma acid-base balance remains to be confirmed.

Following IMT dyspnoea was reduced during hyperpnoea by around 30%. IMT-induced reductions in dyspnoea have been reported previously during whole body exercise (Romer et al. 2002a, Volianitis et al. 2001) and during breathing challenges following respiratory muscle training (Verges et al. 2007b). Whilst the target $\dot{V}_E$ and breathing pattern were identical pre- and post- intervention the relative load was presumably lower due to the training-induced conditioning of the inspiratory muscles. Therefore dyspnoea may have fallen due to a reduced central corollary discharge for a given absolute $\dot{V}_E$ (Kellerman et al. 2000; McConnell and Romer 2004b; Redline et al 1991). It is also possible that repeated training bouts may desensitise mechanosensitive type III and chemosensitive type IV afferents (Revelette and Wiley 1987; Sinoway et al. 1993, 1996). Thus, afferent feedback originating in the inspiratory muscles may have been attenuated thereby reducing the sensations of respiratory effort.

We also observed a reduction in HR during hyperpnoea after IMT. Similar reductions have been reported during constant power exercise (Gething et al. 2004a, b) and fatiguing resistive inspiratory muscle loading (Witt et al. 2007). The reduced relative intensity at which the inspiratory muscles were operating may have attenuated the activity of the centrally-mediated type III mechanoreceptor afferent discharge to a given mechanical stimulus thereby reducing the subsequent sympathetic efferent response and therefore the rise in HR. It has also been suggested (Witt et al. 2007) that increased oxidative capacity of the respiratory muscles following training (Ramírez-Sarmiento et al. 2002) may reduce the firing frequency of mechanically sensitive type III and chemosensitive type IV afferent fibres, possibly due to a reduced metabolite concentration

(Sinoway et al. 1993, 1996), thereby reducing the cardiovascular tone during volitional hyperpnoea. Alternatively, but not exclusively, IMT may offset an increase in EILV and EELV (Romer et al. 2002c). A lower operating tidal flow-volume envelope (i.e. %TLC) would also reduce expiratory pressure swings and thus preserve mean expiratory flow rates. A subsequent lowering in expiratory pressure would promote venous return subsequently increasing stroke volume. Such an increase in cardiac output may attenuate heart rate for a given absolute exercise intensity.

4.5 CONCLUSIONS

In conclusion, these findings extend those of Chapter 3 and previous studies (Verges et al. 2007b) and reinforce the body of evidence indicating that in a young, recreationally active population respiratory muscle work influences exercising $[lac^-]_B$ and that specific training of the respiratory muscles reduces lactate evolution and possibly increases lactate uptake. The findings herein also demonstrate that IMT reduces $[lac^-]_B$ during both steady state exercise and volitional hyperpnoea, thus providing novel evidence that the reductions in $[lac^-]_B$ commonly observed during whole-body exercise following IMT are almost certainly due, in part, to the trained respiratory muscles.

CHAPTER 5

LOADING THE TRAINED INSPIRATORY MUSCLES SPEEDS LACTATE CLEARANCE FOLLOWING MAXIMAL EXERCISE

5.1 INTRODUCTION

Data presented in Chapters 3 and 4 demonstrate that the respiratory muscles are capable of net lactate production when the work of breathing exceeds a critical threshold level (see also Johnson et al. 2006; Verges et al. 2007b) and that specific training of these muscles reduces this efflux. Chapter 4 also shows in agreement with previous literature that reductions in $[lac^-]_B$ occur during exercise following specific RMT (McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al, 1999) suggesting that at moderate levels of pulmonary ventilation, the respiratory muscles may become net lactate consumers (Fregosi and Dempsey 1986). Collectively, these findings suggest an important, previously underestimated role for the respiratory muscles in the regulation of whole body lactate kinetics.

This theme was recently extended by Chiappa et al. (2008b) who found that adding an inspiratory resistance (15 cmH_2O) during recovery from maximal incremental cycling exercise significantly reduced $[lac^-]_B$ (~2.5 $mmol \cdot L^{-1}$) compared to a passive recovery. This intriguing finding suggests that moderate levels of inspiratory muscle work can accelerate lactate clearance by a similar magnitude to that achieved with an active recovery involving locomotor muscles, but with the possible benefit of sparing intramuscular energy stores (Dupont et al. 2003). Given that lactate consumption by the inspiratory muscles may be enhanced by specific training it is attractive to speculate that the findings of Chiappa et al. (2008b) would also be magnified after RMT; this was the focus of the present study.

It is unlikely that increases in $[lac^-]$ alone result in metabolic acidosis and cause skeletal muscle fatigue (Robergs et al. 2004) particularly at physiological temperatures (Westerblad et al. 1997). However, according to the integrated physicochemical systems approach, with which it is possible to quantify the mechanisms accounting for disturbances in acid-base balance during and following exercise, it is proposed that $[lac^-]$ may indirectly affect $[H^+]$ (Stewart et al. 1983). Within a given compartment (e.g. muscle, plasma, erythrocyte) the dependent variables: $[H^+]$ and $[HCO_3^-]$ are determined by the independent

variables: strong ion difference ([SID] = $[Na^+]$ + $[K^+]$ - $[Cl^-]$ + $[lac^-]$), PCO_2 and the total concentration of weak acids ($[A_{tot}^-]$). Therefore, a reduction in $[lac^-]$ in the systemic circulation, assuming all other variables remain constant, may affect the $[H^+]$ by causing a change in [SID] (for reviews: Lindinger 1995; Kowalchuck and Scheuermann 1995). This is especially important given the associations between an elevated $[H^+]$ and / or $[lac^-]$ on some intramuscular processes (Fitts 1994) and subsequent exercise performance (Pilegaard et al. 1994; Thomas et al. 2004).

Accordingly, we hypothesised that inspiratory loading during recovery from maximal exercise would speed lactate clearance and that this would be further increased following specific inspiratory muscle training (IMT). In order to determine the effect of changes in $[lac^-]_B$ on plasma $[H^+]$, the contribution of associated physiological variables to the regulation of plasma acid-base homeostasis were quantified using the integrated physicochemical approach.

5.2 METHODS

5.2.1 PARTICIPANTS

Following ethical approval and written informed consent, 18 healthy non-smoking participants with normal lung function volunteered for the study (Table 5.1). Throughout the study subjects were instructed to adhere to their usual training regimen and followed pre-exercise instructions outlined in section 2.1. All exercise trials were performed using an electromagnetically-braked cycle ergometer (see section 2.2) and in similar laboratory conditions (temperature: 21.1 ± 2.7˚C; relative humidity: 46.6 ± 14.4%).

Table 5.1. Descriptive characteristics of the subjects.

	Control (n = 9)	IMT (n = 9)
Age (years)	27.1 ± 3.7	32.2 ± 6.3 *
Body mass (kg)	81.3 ± 8.0	78.9 ± 16.6
Height (cm)	183.3 ± 6.6	177.0 ± 9.5
FVC (L)	6.03 ± 0.92 (109 ± 14)	5.22 ± 1.03 (107 ± 9)
FEV_1 (L)	4.77 ± 0.63 (103 ± 11)	4.11 ± 0.76 (101 ± 7)
FEV_1/FVC (%)	79.5 ± 5.2 (97 ± 7)	79.3 ± 6.7 (96 ± 7)
MVV_{10} ($L \cdot min^{-1}$)	198.5 ± 23.2 (105 ± 14)	176.6 ± 29.4 (109 ± 9)
MIP (cmH_2O)	148.0 ± 35.7 (114 ± 4)	120.1 ± 27.3 (109 ± 7)
$\dot{V}O_2$ max ($L \cdot min^{-1}$)	4.27 ± 0.49	4.13 ± 0.83
$\dot{W}$max (W)	386 ± 44	378 ± 57

Values are expressed as means ± SD. Values in parentheses represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984). * P<0.05 between groups.

5.2.2 EXPERIMENTAL PROCEDURE

Subjects attended the laboratory 3 times prior to a 6 wk intervention. During the first laboratory visit subjects completed pulmonary function and MIP tests and were subsequently familiarised with all testing procedures including maximal incremental exercise. On two separate occasions subjects completed a maximal incremental exercise test. Immediately following exercise subjects breathed against either a constant pressure threshold inspiratory resistance (15 cmH_2O) for 20 min (ITL) or recovered passively with spontaneous breathing for 20 min (no inspiratory resistance; PR); the order of these trials were randomised. Following the pre-intervention trials, subjects were matched for $\dot{W}$ max and divided in to an IMT group (n = 9) or a control group (n = 9). Following a 6-wk intervention (IMT or no IMT), subjects repeated the pre-intervention trials. Given the increase in MIP expected following IMT, the IMT group completed a third maximal

incremental exercise test in which the absolute intensity of the pressure threshold inspiratory load during recovery was increased, so that the same fraction of MIP was used before and after the intervention. This subsequent trial was defined as ITL%.

5.2.3 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY MOUTH PRESSURE

Pulmonary function was assessed using a pneumotachograph and a hand-held mouth pressure meter measured MIP as an index of global inspiratory muscle strength according to sections 2.3 and 2.5, respectively. MIP was reassessed following 2, 4 and 6 wk of the intervention using the same protocol in both groups.

5.2.4 MAXIMAL EXERCISE FOLLOWED BY PASSIVE RECOVERY (PR)

Subjects performed a maximal incremental cycling test in which the initial power was 0 W and subsequently increased by 20 $W \cdot min^{-1}$ until exercise could no longer be tolerated ($\dot{W}$ max; Chiappa et al. 2008b). The highest oxygen uptake ($\dot{V}O_2$) recorded in any 30 s period defined $\dot{V}O_2$ max. $[lac^-]_B$ was determined at the cessation of exercise, and every 2 min thereafter; PCO_2 and $[H^+]$ were determined at volitional intolerance and every 5 min thereafter. At the cessation of exercise and following 10 and 20 min, physicochemical variables were determined. Throughout exercise and recovery, subjects wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) connected to an online breath by breath expired gas analyser (see section 2.6). Breath by breath respiratory variables were averaged over the final 30 s of every 2 min interval. Heart rate (HR) was recorded continuously during exercise using short-range telemetry (Polar S610, Polar, Kempele, Finland).

5.2.5 MAXIMAL EXERCISE FOLLOWED BY INSPIRATORY LOADING (ITL)

The ITL trial was identical to PR, however, immediately following exercise, a 1.5 m length of wide bore corrugated tubing was attached to the inspiratory port of a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) and connected distally to a weighted plunger pressure threshold inspiratory muscle loading device based on the design of Nickerson and Keens (1982; Johnson et al. 1997; Figure 5.1). physiological range (see Johnson et al. 1996); a full description of the device is provided elsewhere (Johnson et al. 1996). During ITL, weights were added to the plunger to adjust the threshold opening pressure which was fixed at 15 cmH_2O. For the IMT group and the control group this represented 13 ± 3% and 11 ± 3% MIP (pooled data, n = 18, 12 ± 3% MIP), respectively. Following 6 wk IMT, and due to the training-induced increase in MIP, the opening pressure of 15 cmH_2O represented a smaller resistance relative to MIP (10 ± 2% MIP). Thus in the ITL% trial, the absolute resistance was increased to 20 ± 2 cmH_2O which achieved the same relative resistance as the pre-IMT ITL trial (i.e. 13 ± 3%). The measurement accuracy of the online expired gas analyser during ITL was investigated prior to commencement of the study. Comparisons were made with the Douglas bag method at rest and over a range of exercise intensities. The mean bias ± 95% limits of agreement (2 SD) for $\dot{V}_E$ were -1.91 ± 2.19 $L \cdot min^{-1}$; for $\dot{V}O_2$ were -0.08 ± 0.14 $L \cdot min^{-1}$ and for $\dot{V}CO_2$ were -0.07 ± 0.14 $L \cdot min^{-1}$. These data show that the online expired gas analyser performed satisfactorily despite the negative pressures generated during ITL.

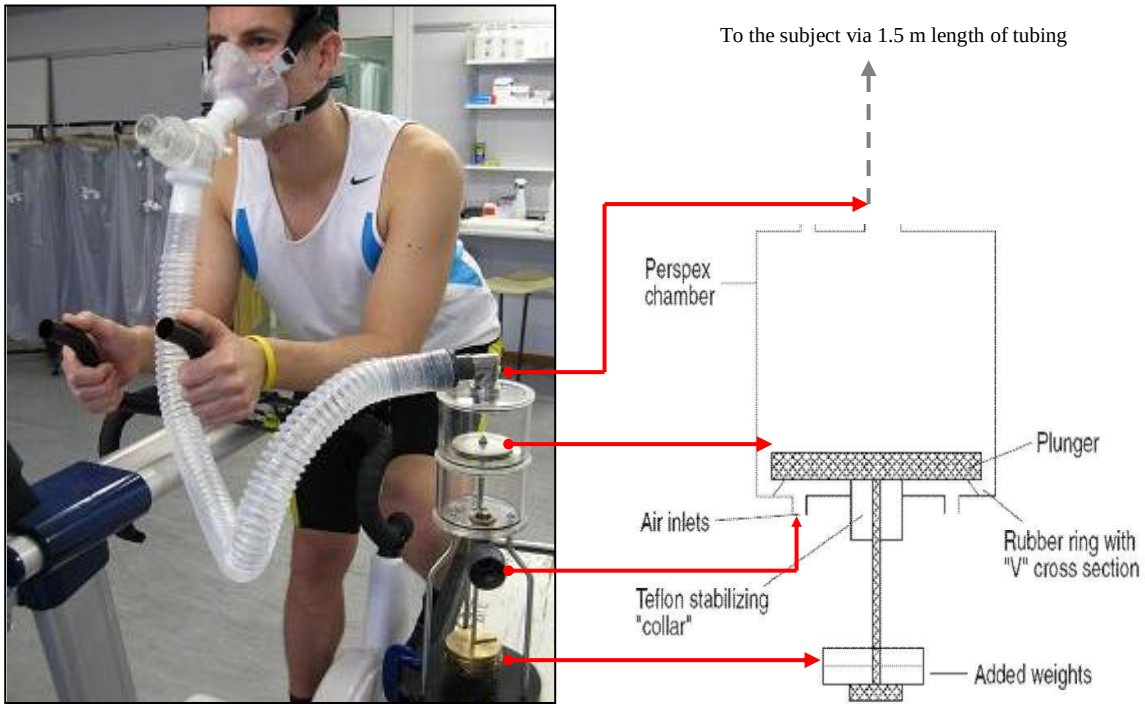

Figure 5.1. Weighted plunger inspiratory pressure threshold loading device (Johnson et al. 1997).

5.2.6 INTERVENTION

IMT was performed using an inspiratory pressure-threshold device as described in section 2.10. Subjects completed a training diary to record IMT adherence and habitual training, which the control group also recorded. The control group continued with their habitual physical training schedule and were not exposed to an intervention. A placebo treatment was not applied to the control group since both $\dot{V}O_2$ max and $\dot{W}$ max are known to be unaffected by RMT (see section 1.16).

5.2.7 BLOOD SAMPLING AND ANALYSIS

During all exercise trials arterialised venous blood was sampled from a dorsal hand vein via an indwelling cannula and analysed immediately for PCO_2, pH, and $[lac^-]_B$ as outlined in section 2.8. Plasma $[HCO_3^-]$ and BE_{ECF} were calculated as described in section 2.8.

To quantify the mechanisms accounting for plasma acid-base disturbances the integrated physicochemical systems approach was used (Stewart 1983). Under resting conditions, at the cessation of maximal exercise and every 10 min thereafter, a 5 ml blood sample was drawn and centrifuged immediately for 10 min at 3000 g. Plasma [Na^+] and [K^+] were measured using inductively coupled plasma optical emission spectrometry (1200DV ICP OES, Perkin Elmer, MA, USA). Plasma [Cl^-] was measured by ion chromatography (DX120, Dionex, CA, USA) and the total concentration of plasma proteins [PPr^-] was assayed in duplicate according to the method of Lowry (1951). The total concentration of weak acids ([A_{tot}^-]) was subsequently calculated as: 2.45 × [PPr^-] (McKenna et al. 1997). Plasma strong ion difference ([SID]) was calculated as the sum of the strong cations minus the sum of the strong anions (Stewart 1983):

$$[SID] = ([Na^+] + [K^+]) - ([Cl^-] + [lac^-]) \quad [1]$$

Free plasma [H^+] was calculated using the empirical relationship derived by Stewart (1983). This equation describes the dependency of the [H^+] within a given compartment on three independent variables ([SID], PCO_2 and [A_{tot}^-]) with respect to the laws of mass action, conservation of mass and electrical neutrality:

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_{tot}^-]) - (K_C\,PCO_2 + K'_W)\} \times [H^+]^2 - \{K_A(K_C\,PCO_2 + K'_W) + (K_3K_C\,PCO_2)\}[H^+] - (K_AK_3K_C\,PCO_2) = 0 \quad [2]$$

The equilibrium constants K_A, K_C, K'_W and K_3 were: $K_A = 3.0 \times 10^{-7}$ equiv·l^{-1}, $K_C = 2.45 \times 10^{-11}$ (equiv·l^{-1})^2, $K'_W = 4.4 \times 10^{-14}$ (equiv·L^{-1})^2 and $K_3 = 6.0 \times 10^{-11}$ equiv·L^{-1}.

The contributions of the independent variables (PCO_2, [SID] and [A_{tot}^-]) to changes in arterialised venous plasma [H^+] were calculated according to Putman et al. (2003) where each independent variable was individually changed to the corresponding exercise value while the remaining two variables were held constant at resting levels (*equation 2*). The resting [H^+] was then subtracted from the resulting [H^+] and the difference expressed as a percentage of the total $\Delta[H^+]$ (Table 5.5).

5.2.8 LACTATE RECOVERY KINETICS

The individual lactate recovery curves prior to and following the intervention were fitted to the following bi-exponential time function using iterative, least squares non-linear regression (Freund and Zouloumian 1981):

$$Lac^-(t) = Lac^-(0) + A_1\left(1 - e^{\gamma_1 \cdot t}\right) + A_2\left(1 - e^{\gamma_2 \cdot t}\right) \quad [3]$$

$Lac^-(t)$ (mmol·L^{-1}) denotes the $[lac^-]_B$ for a given time (t; min) of the recovery period and $Lac^-(0)$ (mmol·L^{-1}) being the $[lac^-]_B$ at the onset of the recovery period. This equation illustrates that blood lactate kinetics following exercise can be described by two mathematical and physiological processes: one with a fast velocity constant (γ_1; ·min^{-1}) describing the appearance of lactate in the arterialised blood or lactate exchange ($A_1 > 0$; mmol·L^{-1}) and an increased $[lac^-]_B$ and a second with a slow velocity constant (γ_2; ·min^{-1}) describing lactate clearance ($A_2 < 0$; mmol·L^{-1}) and a reduction in $[lac^-]_B$. The parameters of the bi-exponential non-linear regression were calculated using SYSTAT (Version 12, SYSTAT software Inc., CA, USA).

5.2.9 STATISTICAL ANALYSES

Statistical analyses of the dependent variables were performed using SPSS (Version 15, SPSS, Chicago, Illinois, USA). Pre- and post-intervention results and group interactions were assessed using one-way or two-way repeated measures ANOVA across groups (IMT vs. Control), trials (PR vs. ITL) and time (20 min recovery duration or Pre- vs. Post-intervention). Following a significant *F*-ratio, Tukey's HSD post-hoc analysis was performed. Pearson product-moment correlation coefficients assessed the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

5.3 RESULTS

5.3.1 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY PRESSURE

Baseline pulmonary function and MIP were all within normal limits (Table 5.1). Training compliance was excellent in the IMT group (92 ± 2%) and inspection of training diaries revealed habitual training remained constant in both groups. MIP was unchanged following the intervention in the control group (pre vs. post: 148.0 ± 35.6 vs. 148.4 ± 37.7 cmH_2O). In contrast, MIP increased from 120.1 ± 27.3 cmH_2O at baseline to 140.0 ± 26.7, 154.8 ± 36.2 and 159.8 ± 34.8 cmH_2O (+34 %; $P<0.001$) following 2, 4 and 6 weeks of IMT, respectively.

5.3.2 RESPIRATORY AND HEART RATE RESPONSES

$\dot{W}$ max, $\dot{V}O_2$ max, breathing pattern and HR responses to maximal exercise prior to the intervention are shown in Table 5.2 for the control and IMT groups, respectively. These responses were similar between trials (PR vs. ITL) and between groups (IMT vs. Control) prior to and following the intervention. There were no within or between group differences in $\dot{V}_E$, f_R, V_T and $\dot{V}O_2$ during incremental exercise both prior to and

following the intervention (Figure 5.2). Transient changes in breathing pattern and $\dot{V}O_2$ throughout recovery from maximal exercise in PR and ITL for the IMT group are shown in Figure 5.3. There were no differences in maximal exercise $\dot{V}_E$, f_R, V_T and T_I/T_{tot} between trials and between groups both prior to and following the intervention. $\dot{V}_E$ during recovery was similar between trials and between groups, however, with ITL, V_T was increased by 0.32 ± 0.16 L and f_R was decreased by 5 ± 2 breaths·min^{-1}; this increased T_I/T_{tot} in both the IMT (absolute increase: 0.020 ± 0.031) and control groups (absolute increase: 0.044 ± 0.047). These responses were similar following the intervention in both groups. HR recovery was similar between trials and between groups. Maximal HR was ~180 beats·min^{-1} and decreased to ~100 beats·min^{-1} following 8 min of recovery which was not different to 20 min. There were no changes in HR in either group following the intervention.

Table 5.2. Pre-intervention responses to maximal incremental cycling exercise prior to 20 min recovery with (ITL) and without (Passive recovery) inspiratory threshold loading.

	Control group		IMT group	
	Passive recovery	ITL	Passive recovery	ITL
$\dot{W}max$	387 ± 44	387 ± 41	378 ± 57	376 ± 57
$\dot{V}O_2max$	4.21 ± 0.66	4.23 ± 0.52	4.10 ± 0.92	4.20 ± 0.75
$\dot{V}_E$	166.6 ± 22.5	170.1 ± 14.0	163.1 ± 32.3	158.8 ± 34.8
f_R	61 ± 13	60 ± 8	60 ± 11	58 ± 11
V_T	2.83 ± 0.62	2.93 ± 0.47	2.90 ± 0.94	2.86 ± 0.93
T_I/T_{tot}	0.50 ± 0.02	0.50 ± 0.01	0.47 ± 0.04	0.50 ± 0.01
HR	177 ± 9	178 ± 11	181 ± 10	181 ± 11

Values are expressed as means ± SD.

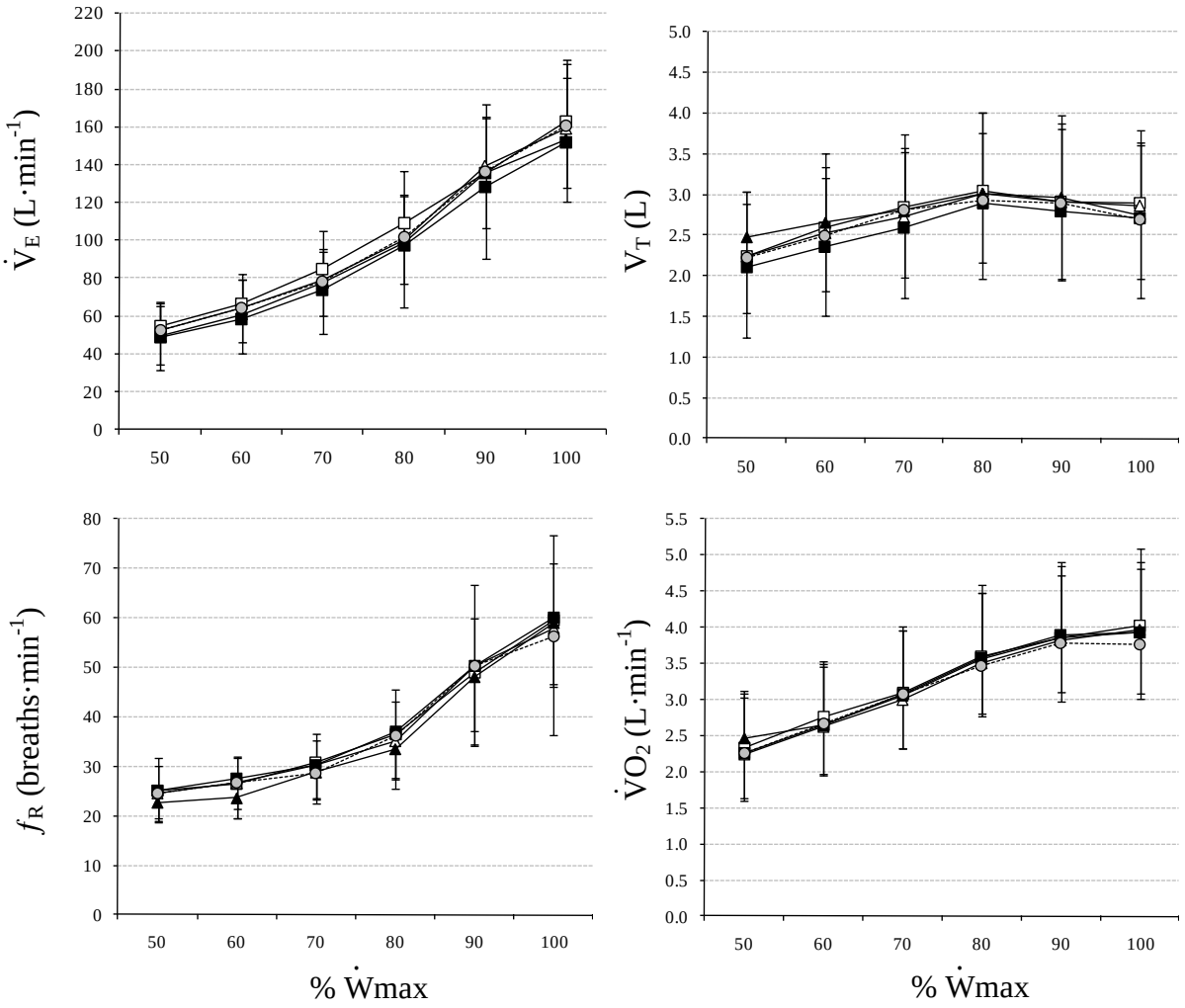

Figure 5.2. Respiratory responses to maximal incremental cycling exercise for the IMT group only prior to and following the 6 wk intervention. ▲, passive recovery trial pre-intervention; □, inspiratory pressure threshold loading trial at 15 cmH_2O pre-intervention; ■, passive recovery trial following the intervention; Δ, ITL trial following the intervention; ●, inspiratory pressure threshold loading trial post-intervention at a higher absolute resistance but the same relative resistance as pre-intervention (ITL%).

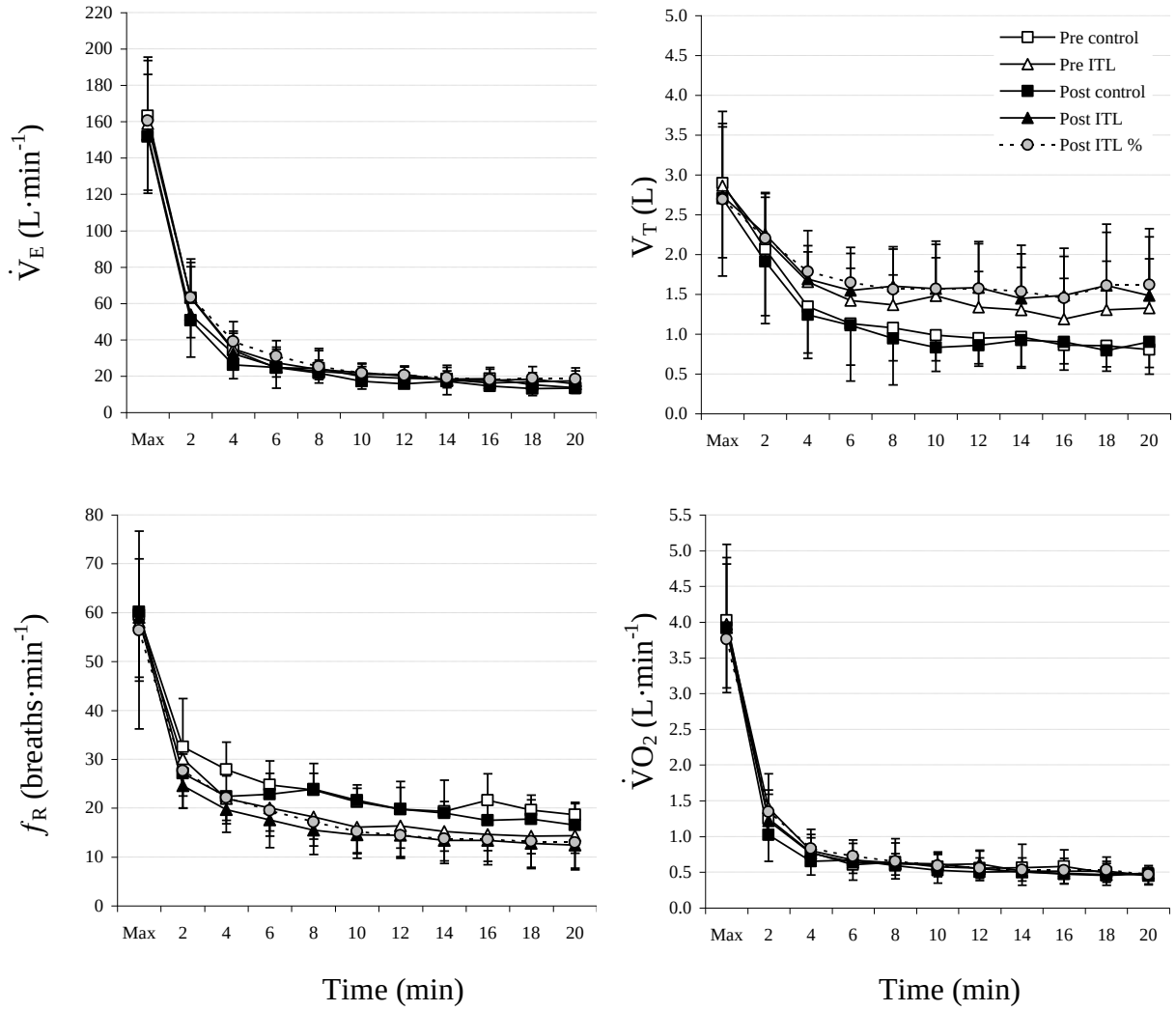

Figure 5.3. Respiratory responses during 20 min recovery from maximal incremental cycling exercise in the IMT group only prior to and following the 6 wk intervention. 'Max' is defined as the point of exercise intolerance. ▲, passive recovery pre-intervention; □, inspiratory pressure threshold loading at 15 cmH_2O pre-intervention; ■, passive recovery following the intervention; Δ, ITL following the intervention; ●, inspiratory pressure threshold loading post-intervention at a higher absolute resistance but the same relative resistance as pre-intervention (ITL%).

5.3.3 LACTATE RECOVERY KINETICS

Pre-intervention, peak and minimum $[lac^-]_B$ were similar in PR and ITL in both groups (Figure 5.4) and were unchanged in the control group following the intervention. Relative to pre-intervention, following IMT the exercise-induced peak and minimum $[lac^-]_B$ were reduced by 1.24 ± 1.32 (P<0.05) and 1.18 ± 1.22 mmol·L^{-1} (P<0.05) in PR and by 1.52 ± 1.26 (P<0.05) and 1.42 ± 1.60 mmol·L^{-1} (P<0.05) in ITL, respectively; these reductions were not different between PR and ITL trials. Following-IMT only, inspiratory pressure threshold loading throughout the 20 min recovery period (mean of 2 to 20 min) reduced $[lac^-]_B$ by 0.66 ± 1.28 mmol·L^{-1} (trial × time interaction effect, P<0.01). When ITL

was performed with the same relative inspiratory pressure threshold load as pre-intervention (ITL%), lactate clearance was not different to the post-IMT trial with passive recovery (Figure 5.4).

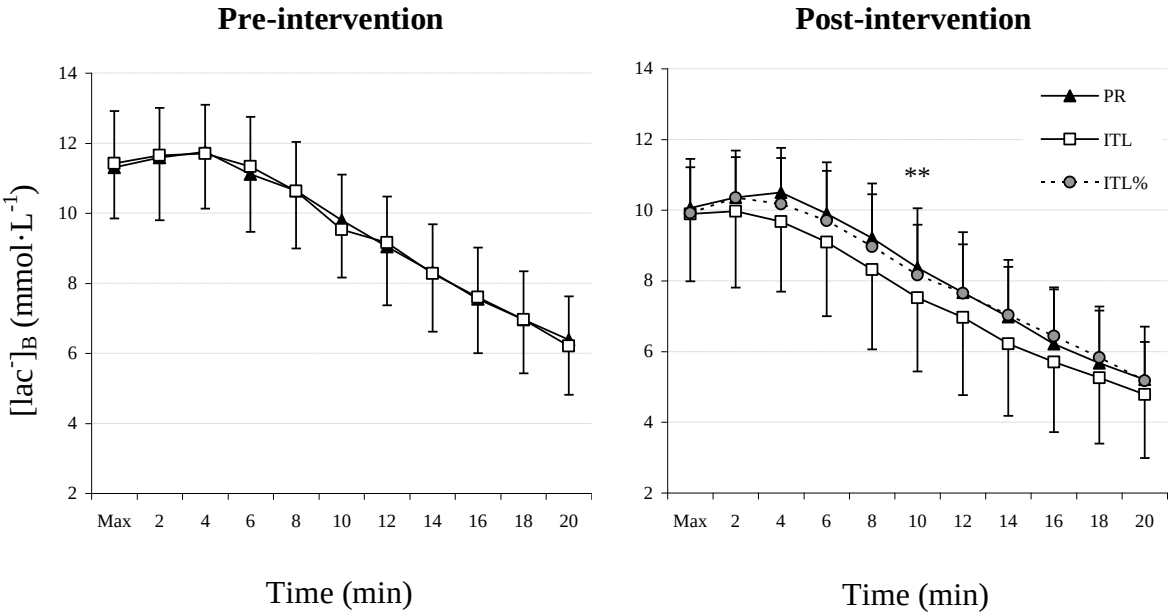

Figure 5.4. Blood lactate concentration ($[lac^-]_B$) during 20 min of recovery from maximal incremental cycling exercise in the IMT group only prior to and following the 6 wk intervention. 'Max' is defined as the point of exercise intolerance. ▲, passive recovery; □, inspiratory pressure threshold loading (15 cmH_2O); ●, inspiratory pressure threshold loading (ITL%). **, Post-intervention: ITL different to PR (P<0.05).

The amplitudes and velocity constants for the lactate recovery curves are shown in Table 5.3. Prior to the intervention, there were no differences between groups or between trials in any parameter, thus ITL throughout recovery failed to affect either lactate exchange or lactate clearance. Following the intervention, all parameters remained unchanged in the control group. Following IMT, relative to the equivalent pre-intervention trial $Lac^-(0)$ and A_2 was reduced in PR (P<0.05). In ITL there was a decrease in A_1 and A_2 and increase in γ_1 and γ_2 (P<0.05); the reduction in A_2 and increase in γ_2 exceeded those of the control group (group × time × trial interaction effect, P<0.05). In ITL%, relative to the pre-intervention ITL trial there was a reduction in $Lac^-(0)$ and A_2 and increase in γ_1 (P<0.05) although relative to the post-intervention ITL trial γ_2 was slower (P<0.05).

Table 5.3. Parameters of the bi-exponential non-linear regression model fitted to 20 min lactate recovery data following maximal incremental exercise with 20 min passive recovery (PR) and inspiratory threshold loading (ITL) for both the control and IMT groups, respectively. Data from the control group were not different following the intervention and have been omitted.

	Control Group		IMT group			
	Pre-intervention		Pre-intervention		Post-intervention	
	PR	ITL	PR	ITL	PR	ITL
La(0)	10.97 ± 1.22	11.11 ± 1.44	11.25 ± 1.53	11.50 ± 1.52	10.12 ± 1.58*	9.91 ± 2.04*
A_1	4.38 ± 1.44	4.38 ± 1.56	3.93 ± 0.39	3.99 ± 1.90	3.36 ± 0.91	2.55 ± 0.66*†
γ_1	0.270 ± 0.246	0.313 ± 0.182	0.296 ± 0.084	0.235 ± 0.076	0.308 ± 0.168	0.463 ± 0.266*†
A_2	-21.76 ± 7.98	-19.16 ± 6.84	-20.62 ± 5.50	-20.17 ± 3.82	-15.16 ± 4.42*	-13.13 ± 3.95*†
γ_2	0.031 ± 0.014	0.037 ± 0.015	0.031 ± 0.011	0.034 ± 0.009	0.036 ± 0.012	0.056 ± 0.025*†

Values are expressed as means ± SD. * P<0.05 vs. pre-intervention; † P<0.05 vs. PR.

In the ITL trial lactate clearance was not correlated with the relative intensity of inspiratory muscle loading (%MIP) prior to the intervention (γ_2; n = 18; see Figure 5.5: Left panel). Following IMT, there was no correlation between the relative intensity of inspiratory loading and γ_2 when data from both the ITL and ITL% trials were combined (n = 9; see Figure 5.5: Right panel).

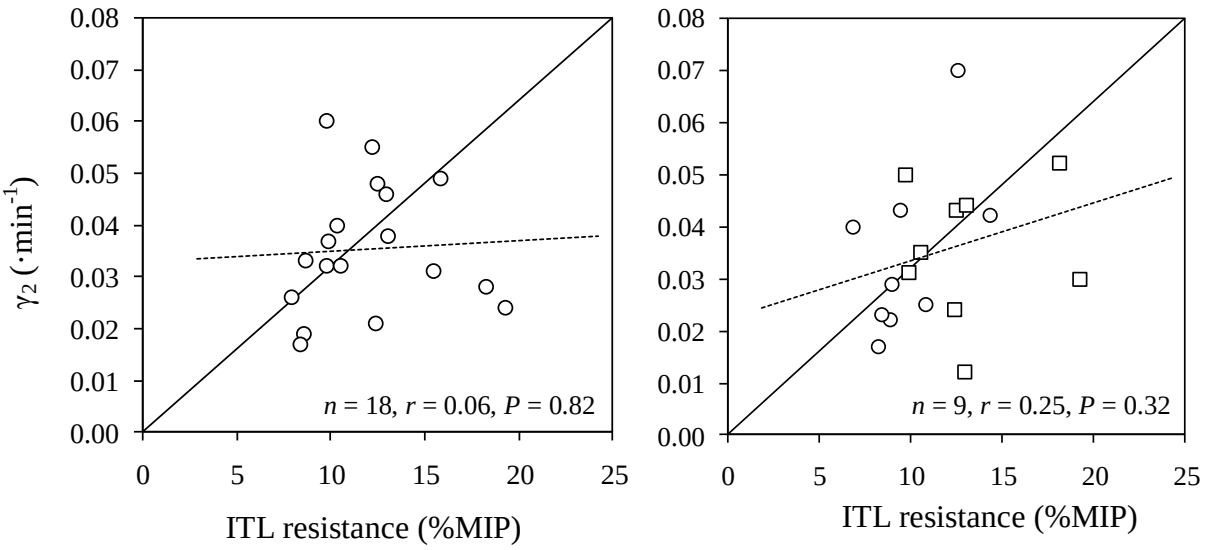

Figure 5.5. Inspiratory pressure threshold load relative to the maximal inspiratory pressure (MIP) versus the slow velocity constant (γ_2; $\cdot min^{-1}$) which describes lactate clearance (A_2 < 0; $mmol \cdot L^{-1}$). Left panel: pre-intervention pooled data of both control and IMT groups. Right panel: post-IMT data from the ITL and ITL% trials; o, ITL data; □, ITL% data. Note: regression line reflects the pooled data from both the ITL and ITL% trials.

5.3.4 ACID-BASE BALANCE: INDEPENDENT VARIABLES

Independent acid-base variables are shown in Table 5.4. At rest [Cl^-] was 101.2 ± 3.6 mmol·L^{-1}, [Na^+] was 138.4 ± 5.9 mmol·L^{-1} and [K^+] was 3.9 ± 0.3 mmol·L^{-1} in the IMT group which was not different to the control group. Immediately following maximal exercise in PR, [Cl^-] and [K^+] increased by 4.4 ± 0.8 and 1.4 ± 0.5 mmol·L^{-1} in the IMT group and by 4.2 ± 0.6 and 2.5 ± 1.3 mmol·L^{-1}, respectively, in the control group (P<0.05), these increases were similar between groups and during both trials. The increases were unchanged after the intervention period in both groups. [Na^+] remained unchanged after maximal exercise and throughout recovery in both groups and in all trials before and after the intervention. During recovery from maximal exercise [K^+] returned to resting values after 10 minutes. [Cl^-] remained higher than rest after 10 minutes of recovery but had returned to resting concentration after 20 minutes. These patterns were similar in both groups and during both PR and ITL trials and were largely unaffected by the intervention period. However, after 20 min of the post-IMT ITL trial [K^+] was greater (absolute increase ~ 0.3 mmol·L^{-1}, P<0.05) than at the same time point of the pre-intervention ITL trial.

Table 5.4. Ion and plasma protein concentrations and independent and dependent plasma acid-base variables immediately following maximal incremental exercise (Max) and after 10 and 20 min recovery in the IMT group only.

			IMT group (n = 9)					
			Passive recovery			15 cmH_2O ITL		
		Rest	Max	10 min	20 min	Max	10 min	20 min
Electrolytes and plasma proteins								
$[lac^-]$ $(mmol \cdot L^{-1})$	Pre	0.7 ± 0.3	11.3 ± 1.4†	9.8 ± 1.6†	6.4 ± 1.6$^{\dagger ab}$	11.4 ± 1.5†	9.5 ± 1.6†	6.2 ± 1.4$^{\dagger ab}$
	Post	-	10.1 ± 1.4*†	8.4 ± 1.7*†	5.1 ± 1.5$^{*\dagger ab}$	9.9 ± 1.9*†	7.5 ± 2.1$^{*\dagger c}$	4.8 ± 1.8$^{*\dagger ab}$
$[Cl^-]$ $(mmol \cdot L^{-1})$	Pre	101.2 ± 3.6	105.6 ± 3.9†	103.0 ± 4.6^{a}	102.1 ± 3.8^{a}	104.9 ± 5.2†	103.6 ± 4.2^{a}	101.4 ± 5.0^{a}
	Post	-	105.8 ± 4.4†	102.3 ± 7.1^{a}	101.9 ± 6.0^{a}	106.6 ± 6.5†	102.9 ± 3.1^{a}	101.6 ± 4.5^{a}
$[Na^+]$ $(mmol \cdot L^{-1})$	Pre	138.4 ± 5.9	139.6 ± 7.1	135.3 ± 6.6	140.3 ± 8.5	140.8 ± 10.0	135.5 ± 6.4	139.3 ± 10.5
	Post	-	143.9 ± 10.2	135.3 ± 8.9	143.0 ± 11.4	142.6 ± 5.0	136.2 ± 6.1	135.7 ± 4.0
$[K^+]$ $(mmol \cdot L^{-1})$	Pre	3.9 ± 0.3	5.4 ± 0.4†	3.7 ± 0.3^{a}	3.9 ± 0.3^{a}	5.3 ± 0.3†	3.9 ± 0.4^{ac}	3.9 ± 0.3^{a}
	Post	-	5.4 ± 0.5†	3.9 ± 0.4^{a}	4.0 ± 0.5^{a}	5.7 ± 0.7†	3.9 ± 0.5	4.2 ± 0.5^{*a}
$[PPr^-]$ $(mmol \cdot L^{-1})$	Pre	66.3 ± 33.8	83.8 ± 22.0	95.0 ± 14.8	79.7 ± 17.7	96.3 ± 39.6	93.4 ± 13.7	76.4 ± 28.2
	Post	-	86.2 ± 12.9	96.9 ± 12.6	87.0 ± 28.9	109.0 ± 53.8	87.8 ± 32.8	90.8 ± 13.6*
Independent acid-base variables								
[SID] $(mmol \cdot L^{-1})$	Pre	40.4 ± 6.2	28.7 ± 7.3	26.2 ± 7.2†	35.8 ± 7.5	33.2 ± 14.4	32.0 ± 13.3	34.0 ± 6.6
	Post	-	32.3 ± 6.7	28.7 ± 6.7†	36.2 ± 11.0	33.1 ± 9.7	28.7 ± 10.1	40.8 ± 11.8*
$[A_{tot}^-]$ $(mmol \cdot L^{-1})$	Pre	16.2 ± 8.2	20.5 ± 5.4	23.3 ± 3.6	19.5 ± 4.3	23.6 ± 9.7	22.9 ± 3.4	18.7 ± 6.9
	Post	-	21.1 ± 3.2	23.7 ± 3.1	21.3 ± 7.1	26.7 ± 13.2^{c}	21.5 ± 8.0	22.2 ± 3.3*
PCO_2 (mmHg)	Pre	39.5 ± 4.1	42.2 ± 8.0	34.4 ± 3.0†	36.1 ± 3.2	44.0 ± 7.7	33.9 ± 4.8	33.2 ± 6.0
	Post	-	42.4 ± 9.0	34.4 ± 2.9	35.7 ± 2.7	42.3 ± 7.4	32.5 ± 4.7†	32.4 ± 5.5
Dependent acid-base variables								
$[H^+]$ $(nmol \cdot L^{-1})$	Pre	37.3 ± 2.2	60.4 ± 7.9†	53.8 ± 5.8†	45.2 ± 4.2$^{\dagger ab}$	63.0 ± 7.3†	51.1 ± 3.8†	41.5 ± 4.0^{ab}
	Post	-	57.0 ± 8.7†	50.6 ± 5.4†	43.2 ± 4.6$^{\dagger ab}$	59.0 ± 10.0†	48.8 ± 7.9^{ab}	40.4 ± 6.3^{ab}
$[HCO_3^-]$ $(mmol \cdot L^{-1})$	Pre	25.3 ± 1.8	16.7 ± 2.0†	15.6 ± 2.1†	19.2 ± 2.4$^{\dagger b}$	16.7 ± 2.1†	15.8 ± 2.0†	19.0 ± 2.4$^{\dagger b}$
	Post	-	17.8 ± 2.5†	16.4 ± 2.1†	19.9 ± 1.9$^{\dagger b}$	17.2 ± 2.1†	16.2 ± 3.0†	19.3 ± 2.7$^{\dagger b}$

Values are expressed as means ± SD. Within trials: †, $P<0.05$ different to rest; ^{a}, $P<0.05$ different to max; ^{b}, $P<0.05$ different to 10 min. Between trials: ^{c}, $P<0.05$ time point different to passive recovery; *, $P<0.05$ pre vs. post.

5.3.5 ACID-BASE BALANCE: DEPENDENT VARIABLES

Table 5.4 shows the changes in dependent variables throughout recovery from maximal exercise and Table 5.5 illustrates the contributions of the independent variables ([SID], $[A_{tot}^-]$ and PCO_2) to changes in plasma $[H^+]$. Before IMT, $[H^+]$ increased significantly from rest (37.3 ± 2.2 nmol·L^{-1}) to maximal exercise (~60 nmol·L^{-1}; P<0.01) in both trials. Similar changes were observed in the control group. During the final 10 min of the recovery period of the PR trial, 84% of the increase in $[H^+]$ above rest was accounted for by a 9.4 mmol·L^{-1} reduction in [SID] with the remaining 16% being due to a 5.2 mmol·L^{-1} increase in $[A_{tot}^-]$. During the recovery period $[H^+]$ was lower by ~3 nmol·L^{-1} in the ITL trial compared to the PR trial however (; P<0.01), this difference was accounted for by the greater hypocapnia (lower PCO_2) observed during the ITL trial since all other independent variables were not different to PR. Similar findings were observed in the control group both prior to and following the intervention.

Compared to pre-intervention values, plasma $[H^+]$ was lower in recovery from maximal exercise in both PR (main effect time, P<0.05) and ITL (main effect time, P<0.05) after IMT in either trial. However, in the same analysis PCO_2 and $[A_{tot}^-]$ after exercise and throughout recovery were not different following IMT. Therefore the reduction in $[H^+]$ was accounted for exclusively by an increased [SID]. The increase in [SID] during PR was accounted for by the reduction in $[lac^-]_B$ and during ITL, by the 1.7 and 0.3 decrease and increase in $[lac^-]_B$ and $[K^+]$, respectively. Between trial differences were not present following-IMT.

Table 5.5. Contributions of the independent variables (PCO_2, [SID] and [A_{tot}^-]) to changes in plasma [H^+] following maximal exercise with 20 min passive recovery (PR) and inspiratory threshold loading (ITL) prior to (Pre-IMT) and following-IMT (Post-IMT). Data are the average of min 10 to 20.

	Concentration			Contribution of independent variables to [H^+] ($nmol \cdot L^{-1}$)		Δ [H^+] = (recovery) – (rest) ($nmol \cdot L^{-1}$)		Percentage contribution to Δ [H^+] (%)	
Variable	Rest	PR	ITL	PR	ITL	PR	ITL	PR	ITL
Pre-IMT									
[H^+] meas. ($nmol \cdot L^{-1}$)	37.3	49.3	46.4	-	-	+12.0	+9.1	-	-
[H^+] calc. ($nmol \cdot L^{-1}$)	36.1	52.0	44.8	-	-	+15.9	8.7	-	-
PCO_2 (mmHg)	39.5	35.2	33.5	32.2	30.9	-3.9	-5.2	-21	-37
[SID] ($mmol \cdot L^{-1}$)	40.4	31.0	33.0	51.9	47.5	+15.8	+11.4	+84	+81
[A_{tot}^-] ($mmol \cdot L^{-1}$)	16.2	21.4	20.8	39.2	38.8	+3.1	+2.7	+16	+19
Post-IMT									
[H^+] meas. ($nmol \cdot L^{-1}$)	37.3	46.9	44.6	-	-	+9.6	+7.3	-	-
[H^+] calc. ($nmol \cdot L^{-1}$)	36.1	49.7	40.8	-	-	+13.6	+4.7	-	-
PCO_2 (mmHg)	39.5	35.1	32.5	32.2	29.6	-3.9	-6.5	-24	-56
[SID] ($mmol \cdot L^{-1}$)	40.4	32.5	34.8	48.8	44.5	+12.7	+8.4	+77	+72
[A_{tot}^-] ($mmol \cdot L^{-1}$)	16.2	22.5	21.9	39.8	39.4	+3.7	+3.3	+23	+28

5.4 DISCUSSION

The primary finding of this study is that the addition of a pressure-threshold inspiratory resistance (15 cmH_2O) during recovery from maximal incremental exercise accelerated blood lactate clearance but only after 6 weeks of specific inspiratory muscle training.

Our finding that pressure-threshold loading of untrained inspiratory muscles immediately following maximal exercise failed to affect systemic lactate clearance (Figure 5.4) disagrees with the findings of Chiappa et al. (2008b, 2009). An explanation for this disagreement is not readily forthcoming as the experimental protocols were identical (including breathing pattern). Our subjects were of notably higher training status ($\dot{W}max$ was around 80 W higher) which may conceivably have influenced the findings. However, breathing endurance is positively related to whole body training status (Eastwood et al. 2001), therefore baseline respiratory muscle conditioning is likely to have been higher in

our subjects and, if this was influential, we might reasonably have expected greater rather than less lactate clearance. Chiappa et al. (2008b) found that during recovery with an inspiratory resistance [H^+] was unaffected by a large (~2.5 mmol·L^{-1}) decrease in $[lac^-]_B$ with no change in PCO_2 . The authors suggest that flux in other strong ions (not measured) may explain the unaltered blood acid-base balance despite the large reduction in $[lac^-]_B$. We found no such changes either before or after IMT in any electrolyte or $[A_{tot}^-]$. Also, lactate clearance is well described by a bi-exponential function following exercise at different intensities (Freund and Zouloumian 1981), with respiratory muscle loading (Perret and Müeller 2007; this study) and following both whole body (Messonnier et al. 2006) and IMT (this study). That this pattern was not observed by Chiappa et al. (2008b) is also difficult to resolve.

Whilst methodologically disparate our (pre-intervention) findings are similar to those of Perret and Mueller (2007) who reported unchanged lactate recovery kinetics following exercise with low intensity isocapnic volitional hyperpnea ($\dot{V}_E$ 61.6 ± 9.3 L·min^{-1}, 30 ± 1% of MVV) compared to PR. Therefore the issue of whether increasing the work of breathing offers a method of accelerating lactate clearance remains equivocal. It is possible that the intensity of inspiratory muscle loading is influential: when ITL was performed at the same relative intensity (an absolute pressure threshold of 20 ± 2 cmH_2O; i.e. ITL%) following IMT lactate clearance was not accelerated relative to PR and ITL. This finding is similar to previous work where relative to high intensity leg exercise (65% $\dot{V}O_2$max), low intensity leg exercise (35% $\dot{V}O_2$max) performed immediately after maximal exercise increased lactate clearance (1984). The blood flow characteristics of different exercise intensities were proposed as an explanation for their findings (1984). Notwithstanding this, the lack of relationship between %MIP of ITL (range: 10% - 19%) and rates of lactate clearance (Figure 5.5) does not support the notion that the ITL intensity is influential. However, it is interesting to speculate whether a lower inspiratory resistance

(<15 cmH_2O) prior to the intervention would have accelerated lactate clearance and further work is warranted to reveal the effects of ITL intensity upon lactate recovery kinetics.

We also report that IMT reduced peak $[lac^-]_B$ by ~1 $mmol \cdot L^{-1}$ after completion of the maximal incremental exercise test. This agrees with previous research which shows reduced $[lac^-]_B$ following both maximal incremental (Spengler et al. 1999) and constant power steady-rate exercise (Chapter 4; McConnell and Sharpe 2005). When comparing $[lac^-]_B$ during the ITL trial pre- and post-IMT the difference was maximal (2.30 $mmol \cdot L^{-1}$) after 8 minutes. Whether such reductions may affect subsequent exercise tolerance remains an intriguing question.

We are the first to report that inspiratory threshold loading after specific inspiratory muscle training can significantly speed lactate clearance following maximal incremental cycling exercise. Following IMT, A_2 which reflects the amplitude concentration of lactate clearance, was reduced during passive recovery from maximal exercise, however, since the velocity constants were unchanged, this is likely to reflect the lower absolute $[lac^-]_B$ throughout recovery relative to pre-intervention (Figure 5.4). Conversely, increasing the work of breathing with ITL immediately following exercise at the same intensity increased the velocity constants and decreased the amplitudes of both exponential terms (Table 5.3). Previous studies have reported similar changes in these parameters following whole-body training (Messonnier et al. 2001, 2006). After IMT, we observed a 68% increase in γ_1 during ITL indicating an improved capacity for lactate exchange between the previously worked muscle(s) and the systemic circulation (Freund and Zouloumian 1981). Due to the specific nature of inspiratory muscle training this is probably achieved by increasing the concentration gradient between the locomotor muscles and the systemic circulation most likely due to increased lactate clearance by the inspiratory muscles (as confirmed by the 71% increase in γ_2). The increase in γ_2 is also similar to that found in whole body exercise training studies in which it was associated with an increase in lactate transport capacity (MCT1, MCT4) and oxidative enzyme activity (Messonnier et al. 2001, 2006). It has been

argued that such adaptations may occur following inspiratory muscle training (cf. McConnell and Sharpe 2005). In support of this argument, oxidative enzyme adaptations have been observed in sheep diaphragm following intense resistive RMT (Akiyama et al. 1996) and muscle biopsy data from the external intercostal muscles following 5 wk IMT similar to that used in the present study have shown an increase in the proportion of type I muscle fibres (Ramírez-Sarmiento et al. 2002). Thus it is likely that loading the trained inspiratory muscles increased their capacity for lactate consumption.

We used the physicochemical approach (Stewart 1983) to quantify the contribution of each of the independent variable to changes in acid-base disturbance. Here, each independent variables was individually changed to the corresponding exercise value while the remaining two variables were held constant at resting levels; the resting $[H^+]$ was then subtracted from the calculated $[H^+]$ and the difference expressed as a percentage of the total $\Delta[H^+]$ (Table 5.5). As reported by others (Putman et al. 2003), there was excellent agreement between the measured and calculated $[H^+]$ (r = 0.925, P<0.001). Prior to the intervention and in both PR and ITL the contribution of the [SID] and hence the influence of $[lac^-]_B$ on plasma $[H^+]$ remained unchanged accounting for 84 and 81% of the total change in $[H^+]$, respectively. Following IMT, the greater reduction in plasma $[H^+]$ with ITL compared to PR was due to an increase in [SID] since both PCO_2 and $[A_{tot}^-]$ and their contribution to the changes in $[H^+]$ were unchanged (Table 5.5).

Previous studies using a similar approach to quantify changes in acid-base balance reported significant reductions in plasma $[H^+]$ following (1 wk) endurance training (2 h·day^{-1}, 60% $\dot{V}O_2$max; Putman et al. 2003). The reduction in plasma $[H^+]$ occurred secondary to adaptations specific to the locomotor muscles which, in turn, affected the concentrations of the strong ions, increasing [SID] (Putman et al. 2003). In the present study, with the exception of a small increase in $[K^+]$ following IMT in ITL, no other strong ion was affected, therefore, the increase in [SID] was almost exclusively accounted for by the reductions in $[lac^-]_B$. These data are the first to show that IMT and ITL are capable of

influencing plasma [H^+] following exercise. The mechanism(s) accounting for this are an IMT-mediated increase in [SID] which was caused by an increase in lactate clearance by the inspiratory muscles. The defence of plasma acid-base homeostasis is considered of great importance during and following exercise (Putman et al. 2003), therefore, IMT and ITL may provide a favourable systemic metabolic environment for subsequent bouts of exercise (Edge et al. 2006).

Whether the present findings suggest that the inspiratory muscles are net consumers of H^+ is equivocal. The increased inspiratory muscle lactate clearance following IMT may occur due to the greater expression of sarcolemmal and mitochondrial bound lactate-H^+ co-transporters (MCT). However, despite this linked transport, the intramuscular [H^+] may remain unchanged due to the reservoir in which the intracellular milieu exists, i.e. water (H_2O), which can provide a sink for both H^+ and OH^-. Rather the H^+ may simply be required to activate the co-transporter proteins, with the movement of lactate and its direct effect on [SID] (or the other independent variables, respectively) *causing* the change in the intramuscular [H^+] (Lindinger 1995; Kowalchuck and Scheuermann 1995). Indeed, in a previous report, the supposed 'efflux' of H^+ from the intramuscular compartment to the extracellular space was four times lower than the efflux of lactate during moderate intensity exercise, suggesting the independent transport of lactate across the muscle membrane (Putman et al. 2003). However, due to the anatomical location of the respiratory muscles this hypothesis is extremely difficult to confirm in exercising human subjects.

5.5 CONCLUSIONS

The present study investigated the effects of ITL and IMT on blood lactate recovery kinetics following maximal incremental exercise. The novel finding of this investigation is that following IMT, ITL accelerates the capacity for whole body lactate exchange and clearance. Furthermore, IMT also reduced plasma [H^+] which was accounted for by the increase in [SID] due almost exclusively to the IMT-mediated reduction in [lac^-

$]_B$. The potential mechanisms affecting lactate recovery kinetics following IMT appear similar to those observed following whole body endurance training and these adaptations may help explain the increase in whole body performance observed following IMT, particularly since superior lactate recovery kinetics are correlated with whole-body performance (Messonnier et al. 1997). Finally, the effects of ITL during recovery from intense exercise on subsequent performance following IMT present novel avenues for future study.

CHAPTER 6

DETERMINANTS OF INSPIRATORY MUSCLE STRENGTH

6.1 INTRODUCTION

Maximal inspiratory mouth pressure (MIP) is a reliable measure of global inspiratory muscle strength in health (Romer and McConnell 2004) and disease (Larson et al. 1993; Smeltzer et al. 1999). Measurement of inspiratory muscle pressure is fundamental to diagnose inspiratory muscle weakness in clinical populations (Steier et al. 2007) and the evaluation of interventions such as IMT (McConnell and Romer 2004a).

In healthy subjects, inspiratory muscle strength varies widely. Reference equations have been published, based largely on age and stature, which identify 'normal' inspiratory muscle strength (e.g. MIP measured at RV for men: $MIP_{PREDICTED} = MIP_{MEASURED}/(142-(1.03\times age)\times 100)$; women: $MIP_{PREDICTED} = MIP_{MEASURED}/(43-(0.71\times height)\times 100)$; Wilson et al. 1984). In competitive cyclists, some studies report inspiratory muscle strength values 137% of predicted (Johnson et al. 2007) whilst others, despite having a similar age, are much lower ~90% (Romer et al. 2002c). Therefore, a large variability exists in MIP between healthy, active persons. Moreover, in this population, there are no published reports of the possible parameters which may predict inspiratory muscle strength. Thus, the aim of Experiment 1 was to determine the possible predictors of MIP which may help explain the disparity in between-subject inspiratory muscle strength in healthy active people.

MIP is routinely measured to identify changes in inspiratory muscle strength during and following IMT. Baseline MIP (i.e. prior to training) may be important in determining the relative improvements in inspiratory muscle strength following IMT (Johnson et al. 2007) as the window for adaptation is reduced in those subjects with a greater baseline strength (Kraemer et al. 1996). In support of this, baseline MIP was negatively correlated with the relative IMT-induced increase in MIP in both Chapters 3 and 4 (r = -0.70, P<0.05 and r = -0.79, P<0.05, respectively). This appears to agree with findings from clinical literature (Duchenne muscular dystrophy; Winkler et al. 2000) where the increases in MIP following 9 months IMT was dose-dependent in those subjects where VC declined by less

than 10% in the year prior to the start of the study. Therefore the aim of Experiment 2 was to confirm the relationship between baseline MIP and the relative IMT-induced increase in MIP with a larger cohort of subjects and investigate whether this relationship also applied to additional measures of dynamic respiratory muscle function.

Following IMT, group mean improvements in MIP range from as little as 10% up to 55% (Leith and Bradley 1976; McConnell and Sharpe 2005; Romer et al. 2002a, b, c; Sonetti et al. 2001; Volianitis et al. 2001; Tong et al. 2008). Hershenson et al. (1988) reported that global inspiratory muscle strength may not be limited by the strength of the diaphragm but rather the relative strengths of the chest wall muscles. Therefore, low inspiratory muscle strength (and thus MIP) may be accounted for by weakness in the chest wall muscles. Consequently, large increases in MIP following IMT may be attributed to increases in the contribution of the chest wall muscles to the generation of inspiratory pressure. Therefore, the aim of Experiment 3 was to firstly evaluate the relationship between the relative contributions of the chest wall inspiratory muscles and the diaphragm to global inspiratory muscle strength and secondly, to investigate the importance of chest wall muscle strength in the IMT-mediated increases in MIP.

6.2 METHODS

6.2.1 PARTICIPANTS

Following ethical approval and written informed consent, 59 non-smoking, recreationally active subjects were recruited for the study. Subjects participated in one of 3 Experiments; the same cohort performed both Experiments 1 and 2. In all trials subjects arrived at the laboratory 2 h post-prandial having abstained from alcohol, caffeine and intense exercise in the 24 h prior to testing.

6.2.2 PULMONARY FUNCTION AND MAXIMAL INSPIRATORY MOUTH PRESSURE

Pulmonary function was assessed using a pneumotachograph and a hand-held mouth pressure meter measured MIP as an index of global inspiratory muscle strength according to sections 2.3 and 2.5, respectively. In Experiments 2 and 3 MIP was re-evaluated throughout the control and intervention periods at 2 wk intervals.

6.3 EXPERIMENT 1: CANDIDATE PREDICTORS OF INSPIRATORY MUSCLE STRENGTH

6.3.1 EXPERIMENTAL PROCEDURES

Thirty eight healthy and athletic (self-report) subjects visited the laboratory on two separate occasions (males n = 22, females n = 16). During the first laboratory visit subjects were familiarised with all testing procedures. During the second visit pulmonary function, inspiratory muscle strength, somatotype and physical characteristics were measured.

6.3.2 SOMATOTYPE AND PHYSICAL CHARACTERISTICS

Body somatotype was assessed using the Heath-Carter method (Carter and Heath 1990) and plotted using specific software (Somatotype, Sweat Technologies, Sweat Technologies, USA). This method quantifies physique through three components: 1) endomorphy, 2) mesomorphy and 3) ectomorphy. Endomorphy refers to relative fatness and was derived from three skinfold measurements including the tricep brachii, subscapular and supraspinal sites using skinfold callipers (Harpenden skinfold callipers, British Indicators, Redhill, UK). Mesomorphy describes the relative muscularity and was determined from the bi-epicondylar femur and humerus widths measured using an anthropometer (Holtain, Crymych, UK) and the arm and calf circumferences measured using a metal tape measure corrected for the site specific skinfold thickness. Ectomorphy refers to the relative linearity of the body shape and is calculated by the stature-body mass ratio ($x / \sqrt[3]{y}$: where x is stature in cm and y is body mass in kg). All measurements were assessed from the right side of the body and repeated in triplicate with the average

used for subsequent analysis. An additional skinfold was measured from the right bicep brachii to determine body density (BD) according to the method of Siri (1961):

$$\mathrm{BD} = 1.1631 - \left(0.0632 \cdot log_{10} \sum x\right)$$

1.1631 and 0.0632 are constants and $\sum x$ is the sum of the four skinfolds.

BD was subsequently used to calculate body fat percentage (%bodyfat; equation *1*), fat mass (FM; equation *2)* and fat free mass (FFM; equation *3)* using the method of Durnin and Wormersley (1974):

$$\%\mathrm{body\,fat} = \left(\left(\frac{495}{\mathrm{BD}}\right) - 450\right) \times 100 \qquad (1)$$

$$\mathrm{FM} = \frac{\%\mathrm{body\,fat}}{100} \times \mathrm{body\,mass} \qquad (2)$$

$$\mathrm{FFM} = \mathrm{FM} - \mathrm{body\,mass} \qquad (3)$$

Body mass index (BMI; equation *4*) and body surface area (BSA; equation *5*) were also calculated:

$$\mathrm{BMI} = \frac{\mathrm{body\,mass}}{\mathrm{stature}^2} \qquad (4)$$

$$\mathrm{BSA} = \frac{\mathrm{stature} \times \mathrm{body\,mass}}{3600} \qquad (5)$$

Finally, handgrip maximal voluntary isometric force was measured from the right arm using a handheld dynamometer (Smedleys, Yagami international trading co Ltd, Nagoya, Japan). Subjects were instructed to fully abduct their arm and then slowly (~5 s) adduct their arm 180° whilst gripping the device as hard as possible. The highest value of three efforts was recorded.

6.3.3 Statistical analyses

Hierarchical regression analysis was used to identify the strongest predictors of MIP. Independent candidate predictors were based on previous research (Carpenter et al. 1999; Enright et al. 1994; Harik-Khan et al. 1998; Vincken et al. 1987) and plausibility. Candidate predictors included somatotype, physical characteristics (stature, body mass, BMI, BSA), hand grip strength and pulmonary function. Statistical significance was set a-priori at $P \leq 0.05$. Data are presented as mean ± SD.

6.4 Experiment 2: Inspiratory muscle strength and inspiratory muscle training: Effects of baseline MIP

6.4.1 Experimental procedures

Prior to and following a 4 wk control period and 4 wk IMT period, thirty eight subjects (see Experiment 1 and Table 6.1) visited the laboratory on two separate occasions. During the first laboratory visit subjects were familiarised with all testing procedures. In the second visit, pulmonary function (see section 2.3), static (see section 2.5) and dynamic inspiratory muscle function and inspiratory muscle endurance were assessed. All female subjects were tested on the same day of each month following the 4 wk control and intervention periods to minimise the possible effects of the menstrual cycle on skeletal muscle contractile characteristics (Sarwar et al. 1996). Evidence suggests that handgrip strength (and thus whole body strength) is greater prior to ovulation due to increased levels

of circulating oestrogen (and possibly testosterone) which may act upon the anabolic receptors (Sarwar et al. 1996; Janse de Jonge et al. 2001).

6.4.2 DYNAMIC INSPIRATORY MUSCLE FUNCTION

Maximal dynamic inspiratory muscle function (Romer et al 2002c; Romer and McConnell 2003) was assessed by performing maximal inspiratory efforts against a pressure threshold inspiratory device (see Figure 1.24). Inspiratory mouth pressure was measured by a differential pressure transducer (± 400 cmH_2O; TSD104A, BIOPAC systems Inc., California, USA) connected to a bridge amplifier (DA100C, BIOPAC systems Inc., California, USA) inserted in to the ceiling of the pressure threshold device through a 2 mm aperture. Inspiratory airflow was measured using a pneumotachograph (TSD160A Fleisch number 3 Pneumotachograph, BIOPAC systems Inc., California, USA) connected distally to the inspiratory port of the pressure threshold device. The pressure and flow signals were passed through an analogue-digital converter and sampled at 200 Hz (Acq*knowledge* version 3.7.3, BIOPAC systems Inc., California, USA).

Maximal inspiratory pressure at zero flow (P_0) was measured by closing the air inlet port of the pressure threshold device and exposing a 1 mm leak to prevent glottic closure. Subsequently, subjects performed 3 inspiratory efforts in random order at approximately 0, 20, 25, 35, 50 and 65% P_0 separated by 30 s. During all manoeuvres, subjects stood, wore a nose clip and received both visual and verbal feedback of voluntary efforts; subjects were encouraged to inspire as fast and hard as possible. Pressure and flow fatigue is negligible following this protocol (Romer et al 2002c; Romer and McConnell 2003).

The maximal value recorded for both pressure and inspiratory flow ($\dot{V}_I$) at each $\%P_0$ was used for analysis, of which the product defined inspiratory muscle power ($\dot{W}_I$). Maximal $\dot{V}_I$ ($\dot{V}max$) was calculated by extrapolation of a linear least squares

representation of the pressure-$\dot{V}_I$ data. A 2nd order polynomial was fitted to the $\dot{W}_I$-$\dot{V}_I$ data and maximal $\dot{W}_I$ ($\dot{W}_I$max) was calculated by differentiation of the quadratic equation. Optimal $\dot{V}_I$ ($\dot{V}_{opt}$ and $\%\dot{V}max = \dot{V}_{opt} / \dot{V}max$) and optimal inspiratory pressure ($\dot{P}_{opt}$ and $\%P_0 = \dot{P}_{opt} / P_0$) were defined as the flow and pressure values corresponding to the asymptote of the $\dot{W}_I$ curve ($\dot{W}_I$max) and the point at which this vertically transected the linear representation of the pressure-flow relationship (Figure 6.1). Maximal rate of inspiratory pressure development (MRPD) was assessed during an inspiratory effort at P_0. MRPD was defined as the positive peak of the pressure derivative as a function of time or the inspiratory pressure commensurate with the greatest ΔPressure (cmH_2O) /Δtime (ms).

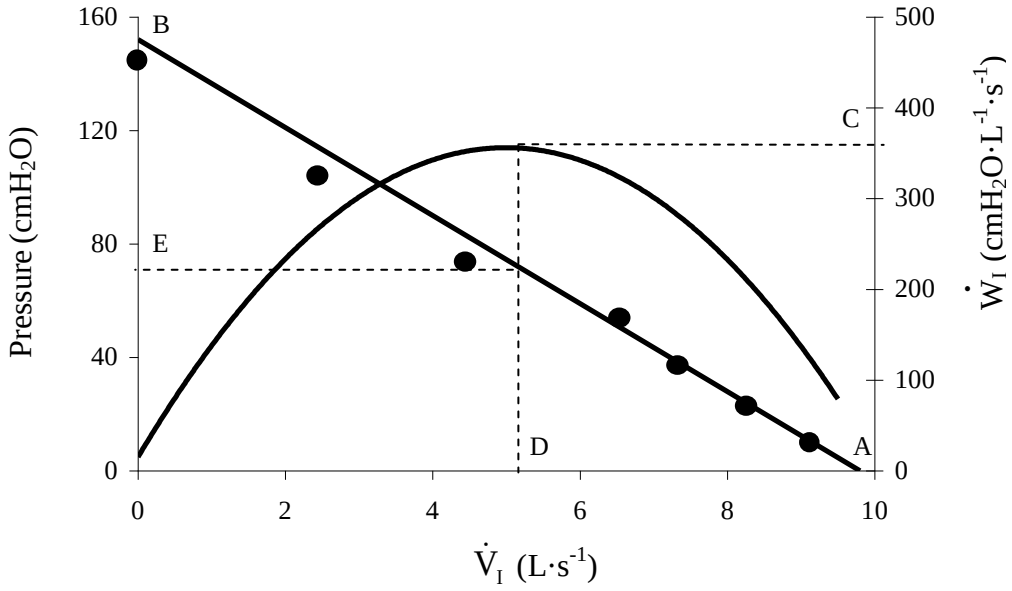

Figure 6.1 Schematic illustration of pressure-flow-work calculations. A, maximal flow at zero pressure ($\dot{V}$max); B, maximal pressure at zero flow (P_0); C, maximal inspiratory muscle power output ($\dot{W}_I$max); D, optimal inspiratory flow rate ($\dot{V}_{opt}$); E, optimal inspiratory pressure ($\dot{P}_{opt}$).

6.4.3 INSPIRATORY MUSCLE ENDURANCE

Inspiratory muscle endurance was assessed on two occasions (the first being a familiarisation session) using a weighted plunger pressure threshold loading device (Johnson et al. 1997; Nickerson and Keens 1982; see section 5.2.4; Figure 5.1). Resistance started at 10 cmH_2O and was increased by adding brass weights (5 cmH_2O) to the plunger

every min until task failure. f_R and T_I/T_{tot} were paced by a custom made audio metronome (f_R = 15 breaths·min^{-1}, T_I/T_{tot} = 0.5). The breathing pattern was chosen to reflect the typical spontaneous breathing pattern adopted during incremental threshold loading using the same device (Johnson et al. 1997). Subjects were seated in an upright position and wore a nose clip. Inspiratory mouth pressure was measured using a differential pressure transducer (TSD104A) inserted into the pressure transducer aperture of the ITL device. V_T was measured continuously during ITL using a Fleisch number 3 pneumotachograph (TSD160A) attached to the inspiratory air inlet port of the ITL device and online integration of inspiratory flow. Subjects were instructed to maintain the prescribed target V_T (which was equal to the resting V_T) as closely as possible. Pilot work indicated that mimicking the target breathing pattern prevented changes in arterialised blood PCO_2. Task failure (endurance time) was defined as the inability to maintain V_T for three consecutive breaths despite verbal encouragement.

6.4.4 STATISTICAL ANALYSES

Pre and post measures of respiratory muscle and pulmonary function were assessed using a paired t-test. Pearson's product moment correlation was used to asses the relationship between baseline MIP and the IMT-mediated improvements in dynamic inspiratory muscle function and endurance. Statistical significance was set a-priori at $P \leq 0.05$. Data are presented as mean ± SD.

6.5 EXPERIMENT 3: TRAINING THE INSPIRATORY MUSCLES AFFECTS THE CONTRIBUTION OF THE CHEST WALL INSPIRATORY MUSCLES AND THE DIAPHRAGM TO GLOBAL INSPIRATORY MUSCLE STRENGTH

6.5.1 EXPERIMENTAL PROCEDURES

Prior to and following a 4 wk intervention 20 subjects (males n = 16, females n = 4) attended the laboratory on two occasions separated by approximately one week. All female subjects were tested on the same day of each month following the 4 wk control and intervention periods to minimise the possible effects of the menstrual cycle on skeletal muscle contractile characteristics (Sarwar et al. 1996).

During the first session participants were familiarised with all procedures. Subsequently pulmonary function was measured according to the procedures outlined in section 2.3. In the second trial subjects arrived at the laboratory at least 2 hr post-prandial to minimise the effects of the gastric contents on transdiaphragmatic pressure (Man et al. 2002), were fitted with oesophageal and gastric balloon catheters and performed 10 maximal Müeller manoeuvres using a mouth pressure meter (see section 2.5). Five efforts were performed from RV and following a 5 min break, 5 were performed from FRC. Measurements of MIP were recorded at RV as recommended by the British Association of Sport and Exercise Sciences (McConnell 2007) and the American Thoracic Society (Green et al. 2002). Measurements were also preformed at FRC since the elastic recoil pressure from the lung and chest wall (>30cmH_2O) are negligible (Green et al. 2002). When performing efforts from FRC, end-expiratory lung volume was controlled by ensuring a similar end expiratory Poe (-2.0 to -5.0 cmH_2O; Romer et al. 2007a). All inspiratory efforts were performed upright and standing to minimise the compressive effects of the mediastinal compartment on Poe (Knowles et al. 1959; Baydur et al. 1982) and with arms and hands relaxed. The mouth pressure meter was secured in place by a table-mounted clamp which was adjusted vertically to align with the mouth (Figure 6.2). Subjects were instructed to inspire maximally and avoid using non-respiratory muscles.

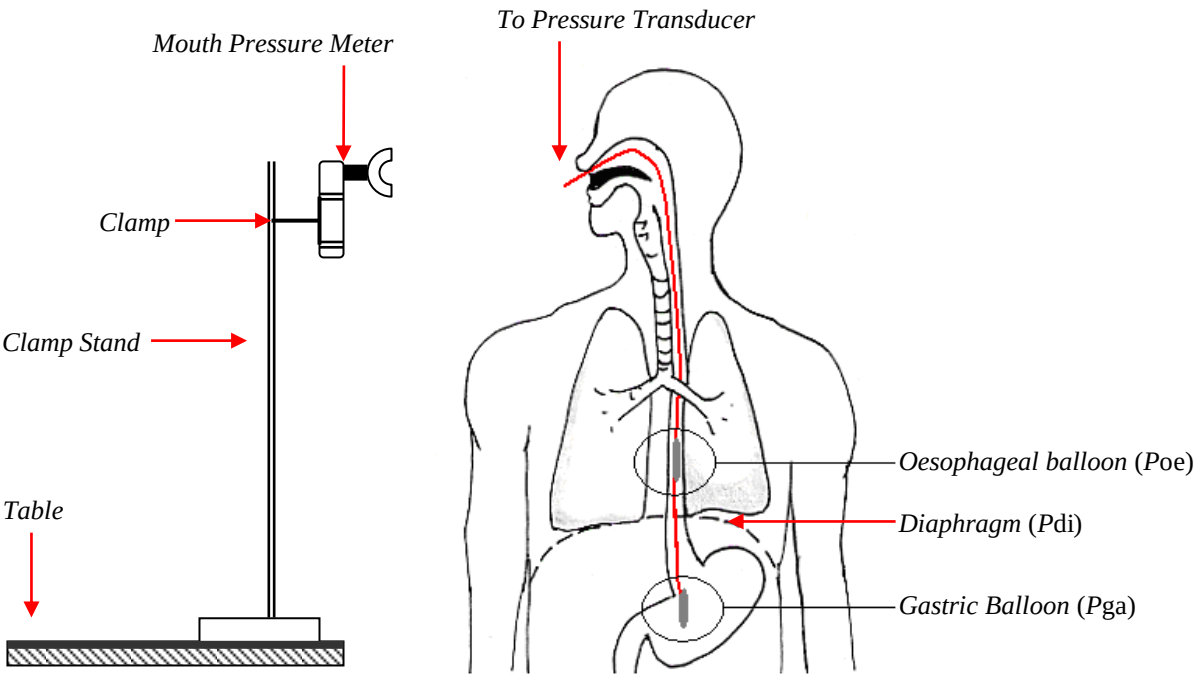

Figure 6.2 Schematic illustration of the placement of nasopharyngeal balloons in the lower third of the oesophagus and the stomach and the table mounted clamp stand and mouth pressure meter.

6.5.2 PRESSURE MEASUREMENTS

Oesophageal (*P*oe) and gastric pressures (*P*ga) were measured using two thin walled (~0.6 mm) latex balloons (10 cm in length) sealed over a single polyethylene catheter (Nspire health, Oberthulba, Germany; Figure 6.2). The balloon catheters were passed through the nasal passage into the stomach and lower one third of the oesophagus following local anaesthesia of the nasal mucosa and posterior pharynx (2% lidocaine; Instillagel®; FARCO-PHARMA GmbH, 50670, Köln, Germany). Using a glass syringe the oesophageal and gastric balloons were filled with 1 and 2 ml of air, respectively, according to their optimal pressure-volume characteristics (Mead et al. 1954; Figure 6.3). In brief, following insertion of the balloon catheter, both balloons were filled with air (0 to 5 ml at 1 ml intervals) using a glass syringe in order to elucidate the optimal balloon volume for intrathoracic pressure measurements.

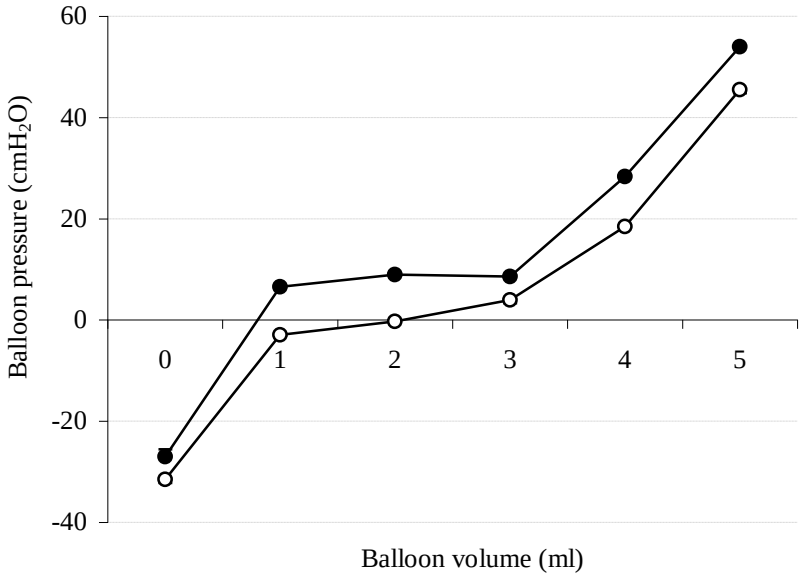

Figure 6.3 Pressure-volume characteristics of the oesophageal (*P*oe; ○) and gastric (*P*ga; ●) balloons measured at resting end-expiratory lung volume (FRC).

Both balloons were initially positioned in the stomach, such that a positive deflection was observed in *P*oe and *P*ga. The catheter was then withdrawn until, during repeated sniffs, *P*oe became negative. Subsequently, the balloon was retracted a further 10 cm, approximately 35 to 45 cm distal to the nares (Baydur et al. 1982; Benditt 2005). Correct positioning of the oesophageal balloon was confirmed with a *P*oe of -2 to -5 cmH_2O at FRC and by close agreement with mouth pressure during dynamic inspiratory efforts and a Müeller manoeuvre (occlusion technique; Baydur et al. 1982; Benditt 2005). Each catheter was connected distally to a differential pressure transducer (TSD104A) and recorded by specific data acquisition software at 200 Hz (Acq*knowledge*). *P*di was calculated by online subtraction of *P*oe from *P*ga.

6.5.3 STATISTICAL ANALYSES

MIP, *P*di, *P*ga and *P*oe were obtained from the inspiratory effort that provided the highest *P*di. The pattern of relative chest wall muscle recruitment was expressed by the *P*oe/*P*di ratio as described by Nava et al. (1993). Pre and post measures of respiratory muscle strength and pulmonary function were assessed using a paired t-test. Pearson's product moment correlation coefficients were calculated to assess the relationship between

baseline MIP and baseline *P*oe/*P*di and the relationship between the IMT-mediated improvements in MIP and *P*oe/*P*di. Statistical significance was set a-priori at $P \leq 0.05$. Data are presented as mean ± SD.

6.6 RESULTS

6.6.1 EXPERIMENT 1

Participants' physical characteristics are shown in Table 6.1 and somatotype is shown in Figure 6.4. Pulmonary function and inspiratory muscle strength are shown in Table 6.4 (see *Baseline* column).

Table 6.1 Physical characteristics of the subjects.

	n = 38	
Age (years)	22.8	(18 – 43)
Body mass (kg)	70.4	(54.0 – 107.0)
Stature (m)	1.72	(1.59 – 1.85)
BMI ($Kg \cdot m^2$)	23.6	(19.4 – 36.1)
BSA (m^2)	3.4	(2.4 – 5.4)
% body fat	16.7	(7.6 – 25.1)
Sum of skinfolds (mm)	41.1	(18.0 – 76.9)
Total fat mass (kg)	11.8	(5.7 – 24.5)
Fat free mass (kg)	58.3	(44.3 – 83.2)
Handgrip strength (kg)	40.7	(26.0 – 65.0)
Somatotype (AU)	3.51 – 4.04 – 2.24	(1.4–6.2) – (1.5–8.0) – (0.7–4.2)

Values are expressed as means (range). Note: Somatotype statistic expressed as: endomorphy – mesomorphy – ectomorphy; AU, arbitrary units.

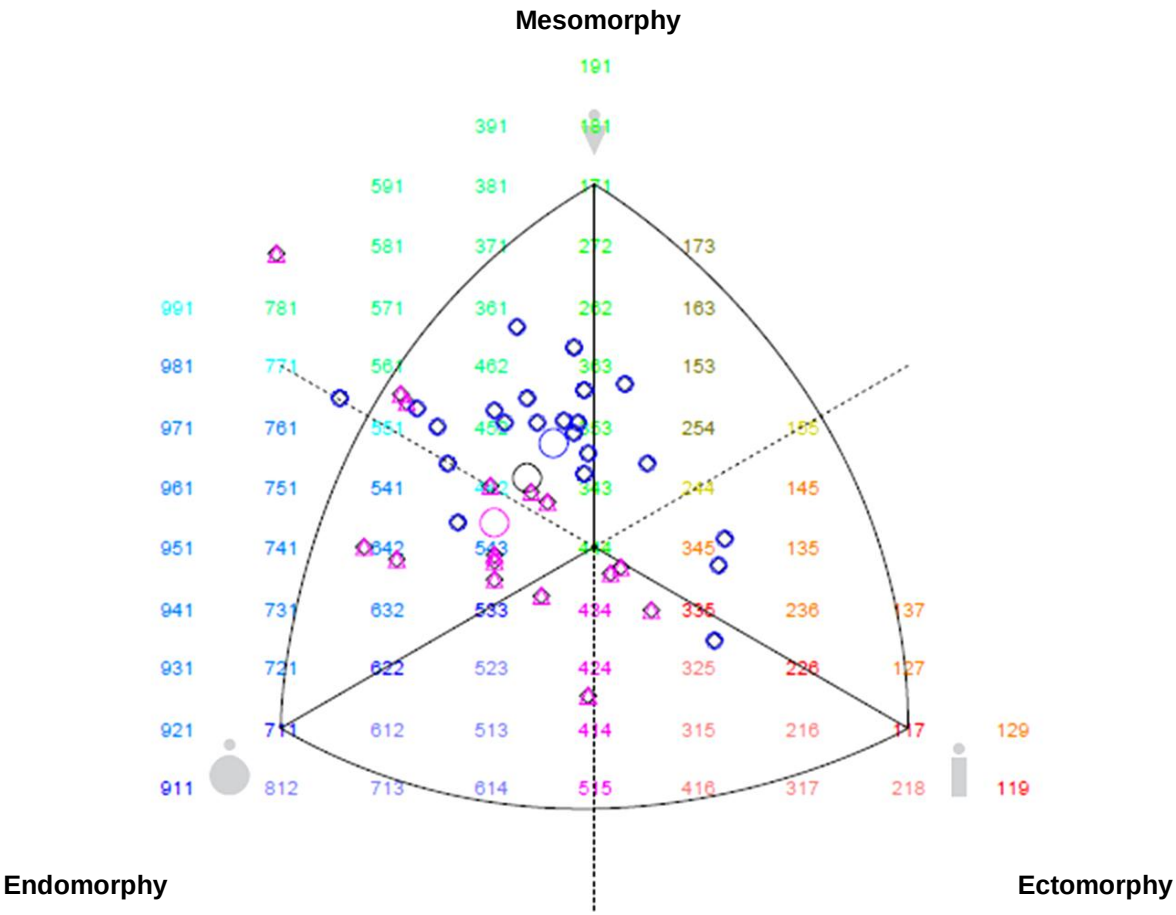

Figure 6.4 Somatoplot of participants. Blue circles, males; pink triangles, females; big blue circle, male group mean; big pink circle, female group mean; big black circle, Pooled group mean. ●, Endomorphy; ▼, mesomorphy; ▌, ectomorphy.

Pearson's product-moment correlations were calculated between MIP and measures of body composition, physical characteristic and pulmonary function (Table 6.2). MIP was significantly correlated with handgrip strength, measures of body fat, height, weight and pulmonary function. The correlation coefficient between MIP and endomorphy and mesomorphy just failed to reach significance.

Table 6.2 Pearson's Product moment correlation coefficients for MIP and physical characteristics

Variable	r	R^2	P	Variable	r	R^2	P
Handgrip strength	0.63	0.40	0.000*	Weight	0.31	0.10	0.030*
Fat free mass	0.42	0.18	0.004*	Height	0.29	0.08	0.040*
% body fat	-0.28	0.08	0.046*	FVC	0.46	0.21	0.002*
Body surface area	0.34	0.12	0.018*	FEV_1	0.47	0.22	0.001*
Endomorphy	-0.26	0.07	0.056	PIF	0.60	0.36	0.000*
Mesomorphy	0.25	0.06	0.061	MVV_{10}	0.66	0.44	0.000*

* $P<0.05$

Using multiple linear regression analysis, significant predictors of MIP were MVV_{10}, age and handgrip strength (Table 6.3 step 3). MVV_{10} (β = .53) and handgrip strength (β = .48) were positive predictors of MIP, where as age (β = -.35) was a negative predictor of MIP ($P<0.05$; Table 6.3). These variables explained 57% of the total variance in MIP. The regression analysis was not different when conducted independently across gender.

Table 6.3 Multiple regression coefficients and constants for predictors of MIP (n = 38).

	b	**SE *b***	**β**
Step 1			
Constant	33.43	22.85	
MVV_{10}	0.76	0.15	.66*
Step 2			
Constant	0.834	22.57	
MVV_{10}	0.536	0.15	.46*
Handgrip strength	1.644	0.50	.41*
Step 3			
Constant	29.12	21.66	
MVV_{10}	0.62	0.13	.53*
Handgrip strength	1.92	0.45	.48*
Age	-2.26	0.68	-.35*

Note: R^2 = .43 for step 1 (P<0.01); ΔR^2 = .13 for step 2 (P<0.01); ΔR^2 = .11 for step 3 (P<0.01). * P<0.01. *b* coefficient; SE *b* standard error of coefficient; β standardised coefficient.

6.6.2 EXPERIMENT 2

MIP, pulmonary and dynamic inspiratory muscle function prior to and following the control and intervention periods are shown in Table 6.4. IMT compliance was good (88 ± 10%). MIP was unchanged following the control period (Table 6.4) and increased 13 ± 13% following IMT (P<0.001, range -7 to +45%). Following 4 wk IMT, the relationship between baseline MIP and the relative increase in MIP just failed to reach significance (P=0.055, n = 38; Figure 6.5A). However, in subjects where training adherence exceeded 93%, a significant negative correlation was observed between baseline MIP and the IMT-induced relative increase in MIP (P=0.007, n = 16; Figure 6.5B). Baseline MIP was also correlated with the relative increase in $\dot{W}_I max$ (r = -0.517, P<0.01; n = 38). Interestingly, 8 of the 38 subjects showed no change in MIP following the intervention (Figure 6.5A; see

below horizontal grey line) of which 3 of these were present in the subgroup of participants with greater than 93% compliance (Figure 6.5B; see below horizontal grey line)

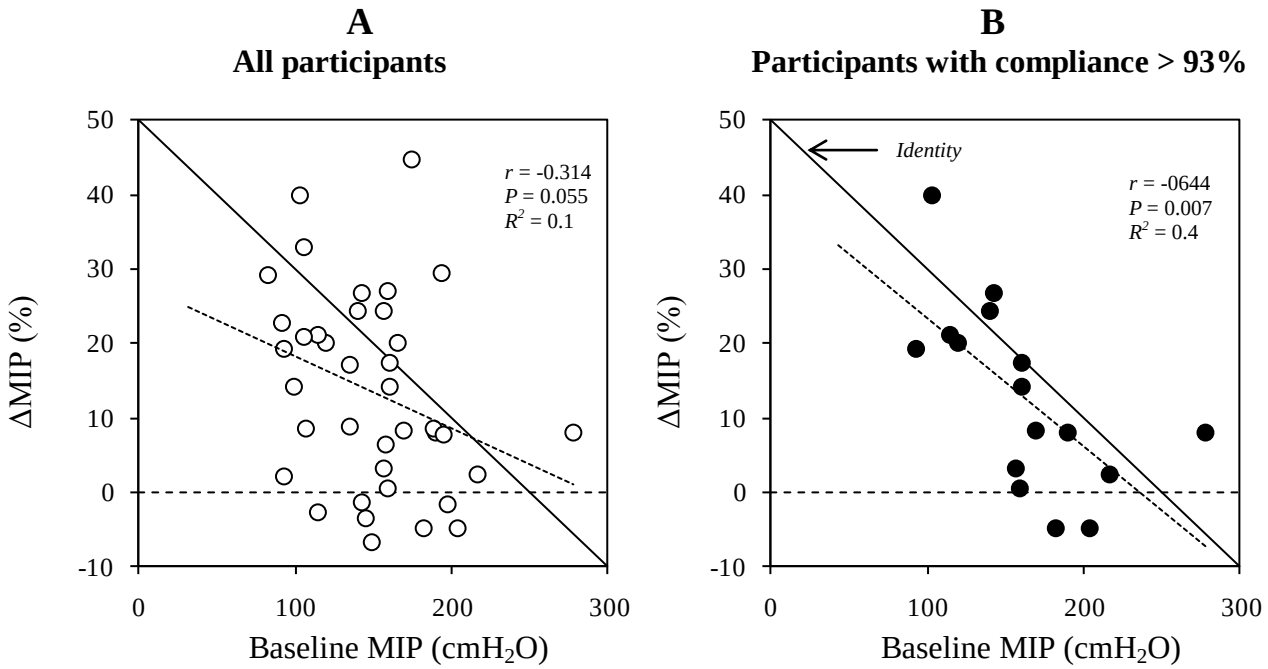

Figure 6.5 Relationship between baseline maximal inspiratory pressure (Baseline MIP) and the relative change in MIP (ΔMIP) following 4 wk inspiratory muscle training. A) n = 38: y = -0.0978x + 28.107; B) n = 16: y = -0.1713x + 40.42).

In addition, the relative change in MIP was positively correlated with the absolute and relative change in $\dot{W}_I max$ (r = 0.585, P<0.01 and r = 0.626, P<0.01, respectively) and the relative change in $\dot{P}_{opt}$ (r = 0.588, P<0.01). The relative change in MIP was also positively correlated with the absolute change in $\dot{V}_{opt}$ (r = 0.516, P<0.01), and $\%\dot{V}max$ (r = 0.562, P<0.01).

Table 6.4 Inspiratory muscle strength, pulmonary and dynamic inspiratory muscle function prior to (Baseline) and following the 4 wk control period (Post-control) and following 4 wk IMT (Post-Intervention).

	Baseline	Post-Control	Post-Intervention
Pulmonary function			
MIP (cmH_2O)	155.8 ± 46.1 (126 ± 35)	149.5 ± 42.0	168.0 ± 45.3
FVC (L)	4.63 ± 0.85 (99.1 ± 11.9)	4.63 ± 0.87	4.65 ± 0.88
FEV_1 (L)	3.85 ± 0.67 (96.2 ± 11.8)	3.78 ± 0.71*	3.80 ± 0.72
FEV_1/FVC (%)	83.6 ± 6.1 (98.0 ± 7.1)	82.1 ± 7.0*	82.0 ± 6.9
PIF ($L \cdot s^{-1}$)	6.84 ± 15.34	6.97 ± 1.66	7.14 ± 1.87
PEF ($L \cdot s^{-1}$)	8.33 ± 1.99 (81.7 ± 15.3)	10.21 ± 12.33	8.38 ± 2.0
MVV_{12} ($L \cdot min^{-1}$)	156.3 ± 33.7 (84.2 ± 19.6)	152.4 ± 36.2	158.2 ± 37.7**
Dynamic inspiratory muscle function and respiratory muscle endurance			
P_I max (cmH_2O)	154.5 ± 40.3	150.6 ± 41.2*	167.8 ± 46.4**
$\dot{V}$max ($L \cdot s^{-1}$)	7.37 ± 1.49	7.30 ± 1.53	7.51 ± 1.34
$\dot{W}_I$max ($cmH_2O \cdot L^{-1} \cdot s^{-1}$)	271.6 ± 95.5	275.6 ± 109.0	319.9 ± 102.6**
$\dot{V}_{opt}$ ($L \cdot s^{-1}$)	3.60 ± 0.76	3.62 ± 0.76	3.77 ± 0.75**
$\dot{P}_{opt}$ (cmH_2O)	67.1 ± 19.7	74.0 ± 21.0	82.1 ± 20.1**
% $\dot{V}$max (%)	49.3 ± 6.9	50.0 ± 6.0	50.2 ± 4.6
% P_I max (%)	43.5 ± 8.4	49.6 ± 7.2	49.8 ± 6.2
MRPD ($cmH_2O \cdot ms^{-1}$)	0.51 ± 0.22	0.48 ± 0.19	0.73 ± 0.62**
ITL (min)	13.48 ± 4.58	13.59 ± 5.34	16.11 ± 4.48**

Values are expressed as means ± SD. * $P<0.05$ vs. baseline; ** $P<0.05$ vs. post-control. Values in parentheses represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984).

6.6.3 EXPERIMENT 3

The physical characteristics, pulmonary function and baseline inspiratory muscle strength of the control and IMT groups are shown in Table 6.5. One subject from the IMT group was omitted from the experiment due to difficulties with balloon catheter insertion.

Table 6.5 Descriptive characteristics of the control and IMT groups.

	Control (n = 11)	IMT (n = 9)
Age (years)	27.0 ± 4.52	21.3 ± 2.9*
Body mass (kg)	75.1 ± 8.2	72.4 ±10.1
Height (cm)	179.9 ± 7.7	175.8 ± 6.0
FVC (L)	5.43 ± 0.92 (103.8 ± 14.1)	4.92 ± 0.66 (99.7 ± 9.04)
FEV_1 (L)	4.22 ± 0.78 (95.5 ± 13.4)	3.92 ± 0.77 (92.0 ± 9.8)
FEV_1/FVC (%)	77.7 ± 7.4 (89.2 ± 8.62)	86.9 ± 23.0 (99.1 ± 25.5)
PIF ($L \cdot s^{-1}$)	9.16 ± 1.58	7.83 ± 1.88 *
PEF ($L \cdot s^{-1}$)	10.04 ± 1.81 (83.4 ± 14.5)	8.43 ± 1.64 (78.5 ± 14.6)
MVV_{10} ($L \cdot min^{-1}$)	186.1 ± 36.4 (103.5 ± 17.2)	172.4 ± 41.0 (105.9 ± 30.5)
MIP (cmH_2O)	155.3 ± 43.8 (142 ± 47)	169.7 ± 48.4 (171 ± 47)

* P<0.05 between groups; values in parentheses represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984)

Global inspiratory muscle strength and intrathoracic pressures at RV and FRC are shown in Table 6.6 for both groups. There were no differences in any measure of pressure in the control group at RV and FRC prior to or following the intervention.

MIP increased significantly following 4 wk IMT by 11 ± 15% (P<0.05). Significant increases were also observed in Poe (14 ± 11%; P<0.01), Pdi (9 ± 9%; P<0.05) and Poe/Pdi (5 ± 5%; P<0.05) at RV and Poe (18 ± 13%; P<0.01), Pdi (15 ± 14%; P<0.05) and Poe/Pdi (3 ± 3%; P<0.05) at FRC (see Table 6.6).

Table 6.6 Pressure responses to maximal inspiratory manoeuvres in the control and IMT groups prior to and following the intervention performed at residual volume (RV) and functional residual capacity (FRC).

	RV pre	RV post	FRC pre	FRC post
Control Group (*n* = 11)				
MIP (cmH_2O)	155.3 ± 43.8	151.7 ± 43.7	147.5 ± 40.2	139.1 ± 40.0
*P*oe (cmH_2O)	-129.3 ± 46.0	-129.7 ± 52.3	-132.0 ± 36.4	-133.2 ± 39.2
*P*ga (cmH_2O)	20.8 ± 24.8	23.8 ± 23.6	29.7 ± 22.0	27.6 ± 18.5
*P*di (cmH_2O)	150.0 ± 40.8	153.6 ± 36.9	161.8 ± 41.8	160.8 ± 43.8
*P*oe/*P*di (%)	85.2 ± 85.1	81.9 ± 18.6	82.2 ± 13.3	83.4 ± 11.8
IMT Group (n = 9)				
MIP (cmH_2O)	174.3 ± 49.6	192.4 ± 55.9*	136.8 ± 30.1	156.4 ± 24.5*
*P*oe (cmH_2O)	-126.3 ± 20.0	-144.6 ± 29.9**	-117.2 ± 26.9	-136.8 ± 31.5**
*P*ga (cmH_2O)	28.8 ± 27.1	24.6 ± 23.9	35.6 ± 24.9	31.2 ± 28.3
*P*di (cmH_2O)	152.1 ± 32.7	166.7 ± 39.9*	147.8 ± 33.3	168.0 ± 35.2*
*P*oe/*P*di (%)	84.4 ± 10.1	88.3 ± 12.2*	80.4 ± 14.0	82.6 ± 14.5*

* $P < 0.05$, ** $P < 0.01$ vs. pre.

Correlations at baseline: In all participants (*n* = 20), baseline MIP was significantly correlated with baseline *P*oe/*P*di at RV (*r* = 0.582, *P* = 0.014) and FRC (*r* = 0.523, *P* = 0.026) (Figure 6.6). Baseline MIP (*n* = 20) was also correlated with *P*di at both RV (*r* = 0.561, *P* = 0.012) and FRC (*r* = 0.515, *P* = 0.024). Neither the baseline MIP nor the IMT-mediated increase in MIP was correlated with any other measure of respiratory pressure.

Correlations post-intervention: Following IMT, the absolute change in MIP was correlated with the absolute (Figure 6.6) and relative change in *P*oe/*P*di at RV (*r* = 0.719, *P* = 0.044 and *r* = 0.742, *P* = 0.035, respectively) and the absolute change in *P*oe/*P*di at FRC (*r* = 0.803, *P* = 0.016). The absolute (*r* = 0.707, *P*<0.05) and relative (*r* = 0.759, *P*<0.05) increase in *P*di was correlated with the absolute increase in *P*oe at RV. At FRC, the

relative change in *P*di was negatively correlated with the absolute (r = -0.813, P<0.05) and relative (r = -0.862, P<0.01) changes in *P*oe/*P*di. Figure 6.6 (Lower right panel) demonstrates the relationship between pre- and post-IMT *P*oe/*P*di, note the tendency for post-IMT *P*oe/*P*di to fall above the line of identity.

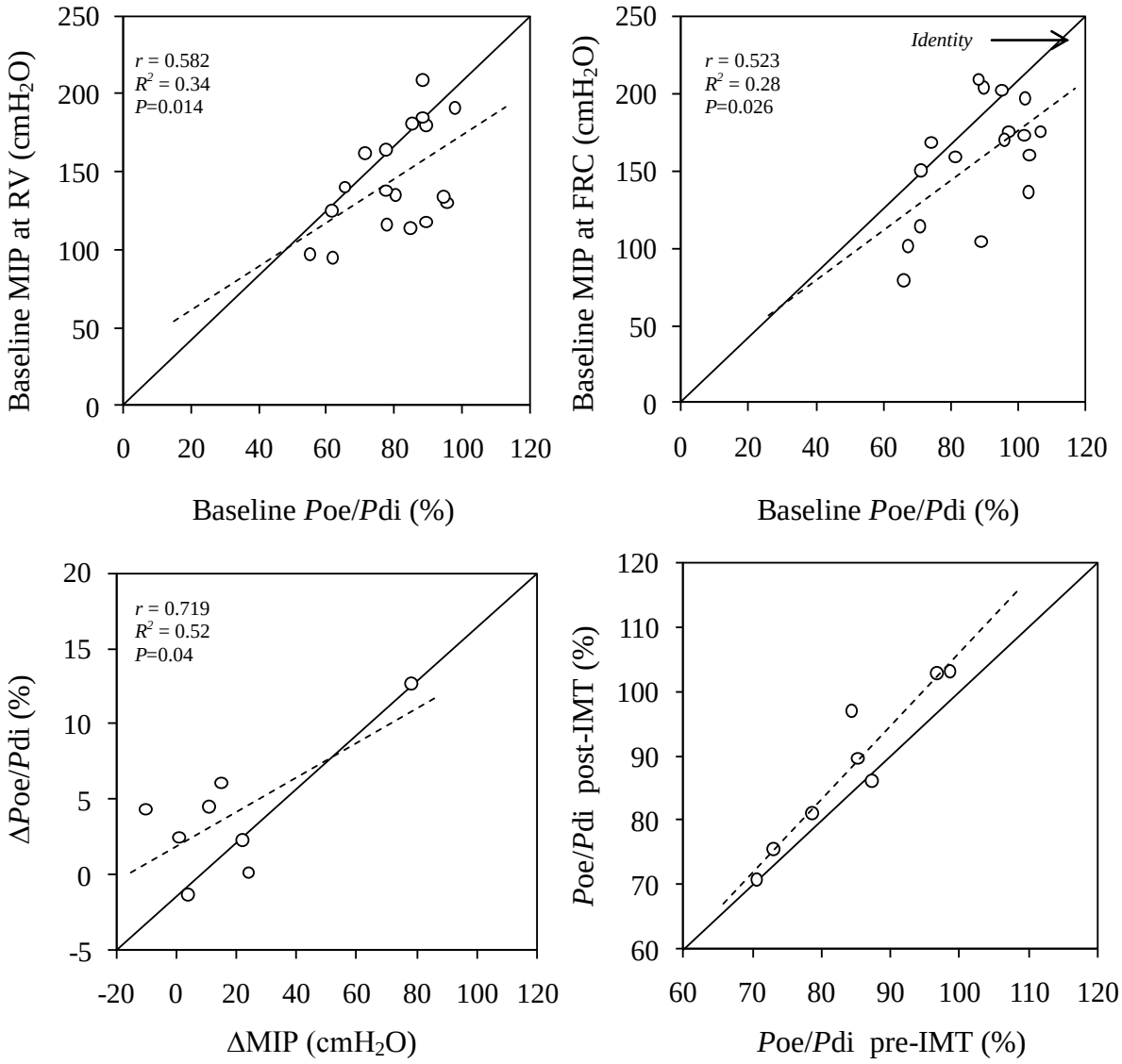

Figure 6.6 Relationship between baseline MIP at RV (Upper left) and baseline MIP at FRC (Upper right) and baseline *P*oe/*P*di, the change in *P*oe/*P*di and the change in MIP following IMT (Lower left) and finally an identity plot of pre- versus post-IMT *P*oe/*P*di (Lower right).

6.7 DISCUSSION

The aim of this study was to investigate the factors which determine inspiratory muscle strength. The main findings were: Experiment 1: in healthy athletic participants, global inspiratory muscle strength was predicted positively by MVV_{10} and handgrip strength and negatively by age. Experiment 2: when training adherence exceeded 93%, a significant negative correlation was observed between the baseline MIP and the IMT-induced increase in MIP. Experiment 3: the increase in MIP following IMT was greatest in subjects who achieved the largest increase in the relative contribution of the chest wall inspiratory muscles to global inspiratory muscle strength.

6.7.1 EXPERIMENT 1

Previous research in elderly patients with cardiovascular disease (n = 2871, age ~65 yr) reported that MIP was positively predicted by sex, FVC, handgrip strength and lean body mass and negatively by age and waist size (Enright et al. 1994). In another study, McConnell and Coopestake (1998) report that in healthy elderly subjects (n = 41, age 71 ± 7 yr) age and weight were negative predictors and stature a positive predictor of MIP. In healthy elderly males (n = 381, age 51.8 ± 17.3 yr), Harik-Khan et al. (1998) also reported that MIP was predicted negatively by age and weight but positively by PEF. In contrast to these studies, Vincken et al. (1987) reported that only age was able to significantly predict MIP (n = 46, age range 16 to 79 yr). Collectively these studies illustrate the large degree of variation in the parameters which predict MIP in elderly and clinical populations and hence, the difficulty when interpreting measures of inspiratory muscle strength in young active participants (this study).

In the present cohort (n = 38, age 22.8 ± 6.4 yr) MVV_{10} was the only measurement of pulmonary function which significantly predicted MIP. Significant correlation coefficients were reported between MIP and PIF (see Table 6.4). The novel relationships observed between MIP and pulmonary function may be due to a whole-body, training-

induced improvement in ventilatory endurance which is greater than untrained subjects (Eastwood et al. 2001). Enright et al. (1994) reported that lean body mass significantly predicted MIP. However, in the present study, no significant correlations were observed between measures of body composition (somatotype) and inspiratory muscle strength. Furthermore, only weak relationships were observed between MIP and %bodyfat, fat free mass and body surface area (see Table 6.4).

In agreement with this, McConnell and Coopestake (1998) identified that MIP is poorly predicted by measures of body composition (in the elderly). However, they did observe a significant influence of physical activity (measured by activity diary and task specific energy expenditure) in the prediction of MIP in a small subgroup of participants (*n* = 10). This may suggest that variables associated with routine physical activity are also likely to determine MIP in younger, healthy athletic populations. In support of this notion, handgrip strength was a significant predictor of MIP in the present study. In previous work, handgrip strength has been related to both aerobic fitness and whole body strength (Bassey and Harries 1993; Kay and Shephard 1969). Additionally, diaphragm thickness and MIP are greater in subjects that perform resistance (DePalo et al. 2004) and yoga training (Madanmohan et al. 2008). Thus, whether a specific measurement of training history and the effect this may have on inspiratory muscle cross sectional area could explain a larger portion of the remaining unique variance in MIP is unknown, but certainly deserves further attention. Collectively, these findings suggest that adaptations associated with chronic whole-body exercise (excluding changes in body composition) may be able to explain a larger portion of the variance in MIP in young healthy populations.

6.7.2 EXPERIMENT 2

The main findings of Experiment 2 were that when training adherence exceeded 93%, a significant relationship was observed between the IMT-mediated increase in MIP and the baseline inspiratory muscle strength. Therefore, these data suggest that a training compliance below this level may not be great enough to elicit maximal adaptations within the inspiratory muscles. However, it is important to note that despite this relationship, in the subgroup of participants with greater than 93% compliance, 3 subjects failed to show an improvement in MIP.

Initial improvements in force generation throughout strength training are a consequence of neuromuscular adaptations with a gradual increase in the contribution of muscular hypertrophy (Kraemer et al. 1996). The physiological scale of adaptation in response to strength training is suggested to be inversely related to the baseline training status, therefore, the closer the individual is to their physiological ceiling, the smaller the window for adaptation (Åstrand et al. 2003; Häkkinen 1994). However, the mechanisms that account for this are not well understood. It has been suggested that this may be due to changes in the hormonal response during training (Ahtiainen et al. 2003) which could delay the increase in muscle fibre size (Kraemer et al. 1996).

The constraining effect of baseline training status on subsequent adaptation appears to apply to the respiratory muscles (see Figure 6.5B) and is similar to findings reported in clinical populations (Winkler et al. 2000). In previous studies, the improvements in global inspiratory muscle strength following IMT have ranged widely from as little as 10% up to 55% (Leith and Bradley 1976; McConnell and Sharpe 2005; Romer et al. 2002a, b, c; Sonetti et al. 2001; Volianitis et al. 2001; Tong et al. 2008). In the studies where large improvements in MIP were observed, participants' baseline MIP was considerably lower (Romer et al. 2002b, c; Volianitis et al. 2001). The present data have also demonstrated this trend, as in Experiments 2 and 3 the improvements in MIP were only 13 and 11% which is likely due to the high baseline MIP (126 and 171% predicted, respectively).

The inspiratory muscles have a number of highly specific functions which are vastly different to other muscle groups. For example, the precise coordination of a number of inspiratory muscles is required to increase thoracic volume with minimal distortion to the chest wall away from the relaxed configuration (Kenyon et al. 1997). It is suggested that the maximal pressure generating capacity of the inspiratory muscles is dependent upon the strength of the chest wall muscles since this muscle group is weaker than the diaphragm (Hershenson et al. 1988). Therefore, the significant negative correlation reported between baseline MIP and the IMT-induced increase in MIP may reflect the superior chest wall muscle strength in those subjects with a high baseline strength and hence a smaller window for chest wall muscle adaptation during IMT. The opposite may be true for subjects with low baseline inspiratory muscle strength and account for their larger increase in MIP. In summary, the findings of Experiment 2 demonstrate that the increase in inspiratory muscle strength following IMT is inversely related to baseline MIP. Data presented in Experiment 3 and discussed in section 6.7.3 support this hypothesis.

It is interesting to note that following 4 wk IMT, 8 of the 38 subjects showed no improvement or even a lower MIP relative to pre-intervention baseline measures. In this sub-group, training adherence was good (88 ± 10%) suggesting that this did not account for the lower MIP. However, baseline MIP was 142 ± 24% of predicted and much higher than the remaining participants (n = 30: 124 ± 37%). This may suggest that the baseline MIP relative to the predicted value may be more important in determining the IMT-mediated improvements in inspiratory muscle strength. Of the 8 subjects within this sub-group, 3 were also studied in Experiment 3. Remarkably, all showed an increase in Poe (n = 3: 21 ± 10 cmH_2O). Why the increase in Poe failed to increase MIP is unknown. One possibility is that following IMT, abdominal compliance was increased. An increased abdominal compliance would lower Pga as the diaphragm descends during the inspiratory effort and attenuate the rise in Pdi. A small reduction in Pga was observed in these subjects following the intervention (~15 cmH_2O) which may support this notion. However, despite

the decrease in *P*ga, improvements were observed in *P*di (9 ± 29 cmH_2O) as the IMT-mediated change in *P*oe exceeded the change in *P*ga. An additional explanation for an increase in *P*oe yet no change in MIP may reside in the nature of volitional tests of inspiratory muscle strength. It is well known that volitional techniques are dependent upon task learning and motivation. Thus, improvements in MIP following IMT may be due to subjects getting better at performing the test (Polkey and Moxham 2004). Thus the subjects may have simply performed the test poorly on the day of measurement. In support of this, it was previously reported that volitional measures of inspiratory muscle pressure development were unchanged despite an altered (non-volitional) muscle function (Johnson et al. 1993) illustrating that volitional measures of intrathoracic pressure may not reflect true changes in muscular strength. Notwithstanding this, examination of MIP in the 8 subjects following 2 wk IMT illustrated a small but significant increase in MIP (3%; n = 3: P=0.017; n = 8: P=0.024). However, it can not be ruled out that some of the participants simply did not perform the prescribed IMT. Indeed, assessing the compliance of the subjects to the IMT is difficult and likely reflects an important flaw in self-report training diaries and unsupervised training sessions. Notwithstanding this, these data suggest that the lack of change in MIP following 4 wk IMT may also have been due to factors related to task learning and the ability to perform the test successfully and / or the baseline MIP relative to the predicted value.

6.7.3 EXPERIMENT 3

The main findings of Experiment 3 were that prior to 4 wk IMT, MIP was significantly correlated with the relative chest wall muscle recruitment as expressed by the *P*oe/*P*di ratio (Nava et al. 1993). Furthermore, following IMT, the absolute increase in MIP was positively correlated with both the absolute and relative increase in *P*oe/*P*di. These findings in combination with the findings presented in Experiment 2, suggest that the baseline status of the inspiratory muscles, specifically the chest wall inspiratory

muscles is a key parameter which may, in part, determine the increase in inspiratory muscle strength following pressure threshold IMT.

Hershenson et al. (1988) hypothesised that during a maximal inspiratory effort, in order to preserve the geometry of the thorax (thoracoabdominal configuration) the force generated by all inspiratory muscles must be equal. If the force generated by the diaphragm exceeded the chest wall muscles, the diaphragm would be shortened and chest wall muscles would be lengthened. This would reduce the mechanical advantage of these muscles, and the rib cage/abdomen would be displaced inward/outward, respectively, i.e. distorting the thoracoabdominal configuration of the thorax. Excessive distortion of the thorax away form the resting configuration would significantly increase the elastic work of breathing (Kenyon et al. 1997).

Hershenson et al. (1988) demonstrated that during a maximal Müeller manoeuvre, diaphragm activation was sub-maximal. This was illustrated by an increase in twitch force superimposed upon a maximal inspiratory effort. This has also been confirmed by a lower diaphragm EMG during a Müeller manoeuvre relative to a combined maximal inspiratory and expulsive effort (Nava et al. 1993). In the study of Hershenson et al. (1988) the pattern of relative chest wall muscle recruitment (*P*oe/*P*di) during the Müeller manoeuvre was 92%. In another trial, the pressure generating capacity of the chest wall muscles was increased by sealing an air tight plastic garment around the thorax and creating a negative body surface pressure using a vacuum cleaner. By increasing the pressure generating capacity of the chest wall muscles (artificially increasing *P*oe), *P*di was greater during subsequent efforts. In this trial, the *P*oe/*P*di increased to 96%. These findings clearly demonstrate that global inspiratory muscle strength was limited by the maximal strength of the chest wall muscles and not the maximal strength of the diaphragm.

The pre-intervention data from Experiment 3 support the findings of Hershenson et al. (1988). Here, a significant positive correlation between MIP and *P*oe/*P*di was observed suggesting that subjects with low inspiratory muscle strength had the weakest chest wall

muscles and the smallest relative contribution of the chest wall muscles (and therefore the diaphragm) to global inspiratory pressure generation. The *P*oe/*P*di ratio reported for both groups in Experiment 3 (~80 to 85%; see Table 6.6) was similar to that reported previously (Fittting et al. 1988; Nava et al. 1993) although lower than those of Hershenson et al. (1988). This is explained by the different methodologies used to measure inspiratory muscle strength. In the study of Hershenson and colleagues, participants performed the Müeller manoeuvre with the glottis closed compared to open in this study. Closing the glottis during a Müeller manoeuvre minimises abdominal muscle recruitment and reduces *P*di.

In the present study, following IMT *P*oe/*P*di increased significantly from 84 and 80% to 88 and 83% at RV and FRC, respectively, and was significantly correlated with the increase in MIP. The increase in the contribution of the chest wall muscles to inspiratory pressure generation (~4%) is similar to that reported by Hershenson et al. (1988) when a negative pressure was applied to the thorax (~4%). These findings are the first to show that the greatest increase in MIP probably occurs in subjects who have the lowest chest wall muscle strength at baseline and the largest increase in chest wall muscle strength following IMT. This is further supported by the increase in post-IMT *P*oe at RV and FRC from -126.3 ± 20.0 and -117.2 ± 26.9 cmH_2O at baseline to -144.6 ± 29.9 and -136.8 ± 31.5 cmH_2O, respectively, and also by the significant post-IMT correlation between the increase in *P*oe and the increase in *P*di.

The notion that the chest wall muscles may receive the largest training stimulus throughout pressure threshold IMT was proposed previously (McConnell et al. 2002) and the present findings are the first to confirm this hypothesis. Although it was not the aim of this study to identify the mechanism(s) accounting for greater chest wall and diaphragm muscle strength following IMT, it may be due to a reduced co-activation of antagonistic muscles and / or improved synchrony of motor unit firing; however these have yet to be quantified following IMT. The plasticity of the chest wall muscles following IMT is,

however, supported by previous studies in which an increase in the size and prevalence of type I and type II muscle fibres was observed following 5 wk IMT (Ramírez-Sarmiento et al. 2002). However, it is important to note that IMT does not target the chest wall muscles *per-se* as significant increases in diaphragm thickness are also observed following IMT (Downey et al. 2007; Chiappa et al. 2008a; Enright et al. 2006). This is supported by the present findings which demonstrate a significant increase in *P*di from 152.1 ± 32.7 and 147.8 ± 33.3 cmH_2O at baseline to 166.7 ± 39.9 and 168.0 ± 35.2 cmH_2O at RV and FRC, respectively, following 4 wk IMT. The precise temporal changes in chest wall and diaphragm adaptation during IMT have yet to be reported. Therefore, whether adaptation of the chest wall with strength training precedes that of the diaphragm or even signals its adaptation is unknown and would certainly be an interesting avenue for future research. It would also be interesting to investigate the effects of negative chest wall pressure (see Hershenson et al. 1988) throughout individual IMT sessions on subsequent global inspiratory muscle strength.

6.8 CONCLUSIONS

Experimental data demonstrates that MIP in young healthy subjects is difficult to predict and that measures of training status and chronic whole-body training-induced alterations in inspiratory muscle function may provide novel parameters relevant to this population for future study. Evidence is provided illustrating that the baseline inspiratory muscle strength limits the IMT mediated increase in MIP. The mechanisms that may account for this relationship appears to be the relative strengths of the chest wall muscles and their proximity with their ceiling for adaptation since following IMT, a large increase in chest wall muscle strength significantly increases global inspiratory muscle strength. These findings highlight the importance of baseline inspiratory muscle strength in determining the magnitude of adaptation and may well explain the disparity in the relative increase in MIP previously reported within the literature.

CHAPTER 7

GENERAL DISCUSSION

7.1 MAIN FINDINGS

The aim of the thesis was to investigate the physiological consequences of the work of breathing during intense endurance exercise and of specific inspiratory muscle training. The main findings demonstrate that:

I) During intense exercise in which pulmonary ventilation increases to near-maximal levels, the respiratory muscles are capable of contributing to the systemic $[lac^-]_B$. This was demonstrated in Chapters 3 and 4 where a ~25% increase in $[lac^-]_B$ was observed when isocapnic volitional hyperpnoea with a breathing pattern matched to that of 85% and 90% $\dot{V}_E$max was mimicked whilst at rest (Chapter 3) and also whilst exercising at the power output corresponding to the MLSS (Chapter 4).

II) Following specific IMT, the volitional hyperpnoea-mediated increases in systemic $[lac^-]_B$ were reduced by ~25% when performed both at rest (Chapter 3) and upon exercise (Chapter 4). A significant reduction in the steady state $[lac^-]_B$ was also observed following IMT during exercise at the MLSS by 8 to 15%. These findings are the first to demonstrate that the inspiratory muscles are the likely source of these reductions and probably explain the reductions in $[lac^-]_B$ often observed during whole-body exercise following RMT.

III) A previous study suggested that the application of a low-intensity pressure threshold inspiratory resistance (15 cmH_2O) following maximal exercise significantly accelerated lactate clearance (Chiappa et al. 2008b, 2009). In stark contrast to this study, using an identical exercise and recovery protocol, we observed no difference in the recovery of lactate following maximal exercise when performed with or without inspiratory muscle loading (Chapter 5). However, in the same study, following 6 wk IMT, relative to pre-intervention, loading the trained inspiratory muscles at the cessation of maximal incremental exercise for 20 min decreased the mean $[lac^-]_B$ by ~20% and

increased inspiratory muscle lactate exchange and clearance capabilities by ~70%. The significant decrease in $[lac^-]_B$ caused an increase in the [SID] which was responsible for a reduction in plasma $[H^+]$.

IV) Finally, this thesis presents novel evidence that the relative gains in inspiratory muscle strength following IMT as measured by the maximal inspiratory pressure generated at the mouth (MIP) is dependent upon the baseline strength (Chapter 6). Specifically, this was due to a lower contribution by the chest wall inspiratory muscles relative to the diaphragm (*P*oe/*P*di) to the evolution of inspiratory muscle pressure (Chapter 6). Consequently, following IMT, the relative increase in *P*oe/*P*di was positively correlated with the relative increase in MIP.

7.2 THE WORK OF BREATHING: A LIMITING FACTOR DURING INTENSE EXERCISE?

7.2.1 THE WORK OF BREATHING

Historically, the respiratory system was not considered a limiting factor of whole-body endurance performance. This was based upon the observation that MVV performed at rest surpassed $\dot{V}_E$max, thus a substantial breathing reserve existed and that the SaO_2 rarely fell below ~98% even during intense exercise in untrained subjects (Dempsey 1986). Furthermore, it was well recognised that the respiratory muscles, in particular the diaphragm, was extremely well evolved for the physiological demands of exercise. During sub-maximal exercise, the respiratory muscles may require ~10% of the available $\dot{Q}$ and whole body $\dot{V}O_2$ (Harms et al. 1997; Aaron et al. 1992a, b). However, when exercise intensity exceeds ~85% $\dot{V}O_2$max, the work of breathing increases dramatically with the respiratory muscles demanding up to 15% of the available $\dot{Q}$ and whole body $\dot{V}O_2$ (Harms et al. 1997; Aaron et al. 1992b). Indeed, during intense whole-body exercise,

respiratory muscle perfusion when expressed relative to muscle mass is greater than all other skeletal muscles (Manohar 1986).

In addition to this large metabolic demand, during intense endurance exercise, in order to maintain high expiratory flow rates, the EELV and EILV are increased to a greater percentage of TLC and f_R is increased by the recruitment of accessory respiratory muscles typically when V_T reaches ~65% of VC (Dempsey 1986). Due to the length-tension and force-velocity relationships of the inspiratory muscles, the greater EILV and f_R increases the length and velocity of shortening of the diaphragm, respectively, reducing the capacity for inspiratory muscle pressure generation (LeBlanc et al. 1988; Johnson et al. 1993). Consequently, the functional weakening of the inspiratory muscles throughout intense exercise and the competition with exercising locomotor muscles for the available $\dot{Q}$ may compromise diaphragmatic perfusion and O_2 transport and ultimately manifest in diaphragm fatigue (Babcock et al. 1998; Johnson et al. 1993).

At exercise intensities which exceed ~85% $\dot{V}O_2$max diaphragm fatigue can occur which may, through a sympathetically mediated reduction in locomotor perfusion, limit locomotor force generation. Thus it is evident that under certain circumstances, most notably those of intense endurance exercise, the work of breathing and its physiological consequences may well contribute to exercise intolerance.

7.2.2 DIAPHRAGM FATIGUE AND THE RESPIRATORY MUSCLE METABOREFLEX: THE QUINTESSENCE OF RESPIRATORY MUSCLE LIMITATION TO EXERCISE?

Exercise-induced diaphragm fatigue is well documented when the exercise intensity meets or exceeds approximately 85% $\dot{V}O_2/\dot{W}$ max and is sustained to the limit of volitional tolerance (Johnson et al. 1993, Babcock et al. 1995, 1996, 1998, 2002). However, the functional importance of diaphragm fatigue is questionable. It has been argued that diaphragm fatigue causes a gradual reduction in the work performed by the diaphragm relative to the entire respiratory system (Figure 7.1), however despite this,

inspiratory accessory muscle recruitment is increased which facilitates further increases in $\dot{V}_E$ and maintains arterial blood gas tensions (Johnson et al. 1993).

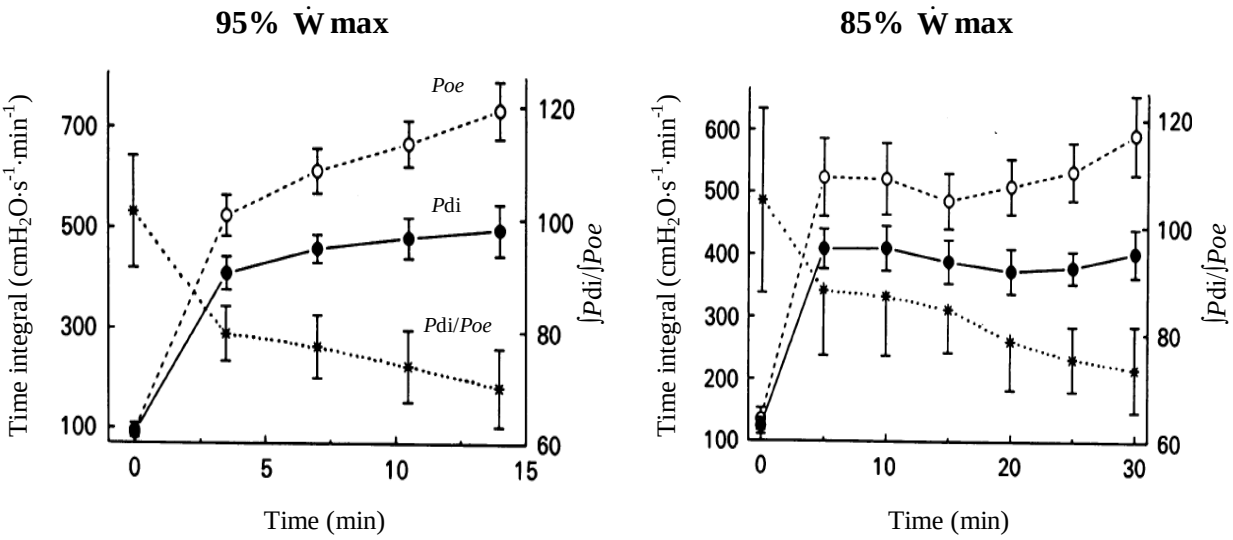

Figure 7.1 Inspiratory chest wall muscle (*P*oe; ○) and diaphragm (*P*di ; ●) work performed throughout exercise to exhaustion at 95% Ẇ max (left) and 85% Ẇ max (right). Also shown in both graphics is the ratio: ∫*P*di/∫*P*oe throughout exercise (···✱···; Johnson et al. 1993).

The physiological importance of exercise-induced diaphragm fatigue appears to be a sympathetically-mediated reflex reduction in locomotor blood flow (Sheel et al. 2001; St. Croix et al. 2000) secondary to an increased limb vascular resistance. This attenuates limb O_2 delivery (Harms et al. 1997) and possibly through an increased reliance upon anaerobic processes increases locomotor muscle fatigue (Romer et al 2006). Interestingly, unloading the inspiratory work of breathing by up to 50% using a mechanical ventilator during intense exercise maintains limb blood flow and performance is improved (Harms et al. 2000). The mechanisms accounting for this improved performance appear to be both centrally and peripherally mediated (Dempsey et al. 2006b). Unloading the inspiratory muscles attenuates peripheral locomotor fatigue possibly by an increased O_2 delivery and decreased reliance upon anaerobic processes (Romer et al. 2006a). It also dampens the intensity of both respiratory and limb effort perception (Harms et al. 1997).

Recently, it was proposed that a respiratory muscle metaboreflex-mediated increase in limb muscle fatigue stimulates chemo and mechanosensitive afferent nerve endings (Dempsey et al. 2006b). The functional significance of this, as illustrated in Figure 7.2, is an increased perception of limb discomfort and consequently, reflex inhibition of central motor output to the locomotor muscles (i.e. an increased central fatigue; Dempsey et al. 2006b). Thus it appears that respiratory muscle fatigue may not only impair performance by exacerbating limb muscle fatigue via a reflex reduction in locomotor perfusion, but may also attenuate motor output from the cortical and / or sub-cortical centres to the locomotor muscles.

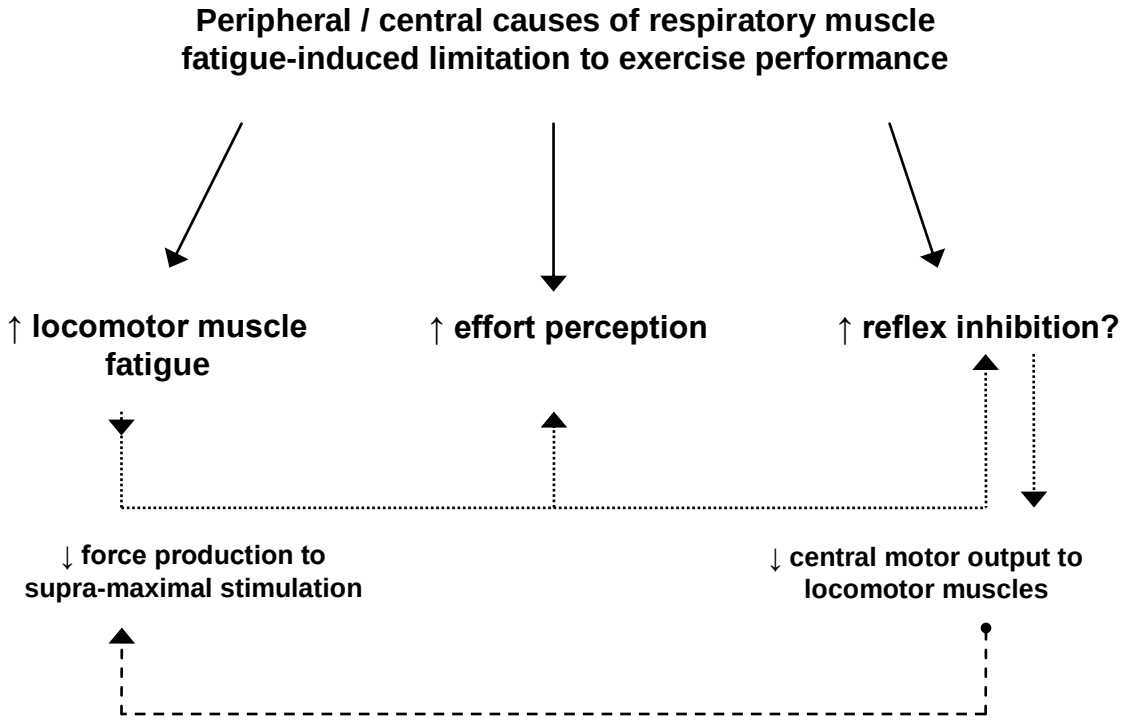

Figure 7.2 Schematic illustration of the possible peripheral and central fatigue influences on exercise tolerance caused by exercise-induced respiratory muscle work. Peripheral locomotor fatigue mediated by fatiguing respiratory muscle work exacerbates effort perception and possibly attenuates central motor output to the exercising limbs (Dempsey et al. 2006b).

Recent evidence supports the notion that locomotor fatigue impairs voluntary descending drive via somatosensory feedback (Amann et al. 2006; Amann et al. 2008b, 2009; Romer et al. 2007b). Prior to a 5 km time-trial, an interspinous ligament injection of anaesthesia in to the epidural space (which reduces the ascending activity of nociceptive

and metaboreceptive group III and IV afferents to the somatosensory cortex) increased both central motor drive and quadriceps fatigue (reduction in quadriceps twitch force: -45%) relative to a placebo control trial (-33%; Amann et al. 2008b). Therefore, it is possible that subsequent to the metaboreflex which may exacerbate locomotor muscle fatigue, this further impairs performance by reducing central motor drive to the exercising limbs.

The role of lactate in the processes outlined above is unknown. A previous study conducted on exercising dogs suggests that lactic acid infusion triggers the metaboreflex (Rodman et al. 2003), buts its precise role in attenuating central motor drive subsequent to the metaboreflex is equivocal. Lactate is known to increase the firing frequency of group III and IV afferent fibres (Balzamo et al. 1992; Graham et al. 1986; Jammes and Balzamo 1992; Jammes et al. 1986). However, following a 5 km cycling time-trial preceded by either high or low-intensity prior exercise, despite a markedly different iEMG (an indicator of central motor drive) yet a similar relative reduction in quadriceps force output and increase in $[lac^-]_B$ was observed (Amann and Dempsey 2008). This suggests a minimal role for lactate as a mediator of central motor drive. It is more likely that lactate has a far more important role in determining exercise tolerance via its effect upon acid-base balance during sub-maximal exercise and possible role for affecting perceived exertion (see 7.3.4 and 7.4.3).

In summary, there is no doubt that central motor output to the locomotor muscles is fundamental to the power output achieved during intense exercise. The respiratory muscle metaboreflex may contribute to exercise intolerance at very high intensities by both a reflex reduction in limb blood flow (and O_2 delivery) which increases peripheral muscle fatigue and the perceptions of breathing and limb discomfort. The latter may increase above a critical sensory tolerance limit and reduce the conscious drive to the locomotor muscles. Thus, the respiratory muscle metaboreflex and its physiological and psychophysical consequences may well be an important determinant of exercise

performance. However, during sub-maximal exercise as is commonly performed during competition, diaphragm fatigue does not occur and limb blood flow is preserved (Wetter et al. 1999). Under these conditions, the respiratory muscle metaboreflex is unlikely to have any bearing upon exercise tolerance. At such intensities, the conscious awareness of respiratory motor drive may well be more important (Jones and Killian 2000; Jones 2008). This notion is supported by significant reductions in dyspnoea which are observed following RMT during whole body-exercise (Edwards et al. 2008; McConnell and Romer 2004a, b; Romer et al. 2002b; Volianitis et al. 2001) and periods of intense pulmonary $\dot{V}_E$ (Chapter 4).

7.3 THE RESPIRATORY MUSCLES AND SYSTEMIC METABOLITES

7.3.1 CONTRIBUTION OF THE RESPIRATORY MUSCLES TO LACTATE TURNOVER

The contribution of the respiratory muscles to systemic lactate kinetics has been investigated for many years. Animal studies suggest the diaphragm has a minimal role in lactate production (Manohar et al. 1988; Manohar and Hassan 1990, 1991) or may engage in lactate consumption (Fregosi and Dempsey 1989). In contrast, human studies suggests that above a critical level of pulmonary $\dot{V}_E$ (~70% MVV), $[lac^-]_B$ increases possibly due to an increased recruitment of the less efficient accessory muscles. This was illustrated in Chapter 3 when intense volitional hyperpnoea was performed at rest (Freedman et al. 1983; Martin et al. 1984; Verges et al. 2007b) and in Chapter 4 when performed upon exercise (Johnson et al. 2006). It is important to note that when mimicking the work performed by the respiratory muscles, the metabolic response will most likely overestimate that of exercise hyperpnoea. Klas and Dempsey (1989) demonstrated that during a voluntary mimic task, the expiratory pressures generated exceed those produced during exercise, probably because of dynamic compression of the airways during expiration and / or sub-maximal airway dilation. Therefore, although the findings reported in Chapters 3 and 4

extend those of previous studies, a study which mimics precisely the work of breathing of very intense exercise hyperpnoea is yet to be completed.

In Chapter 3, whilst at rest, volitional hyperpnoea resulted in a significant 0.96 ± 0.58 mmol·L^{-1} (range: 0.20 to 2.50 mmol·L^{-1}, n = 22) increase in $[lac^-]_B$. In chapter 4, when volitional hyperpnoea was imposed upon cycling exercise at the MLSS, $[lac^-]_B$ increased by 0.99 ± 0.58 mmol·L^{-1} (range: 0.32 to 2.41 mmol·L^{-1}; n = 20). In previous studies which also mimicked the exercise hyperpnoea at rest or when exercising, breathing pattern was not controlled which was shown to significantly overestimate the O_2 cost of breathing relative to exercise hyperpnoea (Coast et al. 1993); subjects were instructed to achieve a fixed f_R (Freedman et al. 1983), V_T (Martin et al. 1984), fraction of MVV (Verges et al. 2007b) or the greatest $\dot{V}_E$ attainable (Johnson et al. 2006). Although the breathing pattern prescribed in Chapters 3 and 4 of the thesis are also likely to overestimate the work of breathing relative to exercise hyperpnoea, the oxidative cost was likely to be similar, therefore these data extend previous findings that when performing volitional hyperpnoea with a breathing pattern matched precisely to that achieved during near-maximal exercise, the respiratory muscles are capable of net lactate production and contribute significantly to the systemic $[lac^-]_B$.

It is clear from the data presented above and in Chapters 3 and 4 that the respiratory muscles contribute significantly to systemic lactate turnover. This is in stark contrast to the notion that the respiratory muscles are unable to alter systemic lactate kinetics (Wetter and Dempsey 2000) and clearly shows that given a high enough level of pulmonary ventilation, the respiratory muscles function anaerobically. The functional significance of this is not clear at present. It is unlikely that respiratory muscle lactate production affects the lactate threshold power output or even $\dot{V}O_2$max. It is predicted that the significance of lactate production by the respiratory muscle may be twofold. Firstly, lactate produced within the fatiguing respiratory muscles is capable of triggering the metaboreflex (Rodman et al. 2003), thus lactate production by the locomotor muscles may not necessarily be required to

initiate this response. Secondly, respiratory muscle lactate efflux may stimulate chemo and mechanosensitive afferent fibres which provide important sensory feedback to the brain regarding the intensity of breathing discomfort. Thus, respiratory muscle lactate production may have a far greater effect upon exercise tolerance during intense exercise than previously recognised.

The application of these findings to clinical populations is equivocal. The ventilatory response to exercise in patients with COPD is well described by an increase in EILV and EELV (hyperinflation) and a tachypnoeic breathing pattern where incomplete emptying of the lungs follows a forceful inspiration (McConnell 2005). Such a breathing pattern reduces the capacity of the inspiratory muscles to generate tension and therefore inspiratory flow (LeBlanc et al. 1988) which further increases central motor output and thus the sensations of respiratory effort through a heightened corollary discharge. Indeed breathlessness is known to be one of the major factors which lead to exercise termination in this population (McConnell and Romer 2004b) and the alleviation of this symptom is recommended for successful pulmonary rehabilitation programmes (Lotters et al. 2002). Importantly, it should be noted that inspiratory muscle strength is lower in patients with COPD relative to healthy individuals (Decramer 1989), but how this effects the respiratory muscle lactate turnover is unclear. Eastwood et al. (2006) investigated the arterial $[lac^-]_B$ response to incremental inspiratory threshold loading prior to and following specific IMT. Prior to training, two of seven subjects demonstrated an increase in $[lac^-]_B$ during ITL (absolute increase, range: 0.57 to 1.03 $mmol \cdot L^{-1}$). Following 8 wk IMT, five of the seven subjects showed an increase in $[lac^-]_B$ during ITL (absolute increase, range: 0.26 to 1.67 $mmol \cdot L^{-1}$). Given that only two of the subjects showed an increase in $[lac^-]_B$ prior to IMT, and that this response was increased after IMT illustrates that structural and oxidative properties of the inspiratory muscles are likely to differ between COPD patients and healthy individuals and that training the inspiratory muscles increased their capacity to perform an absolute level of anaerobic work. Given that RMT is known to improve

exercise tolerance in patients with COPD (Lisboa et al. 1997; Scherer et al. 2000) and alleviate the sensations of dyspnoea, it is likely that the improvement in performance in this population is more closely related to this (i.e. attenuated hyperinflation and central motor output) rather than a mechanism(s) related directly or indirectly to changes in systemic $[lac^-]_{B:}$ since the latter appears to be greater following-IMT.

7.3.2 POSSIBLE SITE(S) OF RESPIRATORY MUSCLE LACTATE EFFLUX

Data reported in chapters 3 and 4 demonstrate that the respiratory muscles make a significant contribution to systemic lactate turnover, however, the precise muscle fibres / muscle mass that are responsible for this production and / or release is unknown. The respiratory muscles are a complex group of highly co-ordinated skeletal muscles. Studies in animals (Fregosi and Dempsey 1989; Manohar and colleagues) and humans (Babcock et al. 1995) suggest the diaphragm does not produce lactate. However, the diaphragm contains both oxidative and glycolytic muscle fibres (Mizuno and Secher 1989). Therefore, with very high ventilations, due to the greater capillary:muscle fibre ratio, smaller diffusion distance and increased mitochondrial density relative to other skeletal muscles, the diaphragm may partake in simultaneous lactate production and consumption which remains undetected within the systemic circulation.

During intense endurance exercise, accessory inspiratory muscle recruitment is increased (Aliverti et al. 1997) as the ratio between the work performed by the diaphragm relative to the intercostal muscles is reduced (Figure 7.1; Johnson et al. 1993). The morphology of the external intercostals (and possibly other accessory muscles) is very similar to the vastus lateralis with up to 40% type II muscle fibres. Given their morphology and the velocity of shortening required during intense volitional hyperpnoea, it is attractive to speculate that these muscles are the source of at least part of the increase in $[lac^-]_B$. This is supported by the significant correlation between the relative increase in $[lac^-]_B$ and the relative breathing intensity of volitional hyperpnoea (Chapter 4; Johnson et al. 2006;

Martin et al. 1984). The expiratory muscles may also contribute to the $[lac^-]_B$ during volitional hyperpnoea. Direct evidence for this is not forthcoming, but fatigue of the abdominal muscles prior to exercise was shown to significantly increase $[lac^-]_B$ relative to a control trial (isotime trial: 7.8 ± 1.7 mmol·L^{-1} vs. expiratory muscle fatigue trial: 9.8 ± 2.0 mmol·L^{-1}; Taylor and Romer 2007) although the greater peak expiratory *P*ga was coupled with a significantly greater f_R and $\dot{V}_E$. Thus it is clear that the hitherto notion which failed to acknowledge the respiratory muscles as significant lactate producers is misleading and that the inspiratory and probably the expiratory muscles are likely sources for both net lactate production and release during intense pulmonary ventilation.

A possibility exists, however, that muscles distal to the respiratory muscle are responsible for the increase in systemic $[lac^-]_B$ during intense volitional hyperpnoea. For example, it may be that the work performed by the respiratory muscles during intense volitional hyperpnoea activates the respiratory muscle metaboreflex. A reflex reduction in limb blood flow would conceivably increase the locomotor glycolytic flux and therefore lactate production. Indeed, 8 min volitional hyperpnoea performed whilst at rest with a similar intensity to that used in this study (70% MVV, breathing pattern not controlled) resulted in diaphragm fatigue (Renggli et al. 2008). However, it is very unlikely that there is an increase in locomotor lactate production secondary to a volitional hyperpnoea-mediated metaboreflex since maximal exercise (with a significantly increased inspiratory muscle work), which causes a reflex reduction in locomotor blood flow does not significantly affect the arterial or femoral venous $[lac^-]_B$ (arterial $[lac^-]_B$: 8.83 ± 0.33 mmol·L^{-1}, femoral venous $[lac^-]_B$: 9.39 ± 0.29 mmol·L^{-1}; Harms et al. 1997). Furthermore, specific breathing challenges such as resistive breathing (30% MIP) and volitional hyperpnoea (V_T: 1.5 L, f_R: 45 breaths·min^{-1}) with near-maximal levels of inspiratory motor output also fail to affect limb vascular resistance, locomotor perfusion and therefore O_2 delivery (Sheel et al. 2002; St. Croix et al. 2000). Collectively these data suggests that the

changes in $[lac^-]_B$ throughout volitional hyperpnoea are not a result of an increased glycolytic flux and lactate release within / from non-respiratory tissues.

7.3.3 INSPIRATORY MUSCLE TRAINING AND SYSTEMIC LACTATE KINETICS

Numerous studies have reported reductions in $[lac^-]_B$ during whole-body exercise following RMT (for example: Griffiths and McConnell 2007; Leddy et al. 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999; Tong et al. 2008 Volianitis et al. 2001). However, whether the respiratory muscles are, in part, the source of these reductions was until now, less well understood.

Chapters 3 and 4 clearly illustrate that IMT attenuates the volitional hyperpnoea-mediated increase in $[lac^-]_B$ and steady state $[lac^-]_B$. In Chapter 5, a significantly lower $[lac^-]_B$ was also observed at the cessation of maximal exercise following IMT. The data presented in this thesis are the first to show (indirectly) that the trained inspiratory muscles are, in part, the source of the reductions in $[lac^-]_B$ observed during volitional hyperpnoea, whole-body exercise and post-exercise recovery. Interestingly, the reductions in $[lac^-]_B$ occur with near-maximal (Chapters 3 and 4: ~135 $L \cdot min^{-1}$) and maximal (Chapter 5: ~160 $L \cdot min^{-1}$) levels of $\dot{V}_E$, suggesting that the reductions were probably because the respiratory muscles produced less lactate. Interestingly however, significant reductions in $[lac^-]_B$ were also observed during steady-state exercise at the MLSS and throughout recovery from exercise where $\dot{V}_E$ was sub-maximal (Chapter 4: ~70 $L \cdot min^{-1}$) and/or similar to rest (Chapter 5: ~20 $L \cdot min^{-1}$), respectively. These findings suggest that the reductions in $[lac^-]_B$ which occur at low ventilations are likely due to net lactate uptake by the respiratory muscles.

7.3.4 POSSIBLE SITE(S) OF RESPIRATORY MUSCLE LACTATE CLEARANCE

The specific site(s) which account for the reductions in $[lac^-]_B$ are unknown. In Chapter 4, a significant correlation was reported between the increase in MIP and the reduction in $[lac^-]_B$ and novel findings presented in Chapter 6 suggests that the greatest improvements in MIP following IMT occur in those subjects with the greatest increase in chest wall muscle strength. Thus it is attractive to speculate that the chest wall inspiratory muscles increase their contribution to lactate turnover following IMT. This is supported by muscle biopsy analyses of the external intercostals following 5 wk IMT (Ramírez-Sarmiento et al. 2002) which show a significant increase in the proportion and size of type I and II muscles fibres, respectively.

Physiological adaptations following IMT do not only target the chest wall muscles. Increases in diaphragm thickness (e.g. Downey et al. 2007) and maximal volitional *P*di (Chapter 6) are observed following IMT. In animal studies, chronic loading of the diaphragm causes an increase in cytochrome-c oxidase activity (Akiyama et al. 1994, 1996) which is fundamental to complex III and IV of the electron transport chain. Thus it appears that accessory inspiratory muscles and the diaphragm are probably responsible for the reductions in $[lac^-]_B$ following IMT, although the relative contribution of each is unknown. Whether expiratory muscle training (EMT) has similar effects upon $[lac^-]_B$ is unlikely since both EMT and combined IMT / EMT do not affect systemic $[lac^-]_B$ during either an incremental or an all-out rowing ergometer test (Griffiths and McConnell 2007).

7.3.5 IMT-MEDIATED REDUCED BLOOD LACTATE CONCENTRATION: EFFECTS UPON EXERCISE TOLERANCE

Reductions in $[lac^-]_B$ following IMT may well contribute to improvements in whole-body exercise tolerance. During intense exercise above 85% $\dot{V}O_2$max, fatiguing diaphragm contractions and the accumulation of respiratory muscle metabolites activate both group III and IV phrenic nerve afferents causing a sympathetically-mediated increase

in limb vascular resistance. The findings of Chapters 3 and 4 demonstrated that high levels of pulmonary ventilation promote respiratory muscle lactate release (and presumably accumulation) which is known to stimulate phrenic nerve afferent fibres (Balzamo et al. 1992; Graham et al. 1986; Jammes and Balzamo 1992; Jammes et al. 1986). In support of this, Rodman et al. (2003) injected a bolus of lactic acid in to the exercising canine diaphragm through the phrenic artery and observed a transient reduction in limb blood flow secondary to an increased vascular conductance. Thus it appears that lactate may well be an important dose-dependent metabolite which contributes to the activation of the respiratory muscles metaboreflex. Therefore, an IMT-mediated reduction in respiratory muscle lactate production (and therefore accumulation) may attenuate phrenic afferent discharge, preserving limb blood flow and improving exercise tolerance (Harms et al. 2000; Romer et al. 2006a).

Notwithstanding this, many performance improvements following IMT occur at sub-maximal intensities (McConnell and Romer 2004a) during which respiratory muscle lactate production is negligible (Chapter 4) and diaphragm fatigue / the metaboreflex does not occur (Wetter et al. 1999). Since following IMT both critical power (Johnson et al. 2007) and MLSS (McConnell and Sharpe 2005; Chapter 4) remain unchanged, the most likely mechanisms by which an IMT-mediated reduction in $[lac^-]_B$ affects whole-body exercise performance are twofold. Firstly, favourable changes in acid-base balance may reduce the stimulation of both locomotor and respiratory muscle afferent nerves and the perception of breathing and locomotor effort. This notion is supported by Romer et al. (2002b) who observed a significant correlation between the IMT-mediated relative reduction in total recovery time throughout a repeated sprint trial and the relative change in RPE, dyspnoea and $[lac^-]_B$. The intensity of locomotor and breathing effort perception is suggested to be a major determinant of sub-maximal exercise tolerance, since despite a different origin, they are both appraised within the sensory cortex (Jones and Killian 2000; Presland et al. 2005). Secondly, it appears that the time taken to reach a metabolic steady

state is faster following IMT which is coupled with a speeding of the pulmonary oxygen uptake kinetics. This suggests that an IMT mediated reduction in $[lac^-]_B$ also reduces part of the metabolic inertia experienced at the onset of exercise. This may provide a possible explanation for the improved performance during whole-body exercise following IMT, particularly in studies where the criterion exercise test has involved repeated bouts of short duration intense sprinting (Romer et al. 2002b; Tong et al. 2008). However, how does this affect the elite athlete? It could be hypothesised that the ergogenic effects of IMT are magnified in highly trained individuals relative to their sedentary counterparts. This notion is based on studies which illustrated that the ventilatory demand of well trained individuals is greater relative to the untrained (Babcock et al. 1996) and that the strength of the inspiratory muscles in unaffected by whole body training (Dempsey 1986). Thus, all other systems devoted to oxygen transport and utilisation are improved with training, yet the respiratory muscle remain surprisingly unchanged. Notwithstanding this, chronic endurance training is positively related to measures of dynamic pulmonary function (Eastwood et al. 2001). Despite this latter finding, given that the strength of the inspiratory muscle remains unchanged following whole body endurance training the greater ventilatory demand of elite athletes would likely result in an increase in $[lac^-]_B$ similar to that presented in the experimental chapters of this thesis with moderate to well trained individuals. It would also be anticipated that these athletes would experience a similar reduction in $[lac^-]_B$ following IMT. Thus, if a change in the systemic $[lac^-]_B$ is responsible, in part, for the improvement in performance (regardless of the specific mechanism[s]) following IMT, this may also be magnified.

7.3.6 Effects of age, sex and nutritional interventions

Non-respiratory skeletal muscle demonstrates functional weakening with age which can be reversed with resistance strength training (Folland and Williams 2007). Non-respiratory, knee extensor muscles show significant improvements in strength and cross

sectional area (>9.8%) following 8 wks high-intensity resistance strength training (Harridge et al. 1999). Similar to the limb muscles, recent evidence also demonstrates that the inspiratory muscles are weakened with age. Britto et al. (2009) revealed that in three groups of subjects matched for height aged from 20 to 59 (group 1), 60 to 69 (group 2) and 70+ yrs (group 3), MIP was significantly greater in group 1 (92 ± 43 cmH_2O) relative to groups 2 and 3 (54 ± 32 cmH_2O). Despite the reduction in MIP with age, Watsford and Murphy (2008) observed a significant increase in MIP following 8 wk IMT (+22%) as well as a 12% improvement in sub-maximal treadmill performance in elderly subjects (age: 60 to 69 yrs). Also a 5% reduction in exercising heart rate and 8% reduction in RPE were observed. These novel findings demonstrate the plasticity and possible ergogenic effects of IMT even in elderly populations. The application of the hypotheses of this thesis (see section 1.9) to elderly populations are however, difficult since no such study has investigated the effects of IMT upon the whole body $[lac^-]_B$ in elderly individuals. It has been suggested that the ageing process promotes fibre type transformation from type II to type I (Maharam et al. 1999) and that whole body endurance training may offset this (Hawkins and Wiswell 2003). Given that the exercising breathing pattern does not change with age (Watsford and Murphy 2008) suggests that so long as the relative ventilatory demand exceeds ~70% MVV and the individual has participated in chronic whole body exercise training requiring high levels of hyperpnoea similar increases in $[lac^-]_B$ relative to those reported in Chapters 3 and 4 are likely to be observed. Furthermore, given the plasticity of skeletal muscles following resistance training in the elderly (Watsford and Murphy 2008) also suggest that any hyperpnoea-mediated increase in $[lac^-]_B$ may also be attenuated following IMT.

The role of IMT in female participants is also equivocal since there is a paucity of studies focusing specifically on this population group. Studies have shown that the relative increase in MIP following IMT is similar between sexes (McConnell and Lomax 2006; Guenette et al. 2006). Guenette et al. (2006) also observed no difference between males or

females in the improvement in time to the limit of tolerance (80% $\dot{W}$ max) following 5 wk IMT (relative improvement in males: 14%; females: 18%, P>0.05). Notwithstanding this, it is well document that when matched for chest volume, females have smaller lung volumes, slower expiratory flow rates, narrower airways and a reduced diffusion distance within the alveoli walls (Sheel et al. 2004). In particular, the smaller airways in females increases the resistive work of breathing for a given absolute ventilatory demand most likely due to a greater f_R (Guenette et al. 2009). Given the smaller lung volumes yet similar inspiratory and expiratory reserve volumes, females present themselves with a unique ventilatory challenge which increases their risk of expiratory flow limitation (McClaran et al. 1998). Expiratory flow limitation promotes hyperinflation and markedly increases the work of breathing and may attenuate cardiac output and thus limb O_2 delivery (Aleverti et al. 2005). Whether IMT would attenuate such effects remains unknown, however, Romer et al. (2002c) reported a non-significant reduction in EELV following IMT throughout maximal incremental exercise; thus, this remains to be confirmed. In addition to an increased prevalence of flow limitation, the smaller lung volumes of females increases the incidence and severity of exercise induced arterial hypoxeamia (EIAH); indeed, flow limitation and EIAH are correlated in female athletes (P<0.05; Walls et al. 2002). The precise cause of EIAH is unknown, although is has been proposed that it is due to i) an increase in the alveolar-arterial O_2 difference likely due to ventilator-perfusion inequality, ii) an insufficient PO_2 in the alveoli due to a relative hypoventilation and/or iii) a rightward shift in the O_2Hb dissociation curve due to metabolic acidosis (Dempsey and Wagner 1999). Of these possible mechanisms, IMT is unlikely to affect the first two. However, it may well indirectly affect the third. A right shift in the O_2Hb dissociation curve is caused by an increase in arterial $[H^+]$ (i.e. a decreased pH) and increased body temperature. If using the physicochemical approach to evaluate such a mechanism, it is possible that IMT would attenuate the systemic $[lac^-]_B$ and thus increase the [SID]. This would attenuate the $[H^+]$ and increase pH, offsetting the right shift in the O_2 dissociation

curve. Whether this hypothetical mechanism would occur in female athletes is unknown, although given that the changes in performance following IMT are similar between males and females supports this notion.

To date, no study has investigate the role of nutritional supplements, such as carbohydrate loading, protein or creatine in conjunction with IMT: thus the effects upon inspiratory muscle strength gains remain unknown. Following exercise, protein breakdown and synthesis are both increased, but protein balance remains negative (Wolfe 2000). Ingestion of carbohydrate stimulates insulin release, which increases protein synthesis at rest and attenuates protein breakdown following exercise (i.e. improving protein balance; Wolfe 2001). However, a positive protein balance is only possible with the provision of amino acids (both prior to, during and following exercise; Tipton and Wolfe 2004). Amino acids promote and provide building blocks for protein synthesis and reduce protein degradation. This would ensure an anabolic rather than catabolic environment enhancing muscle protein accretion (Koopman et al. 2007). Low frequency fatigue (LFF) of the diaphragm is well documented in the human diaphragm (see section 1.5.3), whereby reductions in force are most likely due to structural changes in sarcomere proteins involved in excitation-contraction coupling. Thus the time course of recovery of LFF represents the repair or re-synthesis of these proteins. During IMT where intense inspirations are performed against moderate to high resistances, LFF is likely to occur. This suggests that protein and/or carbohydrate supplementation may well facilitate recovery by improving protein balance, therefore enhancing force output in subsequent training sessions and improving strength gains following IMT. Recent evidence also suggests that protein supplementation immediately prior to resistance training may further improve strength gains possibly because of increased blood flow throughout training, therefore increased transport of amino acids and carbohydrates to the exercising muscles (Candow and Chilibeck 2008). Such a strategy may further improve the recovery of the inspiratory muscles during and following IMT and thus inspiratory muscle strength gains. Creatine monohydrate supplementation has also been shown to improve isotonic force output following resistance training regimens (Bemben and Lamont 2005). This supplement may improve the recovery of

the inspiratory muscles during IMT by increasing the immediate energy stores responsible for resynthesising ATP. Notwithstanding these additional improvements in MIP that may or may not occur when combining nutritional supplements with IMT, the added ergogenic effect on whole body performance is likely to be negligible as improvements in MIP and performance are not correlated (Johnson et al. 2007; Romer et al. 2002c) and may well be dependent upon the strength of the chest wall inspiratory muscles (see Chapter 6). Thus the added benefit of such strategies warrants further attention and clarification.

7.4 ACID-BASE BALANCE

7.4.1 HISTORICAL PERSEPCTIVE

'Traditionally', exercise-induced acid-base disturbances were quantified by the Hendersen-Hasselbalch equation which was first described in 1907 (Jones 2008; Sirker et al. 2002) where $\mathrm{pH} = pK + \log[\mathrm{HCO_3^-}]/[0.03 \times P\mathrm{CO_2}]$. This equation implies that the dissociation constant (*pK*) of carbonic acid is the control system for pH where HCO_3^- and PCO_2 represent the metabolic and respiratory influences upon acid-base balance, respectively (Sirker et al. 2002). However, since $[HCO_3^-]$ is affected by changes in PCO_2, this equation fails to reliably quantify metabolic disturbances. Although the equation expresses a valid quantified relationship, it is more descriptive than mechanistic and does not imply a physiological control system (Jones 2008).

In contrast, the 'modern' physicochemical approach developed by Stewart (1983), quantifies the mechanisms that *cause* changes in pH within a given compartment. During exercise, this model identifies the importance of electrolyte balance, fluid shifts and events which occur within the respiratory, circulatory and skeletal muscle systems (Putman et al. 2003). Within each compartment, $[H^+]$ and $[HCO_3^-]$ are considered dependent variables and are determined by the equilibrium state reached between the independent variables: [SID], PCO_2 and $[A_{tot}^-]$ (Jones 2008). Each independent variable is constrained by physical laws including its disassociation constant in water (*pK*), the laws of conservation of mass

(θ) and electrical neutrality (Q; Figure 7.3). Therefore, to summarise, the physicochemical approach appears to be, at present, the most robust method available to quantify the mechanisms which account for changes in acid-base balance in healthy exercising subjects. Consequently, its application has received a great deal of support from leading physiologists (Gladden 2008; Jones 2008; Kowalchuck and Scheuermann 1995; Lindinger 1995).

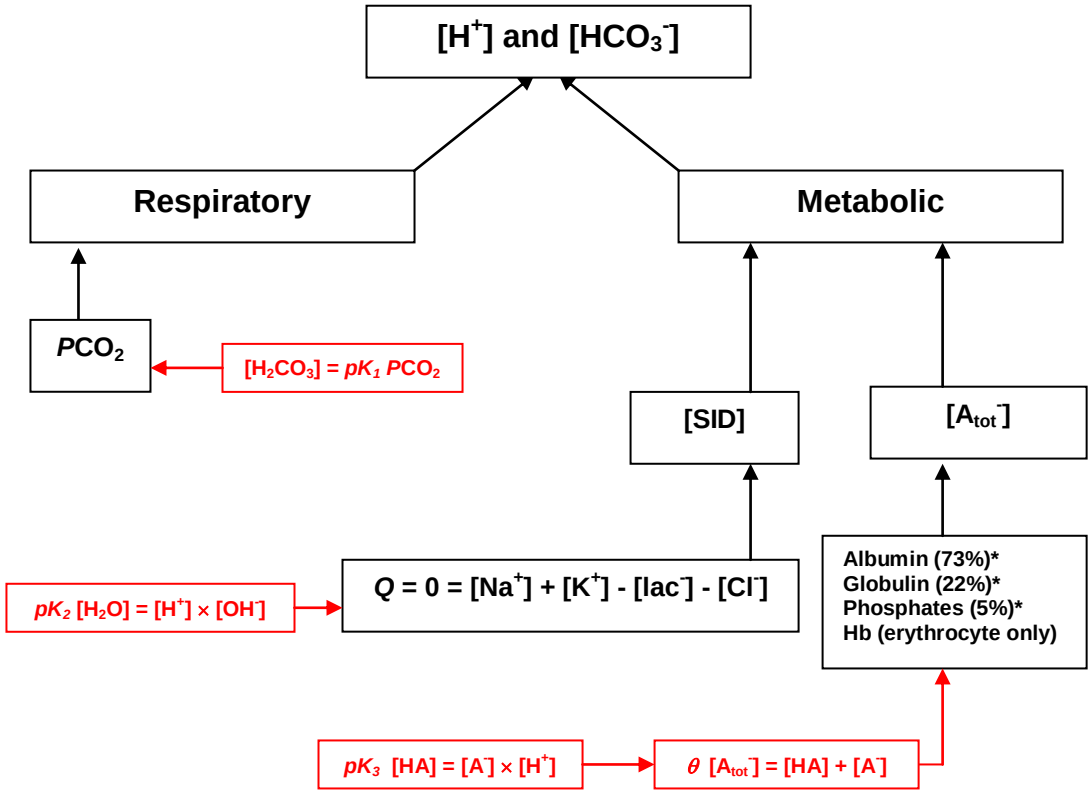

Figure 7.3 Determinants of plasma pH according to Stewart's physicochemical approach (Sirker et al. 2002; Stewart 1983). * from Staempfli and Constable (2003). *pK*, dissociation constant: pK_1, $_2$ and $_3$, dissociation constant for carbonic acid, water and weak acids, respectively; *Q*, net electric charge; θ, conservation of mass; Hb, haemoglobin; H_2CO_3, carbonic acid; H_2O, water.

7.4.2 PHYSICOCHEMICAL INTERACTIONS DURING EXERCISE

At the onset of exercise, many changes occur with respect to fluid volumes, metabolite and ion concentrations. In brief, water, Na^+ and Cl^- ions shift into the muscles in exchange for K^+, lactate and metabolic CO_2; these processes serve to increase intracellular [SID] and attenuate the rise in intramuscular [H^+] (McKenna et al. 1997). The influx of water increases interstitial and muscle volume and dilutes the metabolite and ion

concentrations in these compartments; although this subsequently increases the concentration of these ions and [A_{tot}^-] in the plasma. The erythrocyte also plays an important function with respect to lactate, K^+, Cl^- and PCO_2 storage/exchange (Jones 2008; Putman et al. 2003). During exercise, regulatory mechanisms are activated to restore intracellular and interstitial homeostasis at the expense of the plasma compartment. Thus, efficient regulation of plasma acid-base balance can have a marked effect upon the regulation of intracellular [H^+] (McKenna et al. 1997; Putman et al. 2003).

During recovery from exercise, the exchange of strong ions, CO_2 and water between the intra- and extracellular compartments (Kowalchuck et al. 1988) function to restore acid-base homeostasis (Lindinger et al. 1992). Following exercise, the recovery of plasma electrolytes is relatively fast, with values similar to rest within ~10 min (Kowalchuck et al. 1988; Chapter 5). However, the recovery of lactate is much slower (>90 min) clearly demonstrating its importance in affecting the post-exercise plasma [SID] (Lindinger et al. 1992).

7.4.3 REDUCED SYSTEMIC LACTATE AND PLASMA ACID-BASE BALANCE

There is a dearth of literature reporting the physicochemical mechanisms responsible for changes in acid-base balance following whole-body training (McKenna et al. 1997; Putman et al. 2003). The data presented in Chapter 5 are the first to have examined the mechanisms which are responsible for changes in acid-base balance after maximal exercise following specific IMT. Putman et al. (2003) reported significant changes in ion and water flux regulation following just 1 wk of sub-maximal endurance training 2 h·day^{-1} at 60% $\dot{V}O_2$max. After-training and during exercise at 75% $\dot{V}O_2$max, arterial and venous [H^+] was lower relative to pre-training values by ~5 nmol·L^{-1} due to a smaller decrease in [SID] and a lower [A_{tot}^-]. At this intensity, the smaller decrease in [SID] was accounted for by a lower intramuscular lactate concentration, K^+ efflux from the

intramuscular space and greater erythrocyte lactate and Cl^- uptake. The lower arterial and venous [A_{tot}^-] was accounted for by a lower influx of water.

The data presented in Chapter 5 are in stark contrast to those of Putman et al. (2003) as following IMT, lactate was the only physicochemical parameter which changed. As a consequence, the increase in [SID] (and lower [H^+]) throughout recovery from maximal exercise following IMT was almost exclusively attributed to the lower [$lac^-]_B$. Therefore, it appears that following IMT, reductions in [$lac^-]_B$ during and following exercise have an important role in determining plasma acid-base balance since there does not appear to be any changes in any other independent variable within the plasma. Whether there are changes in any other physicochemical parameter within the respiratory muscles *per-se* remains to be investigated, although ethical and methodological constraints would limit such studies to animal models. These data therefore present an important milestone in the understanding of the role of IMT and changes in systemic [$lac^-]_B$ in the regulation of whole-body acid-base homeostasis.

The wider implications of these findings may be applicable to sports performance. A lower [$lac^-]_B$ and [H^+] are associated with improved performance (Edge et al. 2006). Therefore, following IMT, the use of a low intensity inspiratory resistance may speed recovery and facilitate subsequent performance. Such a technique may be applicable to athletes who have limited space to recover actively following exercise. This may include sports such as swimming or when athletic heats are performed. In addition, this technique may be relevant to wheelchair bound sports. Furthermore, that following IMT, reductions in [$lac^-]_B$ are correlated with reductions in recovery time during a repeated sprint test (Romer et al. 2002b), accelerating recovery processes by using inspiratory loading may also be beneficial to speed recovery between repeated sprint exercise. This would be applicable for intermittent sports such as hockey where rolling substitutions are used throughout or soccer and rugby, where a fixed duration half-time break is employed.

7.5 EFFECTS OF BASELINE INSPIRATORY MUSCLE STRENGTH

Following inspiratory muscle training, some studies report large improvements in inspiratory muscle strength (~30 to 40%; Romer et al. 2002b; Volianitis et al. 2001), where as others are far more modest (~10 to 20%; Johnson et al. 2007; Sonetti et al. 2001). In Chapter 6 (Experiment 2), approximately 40% of the between-subject variation in the improvements in MIP was explained by the baseline status, which in itself was determined by the pattern of relative chest wall muscle recruitment (expressed by the ratio: *P*oe/*P*di). Furthermore, following 4 wk IMT a significant positive correlation was observed between the relative increase in *P*oe/*P*di and the increase in MIP illustrating that the greater maximal pressure generating capacity of the chest wall inspiratory muscles permitted a greater pressure generation by the diaphragm (Hershenson et al. 1988). It also appears that the IMT-mediated improvements in MIP are determined by the degree to which the baseline inspiratory muscle strength exceeds its predicted value. In Chapter 6, 8 of 38 subjects failed to demonstrate an improvement in MIP following 4 wk IMT.

The functional importance of these relationships is unknown particularly since the contribution of the diaphragm to inspiratory pressure development is inversely related to the exercise intensity (Aliverti et al. 1997; Johnson et al. 1993). Although subjects with weaker chest wall inspiratory muscles (and thus MIP) show the largest improvement in MIP following IMT, the likelihood of this translating into a superior performance gain is small, since performance improvements following IMT and increases in MIP are not correlated (Johnson et al. 2007; Romer et al. 2002c). Furthermore, whether having weaker chest wall inspiratory muscles increases the possibility of a relative hypoventilation during exercise and the development of conditions such as exercise induced arterial hypoxemia is also unknown. However, in Chapter 4 a significant correlation was reported between the IMT-mediated increase in MIP and the reductions in $[lac^-]_B$ during volitional hyperpnoea and in Chapter 5, between the relative increase in MIP and the increase in [SID] at the

point of maximal exercise termination. Therefore, this relationship may well be more important for the RMT-mediated changes in systemic acid-base balance.

Collectively these findings suggests that future studies who aim to reveal a homogenous response to IMT with regard to changes in MIP or other dependent variables should strive to employ a group with similar levels of inspiratory muscle strength. Furthermore, when designing an IMT study, in order to maximise the potential of observing a positive response and avoiding a type II error, recruiting a cohort of subjects with a low MIP may be more beneficial than a group with a high baseline MIP since their capacity for adaptation may be limited.

7.6 LIMITATIONS AND FUTURE PERSPECTIVES

In Chapters 3 and 4 volitional hyperpnoea was imposed upon exercise under resting conditions and superimposed upon cycling exercise at the MLSS. Throughout volitional hyperpnoea, subjects were instructed to mimic their spontaneous exercise breathing pattern. Although, the methods used within Chapters 3 and 4 are more applicable to endurance exercise than those of previous studies, it is likely that physiological responses were overestimated (see section 7.3.1). Future studies should attempt to precisely mimic during volitional hyperpnoea the inspiratory and expiratory pressure swings and the operating lung volumes achieved during exercise to identify the true respiratory muscle contribution to systemic lactate kinetics.

The pre-intervention data presented in Chapters 3 and 4 show that intense volitional hyperpnoea increases $[lac^-]_B$, however, the precise source(s) of this increase remains unknown. Since expiratory muscle work is artificially increased during volitional hyperpnoea (Klas and Dempsey 1989), breathing with and without a less dense gas mixture such as heliox would allow the quantification of the contribution of abdominal work (and the associated increase in flow limitation) to changes in $[lac^-]_B$ during intense endurance exercise. Mimicking independently the pressures generated by the diaphragm, chest wall

and / or abdominal muscles that were achieved during intense exercise and measuring the subsequent metabolic response may also provide insight into their individual contribution to lactate turnover. During volitional hyperpnoea and with inspiratory muscle loading following exercise, sampling blood from an artery distal to the respiratory muscles and the phrenic, intercostal and deep circumflex vein / vena cava would provide useful information regarding the contribution of these muscles to systemic lactate turnover. A negative or positive difference in the venous and arterial $[lac^-]_B$ would indicate net lactate release or consumption, respectively. Additionally, injection and analyses of stable (lactate) isotopes would also provide invaluable information regarding respiratory muscle lactate production and oxidation during volitional hyperpnoea and recovery both prior to and following RMT. For example, administration of labelled isotopes such as [1-^{14}C]lactate and subsequent analysis of uniformely radiolabelled $\dot{V}^{14}CO_2$ can provide a reliable measure of lactate oxidation (Brooks and Gaesser 1980).

In Chapters 3, 4 and 5, significant reductions in systemic $[lac^-]_B$ were observed following IMT. It is likely that these reductions were mediated by a reduction in lactate production with a high $\dot{V}_E$ and / or by lactate consumption with low $\dot{V}_E$. The precise mechanisms which result in these changes, however, remain unknown. Future study would benefit from muscle biopsy analyses of the respiratory muscles following IMT with immuno-histochemical analyses of muscle fibre types, enzyme activity and MCT protein expression. Although tissue sampling techniques for the respiratory muscles are inherently restricted by their small size and proximity to essential organs, techniques are available to achieve such goals for both the human diaphragm (Nguyen et al. 2000) and external intercostals (Ramírez-Sarmiento et al. 2002). Such analyses may provide further evidence of respiratory muscle plasticity following IMT and the possible enzymatic and morphological mechanisms which contribute to a lower $[lac^-]_B$.

The protection of plasma acid-base homeostasis is considered essential for whole body exercise tolerance (Putman et al. 2003). In Chapter 5, a significant reduction in

plasma [H^+] was observed following IMT when low intensity inspiratory muscle loading was performed which was accounted for by the increase in [SID]. It would be interesting to investigate whether similar observations occur with expiratory muscle loading since the muscle mass of the expiratory muscles is far greater than their inspiratory counterparts. Furthermore, given the inverse relationship between [H^+] and performance, an-IMT mediated reduction in [H^+] during recovery from bouts of high-intensity exercise with the addition of a low-intensity inspiratory resistance may serve to improve subsequent performance. This hypothesis certainly provides a novel avenue for future study.

The mechanisms responsible for the improvements in whole-body exercise performance following RMT are not well understood. One possible mechanism is an IMT-mediated attenuation of exercise-induced diaphragm fatigue and subsequent activation of the respiratory muscle metaboreflex. Such a mechanism may improve locomotor muscle perfusion and exercise tolerance. Previous studies have reported a reduction in exercise-induced diaphragm fatigue following RMT (Romer et al. 2002a; Verges et al. 2007b). IMT was also reported to attenuate the sympathetic efferent response triggered by fatiguing diaphragm work (McConnell and Lomax 2006; Witt et al. 2007). Notwithstanding this, one key question which remains is whether specific IMT attenuates locomotor muscle fatigue following intense exercise. Future studies should be performed to investigate the role of IMT upon locomotor muscle fatigue following time-trial exercise and intense exercise to exhaustion as assessed by supramaximal stimulation of the femoral nerve.

Finally, in Chapter 6, a significant relationship was observed between the relative strengths of the chest wall muscles and the global inspiratory muscle strength (MIP). In other words, the lower the strength of the chest wall muscles, the lower the global force generating capacity of the inspiratory muscles. Similarly, following IMT, the largest increases in MIP were observed in subjects who enjoyed the greatest increase in the contribution of relative chest wall muscle recruitment. Although the ratio of *P*oe/*P*di provides an indication of the pressure generated between diaphragm vs. non-diaphragmatic

muscles, it does not provide information regarding the precise non-diaphragmatic muscles which are active throughout the inspiratory effort. For example, it is well known that accessory muscles within the neck (i.e. the scalenes and sternocleidomastoids) are tonically active during a Müeller manoeuvre. The change in muscle recruitment and / or synchrony in the chest and neck muscles would also be an interesting avenue for future research. Attempts to measure inspiratory muscle EMG were made in Chapter 6 (data not shown), however, the between-day coefficient of variation was poor (>30%) and did not provide an insight into IMT-mediated changes in inspiratory muscle recruitment. Whether the relationships observed between the strength of the chest wall inspiratory muscles and MIP determine the ergogenicity and / or physiological consequences of RMT remains equivocal. However, if this were a functional relationship, future study should aim to recruit participants with a similar baseline MIP and relative chest wall activation thus revealing the true effects of baseline inspiratory muscle strength and RMT upon exercise tolerance. Indeed, a large scale study recruiting participants who have a variety of baseline MIP measures is warranted to determine whether there are responders and non-responders to IMT and the mechanisms that may potentially explain this phenomenon.

REFERENCES

REFERENCES

Aaker, A. and Laughlin, M.H. (2002a). Diaphragm arterioles are less responsive to α_1-adrenergic constriction than gastrocnemius arterioles. *Journal of Applied Physiology*, **92**, 1808-1816.

Aaker, A. and Laughlin, M.H. (2002b). Differential adenosine sensitivity of diaphragm and skeletal muscle arterioles. *Journal of Applied Physiology*, **93,** 848-856.

Aaron, E.A., Johnson, B.D., Seow, K.C. and Dempsey, J.A. (1992a). Oxygen cost of exercise hyperpnoea: measurement. *Journal of Applied Physiology*, **72,** 1810-1817.

Aaron, E.A., Seow, K.C., Johnson, B.D. and Dempsey, J.A. (1992b). Oxygen cost of exercise hyperpnoea: implications for performance. *Journal of Applied Physiology*, **72,** 1818-1825.

Abbrecht, P.H., Rajagopal, K.R. and Kyle, R.R. (1991). Expiratory muscle recruitment during inspiratory flow-resistive loading and exercise. *American Review of Respiratory Disease*, **144**, 113-120.

Agostoni, E. (1986). Mechanics of the pleural space. In: *Section 3: The Respiratory System*. Fishman, A.P., Macklem, P.T., Mead, J. and Geiger, S.R. (Eds.), The Williams and Wilkins Company: Baltimore, pp. 531-559.

Ahtiainen, J.P., Pakarinen, A., Alen, M., Kraemer, W.J. and Häkkinen, K. (2003). Muscle hypertrophy, hormonal adaptations and strength development during strength training in strength-trained and untrained men. *European Journal of Applied Physiology*, **89**, 555-563.

Akiyama, Y., Garcia, R.E., Prochaska, L.J. and Bazzy, A.R. (1994). Effect of chronic respiratory loading on the subunit composition of cytochrome c oxidase in the diaphragm. *American Journal of Physiology, Lung, Cellular and Molecular Physiology*, **267**, L350-L355.

Akiyama, Y., Garcia, R.E. and Bazzy, A.R. (1996) Effect of inspiratory training on mitochondrial DNA and cytochrome-c oxidase expression in the diaphragm. *American Journal of Physiology, Lung, Cellular and Molecular Physiology*, **271**, L20-25.

Aliverti, A., Cala, S.J., Duranti, R., Ferrigno, G., Kenyon, C.M., Pedotti, A., Scano, G., Sliwinski, P., Macklem, P.T. and Yan, S. (1997). Human respiratory muscle actions and control during exercise. *Journal of Applied Physiology*, **83**, 1256-1269.

Aliverti, A., Dellacà, R.L., Lotti, P., Bertini, S., Duranti, R., Scano, G., Heyman, J., Lo Mauro, A., Pedotti, A. and Macklem, P.T. (2005). Influence of expiratory flow-limitation during exercise on systemic oxygen delivery in humans. *European Journal of Applied Physiology*, **95**, 229-242.

Allen, D.G., Lamb, G.D. and Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiological Reviews*, **88**, 287-332.

Amann, M., Romer, L.M., Pegelow, D.F., Jacques, A.J., Hess, C.J. and Dempsey, J.A. (2006). Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *Journal of Applied Physiology*, **101**, 119-127.

Amann, M. and Dempsey, J.A. (2008). Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *Journal of Physiology*, **586**, 161-173.

Amann, M., Hopkins, W.G. and Marcora, S.M. (2008a). Similar sensitivity of time to exhaustion and time-trial time to changes in endurance. *Medicine and Science in Sports and Exercise*, **40**, 574-578.

Amann, M., Proctor, L.T., Sebranek, J.J., Eldridge, M.W., Pegelow, D.F. and Dempsey, J.A. (2008b). Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *Journal of Applied Physiology*, **105**, 1714-1724.

Amann, M., Proctor, L.T., Sebranek, J.J., Pegelow, D.F. and Dempsey, J.A. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *Journal of Physiology*, **587**, 271-283.

Arora, N.S. and Rochester, D.F. (1982). Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area. *Journal of Applied Physiology*, **52,** 64-70.

Åstrand, P-O., Rodahl, K., Dahl, H.A. and Stromme, S.B. (2003). *Textbook of Work Physiology: Physiological Bases of Exercise.* 4th ed. Leeds: Human Kinetics.

Aunola, S. and Rusko, H. (1992). Does anaerobic threshold correlate with maximal lactate steady-state? *Journal of Sports Sciences*, **10**, 309-323.

Babcock, M.A., Pegelow, D.F., McClaran, S.R., Suman, O.E. and Dempsey. J.A. (1995). Contribution of diaphragmatic power output to exercise-induced diaphragm fatigue. *Journal of Applied Physiology*, **78,** 1710-1719.

Babcock, M.A., Pegelow, D.F., Johnson, B.D. and Dempsey, J.A. (1996). Aerobic fitness effects on exercise-induced low-frequency diaphragm fatigue. *Journal of Applied Physiology*, **81**, 2156-2164.

Babcock, M.A., Pegelow, D.F., Taha, B.H. and Dempsey, J.A. (1998). High frequency diaphragmatic fatigue detected with paired stimuli in humans. *Medicine and Science in Sports and Exercise*, **30**, 506-511.

Babcock, M.A., Pegelow, D.F., Harms, C.A. and Dempsey, J.A. (2002). Effects of respiratory muscle unloading on exercise-induced diaphragm fatigue. *Journal of Applied Physiology*, **93**, 201-206.

Bacon, L. and Kern, M. (1999). Evaluating a test protocol for predicting maximum lactate steady state. *Journal of Sports Medicine and Physical Fitness*, **39**, 300-308.

Baker, S.K., McCullagh, K.J. and Bonen, A. (1998). Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. *Journal of Applied Physiology*, **84**, 987-994.

Balzamo, E., Lagier-Tessonnier, F. and Jammes, Y. (1992). Fatigue-induced changes in diaphragmatic afferents and cortical activity in the cat. *Respiration Physiology*, **90**, 213-226.

Baron, B., Dekerle, J., Robin, S., Neviere, R., Dupont, L., Matran, R., Vanvelcenaher, J., Robin, H. and Pelayo, P. (2003). Maximal lactate steady state does not correspond to a complete physiological steady state. *International Journal of Sports Medicine*, **24**, 582-587.

Bassett, D.R. Howley, E.T., Thompson, D.L., King, G.A., Strath, S.J., McLaughlin, J.E. and Parr, B.B. (2001). Validity of inspiratory and expiratory methods of measuring gas exchange with a computerized system. *Journal of Applied Physiology*, **91,** 218-224.

Bassey, E.J. and Harries, U.J. (1993). Normal values for handgrip strength in 920 men and women aged over 65 years, and longitudinal changes over 4 years in 620 survivors. *Clinical Science*, **84**, 331-337.

Baydur, A., Behrakis, P.K., Zin, W.A., Jaeger, M. and Milic-Emili, J. (1982). A simple method for assessing the validity of the esophageal balloon technique. *American Review of Respiratory Disease*, **126**, 788-791.

Bazzy, A.R., Pang, L.M., Akabas, S.R. and Haddad, G.G. (1989). O_2 metabolism of the sheep diaphragm during flow resistive loaded breathing. *Journal of Applied Physiology*, **66,** 2305-2311.

Beelen, A. and Sargeant, A.J. (1993). Effect of prior exercise at different pedalling frequencies on maximal power in humans. *European Journal of Applied Physiology*, **66,** 102-107.

Bellemare, F., Wight, D., Lavigne, C.M. and Grassino. A. (1983). Effect of tension and timing of contraction on the blood flow of the diaphragm. *European Journal of Applied Physiology*, **54,** 1597-1606.

Bellemare, F. and Bigland-Ritchie, B. (1987). Central components of diaphragmatic fatigue assessed by phrenic nerve stimulation. *Journal of Applied Physiology*, **62,** 1307-1316.

Bemben, M.G. and Lamont, H.S. (2005). Creatine supplementation and exercise performance: recent findings. *Sports Medicine*, **35**, 107-25.

Benditt, J.O. (2005). Esophageal and gastric pressure measurement. *Respiratory Care*, **50**, 68-77.

Black, L.F. and Hyatt, R.E. (1969). Maximal respiratory pressures: normal values and relationship to age and sex. *American Review of Respiratory Disease*, **99**, 696-702.

Bland, J.M. and Altman, D.G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, **1**, 307-10.

Borg, G.A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, **14**, 377-381.

Boussana, A., Matecki, S., Galy, O., Hue, O., Ramonatxo, M. and Le Gallais, D. (2001). The effect of exercise modality on respiratory muscle performance in triathletes. *Medicine and Science in Sports and Exercise*, **33**, 2036-2043.

Boutellier, U., Buchel, R., Kundert, A. and Spengler, C. (1992). The respiratory system as an exercise limiting factor in normal trained subjects. *European Journal of Applied Physiology*, **65**, 347-353.

Boutellier, U. and Piwko, P. (1992). The respiratory system as an exercise limiting factor in normal sedentary subjects. *European Journal of Applied Physiology*, **64**, 145-152.

Bramble, D.M. and Carrier, D.R. (1983). Running and breathing in mammals. *Science*, **219**, 251-256.

Braun, N.M., Arora, N.S. and Rochester, D.F. (1982). Force-length relationship of the normal human diaphragm. *Journal of Applied Physiology*, **53**, 405-412.

Britto, R.R., Zampa, C.C., de Oliveira, T.A., Prado, L.F. and Parreira, V.F. (2009). Effects of the Aging Process on Respiratory Function. *Gerontology*, DOI: 19713688.

Brooks, G.A. (1986). The lactate shuttle during exercise and recovery. *Medicine and Science in Sports and Exercise*, **18**, 360-368.

Brooks, G.A. (2000). Intra- and extra-cellular lactate shuttles. *Medicine and Science in Sports and Exercise*, **32**, 790-799.

Brooks, G.A. (1991). Current concepts in lactate exchange. *Medicine and Science in Sports and Exercise*, **22**, 895-906.

Brooks, G.A. (1999). Active muscle and whole body lactate kinetics after endurance training in men. *Journal of Applied Physiology*, **87**, 1684-1696.

Brooks, G.A. and Gaesser, G.A. (1984). End points of lactate and glucose metabolism after exhausting exercise. *Journal of Applied Physiology*, **49**, 1057-1069.

Brooks, G.A., Brown, M., Butz, C.E., Sicurello, J.P. and Dubouchaud, H. (1999). Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *Journal of Applied Physiology*, **87**, 1713-1718.

Burgomaster, K.A., Cermak, N.M., Phillips, S.M., Benton, C.R., Bonen, A. and Gibala, M.J. (2007). Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, **292**, R1970-R1976.

Bye, P.T., Esau, S.A., Walley, K.R., Macklem, P.T. and Pardy, R.L. (1984). Ventilatory muscles during exercise in air and oxygen in normal men. *Journal of Applied Physiology*, **56**, 464-471.

Caine, M.P. and McConnell, A.K. (2000). Development and evaluation of a pressure threshold inspiratory muscle trainer for use in the context of sports performance. *Sports Engineering*, **3**, 149-159.

Cala, S.J., Kenyon, C.M., Ferrigno, G., Carnevali, P., Aliverti, A., Pedotti, A., Macklem, P.T., Rochester, D.F. (1996). Chest wall and lung volume estimation by optical reflectance motion analysis. *Journal of Applied Physiology*, **81**, 2680-2689.

Candow, D.G. and Chilibeck, P.D. (2008). Timing of creatine or protein supplementation and resistance training in the elderly. *Applied Physiology, Nutrition and Metabolism*, **33**, 184-190.

Cappello, M. and De Troyer, A. (2002). On the respiratory function of the ribs. *Journal of Applied Physiology*, **92**, 1642-1646.

Carpenter, M.A., Tockman, M.S., Hutchinson, R.G., Davis, C.E. and Heiss, G. (1999). Demographic and anthropometric correlates of maximum inspiratory pressure: The Atherosclerosis Risk in Communities Study. *American Journal of Respiratory and Critical Care Medicine*, **159**, 415-422.

Carter, J.E.L. and Heath, B.H. (1990). *Somatotyping: development and applications*. Cambridge University Press, Cambridge.

Chatham, K., Baldwin, J., Griffiths, H., Summers, L. and Enright, S. (1999). Inspiratory muscle training improves shuttle run performance in healthy subjects. *Physiotherapy*, **85**, 676-683.

Chevrolet, J.C., Tschopp, J.M., Blanc, Y., Rochat, T. and Junod, A.F. (1993). Alterations in inspiratory and leg muscle force and recovery pattern after a marathon. *Medicine and Science in Sports and Exercise*, **25**, 501-507.

Chiappa, G.R., Roseguini, B.T., Vieira, P.J., Alves, C.N., Tavares, A., Winkelmann, E.R., Ferlin, E.L., Stein, R. and Ribeiro, J.P. (2008a). Inspiratory muscle training improves blood flow to resting and exercising limbs in patients with chronic heart failure. *Journal of the American College of Cardiology*, **29**, 1663-1671.

Chiappa, G.R., Roseguini, B.T., Alves, C.N., Ferlin, E.L., Neder, J.A. and Ribeiro, J.P. (2008b). Blood lactate during recovery from intense exercise: impact of inspiratory loading. *Medicine and Science in Sports and Exercise*, **40**, 111-116.

Chiappa, G.R., Ribeiro, J.P., Alves, C.N., Vieira, P.J., Dubas, J.O., Queiroga, F., FBatista, L.D. and Silva, A.C. (2009). Inspiratory resistive loading after all-out exercise improves subsequent performance. *European Journal of Applied Physiology*, **106**, 297-303.

Ciufo, R., Dimarco, A., Stofan, D., Nethery, D. and Supinski, G. (2001). Dichloroacetate reduces diaphragmatic lactate formation but impairs respiratory performance. *American Journal of Respiratory and Critical Care Medicine*, **164**, 1669-1674.

Coast, J.R., Clifford, P.S., Henrich, T.W., Stray-Gundersen, J. and Johnson, R.L. Jr. (1990). Maximal inspiratory pressure following maximal exercise in trained and untrained subjects. *Medicine and Science in Sports and Exercise*, **22**, 811-815.

Coast, J.R., Rasmussen, S.A., Krause, K.M., O'Kroy, J.A., Loy, R.A. and Rhodes J. (1993). Ventilatory work and oxygen consumption during exercise and hyperventilation. *Journal of Applied Physiology*, **74**, 794-798.

Cobb, M.A., Schutt, W.A. and Hermanson, J.W. (1994). Morphological, histochemical, and myosin isoform analysis of the diaphragm of adult horses, Equus caballus. *The Anatomical Record*, **238**, 317-325.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. Academic Press: New York , USA.

Comtois, A., Gorczyca, W. and Grassino, A. (1987). Anatomy of diaphragm circulation. *Journal of Applied Physiology*, **62**, 238-244.

Costill, D.L., Coyle, E.F., Fink, W.F., Lesmes, G.R. and Witzmann, F.A. (1979). Adaptations in skeletal muscle following strength training. *Journal of Applied Physiology*, **46**, 96-99.

Cotes, J.E. (1993). *Lung Function: Assessment and Application in Medicine*. 5th ed. Blackwell Scientific Publications: London.

Crowther, G.J., Carey, M.F., Kemper, W.F. and Conley, K.E. (2002). Control of glycolysis in contracting skeletal muscle. I. Turning it on. *American Journal of Physiology, Endocrinology and Metabolism*, **282**, E67-E73.

Dallal, G.E. (1990). PC-SIZE: consultant. A program for sample size determination. *The American Statistician*, **44**, 243.

Davies, S.F., Iber, C., Keene, S.A., McArthur, C.D. and Path, M.J. (1986). Effect of respiratory alkalosis during exercise on blood lactate. *Journal of Applied Physiology*, **61**, 948-952.

Davison, R.C.R., Coleman, D., Balmer, J., Nunn, M., Theakston, S., Burrows, M. and Bird, S. (2000). Assessment of blood lactate: practical evaluation of the Biosen 5030 lactate analyzer. *Medicine and Science in Sports and Exercise*, **32**, 243-247.

Decramer, M. and De Troyer, A. (1984). Respiratory changes in parasternal intercostal length. *Journal of Applied Physiology*, **57**, 1254-1260.

Decramer, M. (1984). Effects of hyperinflation on the respiratory muscles. *European Respiratory Journal*, **2**, 299-302.

Delhez, L., Bottin, R., Thonon, A. and Vierset, J. (1966). Modifications of maximum pressure-volume diagram of the thoracic-pulmonary system after training of the respiratory muscles by static exercises. *Archives Internationales de Physiologie et de Biochimie*, **74**, 335-336.

Dempsey, J.A. (1986). Is the lung built for exercise? *Medicine and Science in Sports and Exercise*, **18**, 143-155.

Dempsey, J.A. and Wagner, P.D. (1999). Exercise-induced arterial hypoxemia. *Journal of Applied Physiology*, **87**, 1997-2006.

Dempsey, J.A., Harms, C.A. and Ainsworth, D.M. (1996). Respiratory muscle perfusion and energetics during exercise. *Medicine and Science in Sports and Exercise*, **28**, 1123-1128.

Dempsey, J.A., Miller, J.D. and Romer, L.M. (2006a). *The respiratory system*. In: Tipton, C.M., Sawka, M.N. and Tate, C.A. Terjung RL. (Eds.). ACSM's advanced exercise physiology. USA: Lippincott, Williams and Wilkins: pp 246-298.

Dempsey, J.A., Romer, L., Rodman, J., Miller, J. and Smith, C. (2006b). Consequences of exercise-induced respiratory muscle work. *Respiratory Physiology and Neurobiology*, **151**, 242-50.

Dempsey, J.A., Amann, M., Romer, L.M. and Miller, J.D. (2008). Respiratory system determinants of peripheral fatigue and endurance performance. *Medicine and Science in Sports and Exercise*, **40**, 457-461.

DePalo, V.A., Parker, A.L., Al-Bilbeisi, F. and McCool, F.D. (2004). Respiratory muscle strength training with nonrespiratory maneuvers. *Journal of Applied Physiology*, **96**, 731-734.

De Troyer, A. and Kelly, S. (1984). Action of neck accessory muscles on rib cage in dogs. *Journal of Applied Physiology*, **56**, 326-332.

De Troyer, A. and Estenne, M. (1984). Coordination between rib cage muscles and diaphragm during quiet breathing in humans. *Journal of Applied Physiology*, **57**, 899-906.

De Troyer, A. and Estenne, M. (1988). Functional anatomy of respiratory muscles. *Clinics in Chest Medicine*, **9**, 175-193.

De Troyer, A. and Farkas, G.A. (1989). Inspiratory function of the levator costae and external intercostal muscles in the dog. *Journal of Applied Physiology*, **67**, 2614-2621.

De Troyer, A., Legrand, A., Gevenois, P.A. and Wilson, T.A. (1998). Mechanical advantage of the human parasternal intercostal and triangularis sterni muscles. *Journal of Physiology*, **513**, 915-925.

De Troyer, A., Estenne, M., Ninane, V., Van Gansbeke, D. and Gorini, M. (1990). Transversus abdominis muscle function in humans. *Journal of Applied Physiology*, **68**, 1010-1016.

De Troyer, A., Kirkwood, P.A. and Wilson, T.A. (2005). Respiratory action of the intercostal muscles. *Physiological Reviews*, **85**, 717-756.

Derchak, P.A., Sheel, A.W., Morgan, B.J. and Dempsey, J.A. (2002). Effects of expiratory muscle work on muscle sympathetic nerve activity. *Journal of Applied Physiology*, **92**, 1539-1552.

Dodd, S., Powers, S.K., Callender, T. and Brooks, E. (1984). Blood lactate disappearance at various intensities of recovery exercise. *Journal of Applied Physiology*, **57**, 1462-1465.

Dodd, S.L., Powers, S.K., Thompson, D., Landry, G. and Lawler, J (1989). Exercise performance following intense, short-term ventilatory work. *International Journal of Sports Medicine*, **10**, 48-52.

Downey, A.E., Chenoweth, L.M., Townsend, D.K., Ranum, J.D., Ferguson, C.S. and Harms, C.A. (2007). Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. *Respiratory Physiology and Neurobiology*, **156**, 137-146.

Dubouchaud, H., Butterfield, G.E., Wolfel, E.E., Bergman, B.C. and Brooks, G.A. (2000). Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *American Journal of Physiology, Physiology, Endocrinology and Metabolism*, **278**, E571-E579.

Dupont, G., Blondel, N. and Berthoin, S. (2003). Performance for short intermittent runs: active recovery vs. passive recovery. *European Journal of Applied Physiology*, **89**, 548-554.

Durnin, J.V. and Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, **32**, 77-97.

Eastwood, P.R., Hillman, D.R. and Finucane, K.E. (2001). Inspiratory muscle performance in endurance athletes and sedentary subjects. *Respirology*, **6,** 95-104.

Eastwood, P.R., Van der Touw, T.J., Sturdy, G.A., Jenkins, S.C. and Hillman, D.R. (2006). Anaerobic metabolism of inspiratory muscles in COPD. *Respirology*, **11,** 32-40.

Edge, J., Hill-Haas, S., Goodman, C. and Bishop, D. (2006). Effects of resistance training on H^+ regulation, buffer capacity, and repeated sprints. *Medicine and Science in Sports and Exercise,* **38**, 2004-2011.

Edwards, R.H. and Clode, M. (1979). The effect of hyperventilation on the lactacidaemia of muscular exercise. *Clinical Science*, **38**, 269-276.

Edwards, A.M. and Cooke, C.B. (2004). Oxygen uptake kinetics and maximal aerobic power are unaffected by inspiratory muscle training in healthy subjects where time to exhaustion is extended. *European Journal of Applied Physiology*, **93**, 139-144.

Edwards, A.M., Wells, C. and Butterly, R. (2008). Concurrent inspiratory muscle and cardiovascular training differentially improves both perceptions of effort and 5000 m running performance compared with cardiovascular training alone. *British Journal of Sports Medicine*, **42**, 523-527.

Eldridge, F. (1966). Anaerobic metabolism of the respiratory muscles. *Journal of Applied Physiology*, **21**, 853-857.

Eldridge, F.L., Millhorn, D.E, and Waldrop, T.G. (1981). Exercise hyperpnea and locomotion: parallel activation from the hypothalamus. *Science*, **20**, 844-846.

El-Manshawi, A., Killian, K.J., Summers, E. and Jones, N.L. (1986). Breathlessness during exercise with and without resistive loading. *Journal of Applied Physiology*, **61**, 896-905.

Engelen, M.P., Casaburi, R., Rucker, R. and Carithers, E. (1995). Contribution of the respiratory muscles to the lactic acidosis of heavy exercise in COPD. *Chest*, **108**, 1246-1251.

Enright, P.L., Kronmal, R.A., Manolio, T.A., Schenker, M.B. and Hyatt, R.E. (1994). Respiratory muscle strength in the elderly. Correlates and reference values. Cardiovascular health study research group. *American Journal of Respiratory and Critical Care Medicine*, **149**, 430-438.

Enright, S.J., Unnithan, V.B., Heward, C., Withnall, L. and Davies, D.H. (2006). Effect of high-intensity inspiratory muscle training on lung volumes, diaphragm thickness, and exercise capacity in subjects who are healthy. *Physical Therapy*, **86**, 345-354

Fairbarn, M.S., Coutts, K.C., Pardy, R.L. and McKenzie, D.C. (1991). Improved respiratory muscle endurance of highly trained cyclists and the effects on maximal exercise performance. *International Journal of Sports Medicine*, **12**, 66-70.

Ferguson, R.A., Ball, D., Krustrup, P., Aagaard, P., Kjaer, M., Sargeant, A.J., Hellsten, Y. and Bangsbo, J. (2001). Muscle oxygen uptake and energy turnover during dynamic exercise at different contraction frequencies in humans. *Journal of Physiology*, **536**, 261-271.

Field, A. (2008). Discovering statistics using SPSS – and sex drugs and rock and roll. London, UK: Sage Publications.

Fitting, J.W., Bradley, T.D., Easton, P.A., Lincoln, M.J., Goldman, M.D. and Grassino, A. (1988). Dissociation between diaphragmatic and rib cage muscle fatigue. *Journal of Applied Physiology*, **64**, 959-965.

Fitts, R.H. (1994). Cellular mechanisms of muscle fatigue. *Physiological Reviews,* **74**, 49-94.

Folland, J.P. and Williams, A.G. (2007). The adaptations to strength training. Morphological and neurobiological contributions to increased strength. *Sports Medicine*, **32**, 145-168.

Folland, J.P., Wakamatsu, T. and Fimland, M.S. (2008). The influence of maximal isometric activity on twitch and H-reflex potentiation, and quadriceps femoris performance. *European Journal of Applied Physiology*, **104**, 739-748.

Forster, H.V., Dempsey, J.A., Thomson, J., Vidruk, E. and DoPico, G.A. (1972). Estimation of arterial PO_2, PCO_2, pH, and lactate from arterialized venous blood. *Journal of Applied Physiology*, **32**, 134-137.

Freedman, S., Cooke, N.T. and Moxham, J. (1983). Production of lactic acid by respiratory muscles. *Thorax*, **38**, 50-54.

Fregosi, R.F. and Dempsey, J.A. (1986). Effects of exercise in normoxia and acute hypoxia on respiratory muscle metabolites. *Journal of Applied Physiology*, **60**, 1274-1283.

Freund, H. and Zouloumian, P. (1981). Lactate after exercise in man: I. Evolution kinetics in arterial blood. *European Journal of Applied Physiology*, **46**, 121-133.

Fukuba, Y. and Whipp, B.J. (1999). A metabolic limit on the ability to make up for lost time in endurance events. *Journal of Applied Physiology*, **87**, 853-861.

Gandevia, S.C., Killian, K.J. and Campbell, E.J. (1981). The effect of respiratory muscle fatigue on respiratory sensations. *Clinical Science,* **60**, 463-466.

Gandevia, S.C., Leeper, J.B., McKenzie, D.K. and De Troyer, A. (1996). Discharge frequencies of parasternal intercostal and scalene motor units during breathing in normal and COPD subjects. *American Journal of Respiratory and Critical Care Medicine*, **153**, 622-628.

Gandevia, S.C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiological Reviews*, **81**, 1725-1789.

Gething, A.D., Passfield, L. and Davies, B. (2004a). The effects of different inspiratory muscle training intensities on exercising heart rate and perceived exertion. *European Journal of Applied Physiology,* **92**, 50-55.

Gething, A.D., Williams, M. and Davies, B. (2004b). Inspiratory resistive loading improves cycling capacity: a placebo controlled trial. *British Journal of Sports Medicine*, **38**, 730-736.

Gladden, L.B. (2008). Comments on Point:Counterpoint "Lactic acid is/is not the only physiochochemical contributor to the acidosis of exercise: Cause and Effect?. *Journal of Applied Physiology*, **105**, 364.

Graham, R., Jammes, Y., Delpierre, S., Grimaud, C. and Roussos, C. (1986). The effects of ischemia, lactic acid and hypertonic sodium chloride on phrenic afferent discharge during spontaneous diaphragmatic contraction. *Neuroscience Letters*, **67**, 257-262.

Green, M., Road, J., Sieck, G.C. and Similowski, T. (2002). American Thoracic Society/European Respiratory Society. ATS/ERS Statement on respiratory muscle testing. 2 Tests of respiratory muscle strength. *American Journal of Respiratory and Critical Care Medicine*, **166**, 518-624.

Griesenbach, U., Geddes, D.M. and Alton, E.W.F.W. (2004). Gene therapy for cystic fibrosis: an example for lung gene therapy. *Gene Therapy*, **11**, 43-50.

Griffiths, L.A. and McConnell, A.K. (2007). The influence of inspiratory and expiratory muscle training upon rowing performance. *European Journal of Applied Physiology*, **99**, 457-466.

Guenette, J.A., Martens, A.M., Lee, A.L., Tyler, G.D., Richards, J.C., Foster, G.E., Warburton, D.E. and Sheel, A.W. (2006). Variable effects of respiratory muscle training on cycle exercise performance in men and women. *Applied physiology, Nutrition and Metabolism*, **31**, 159-166.

Guenette, J.A., Querido, J,S., Eves, N.D., Chua, R. and Sheel, A.W. (2009). Sex differences in the resistive and elastic work of breathing during exercise in endurance-trained athletes. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology*, **297**, R166-175.

Guleria, R., Lyall, R., Hart, N., Harris, M.L., Hamnegård, C.H., Green, M., Moxham, J. and Polkey, M.I. (2002). Central fatigue of the diaphragm and quadriceps during incremental loading. *Lung*, **180**, 1-13.

Häkkinen, K. (1994). Neuromuscular adaptation during strength training, aging, detraining and immobilization. *Critical Reviews in Physical and Rehabilitation Medicine*, **63**, 161-198.

Hanel, B. and Secher, N.H. (1991). Maximal oxygen uptake and work capacity after inspiratory muscle training: a controlled study. *Journal of Sports Sciences*, **9**, 43-52.

Haouzi, P. (2006). Theories on the nature of the coupling between ventilation and gas exchange during exercise. *Respiratory Physiology and Neurobiology*, **28**, 267-279.

Howlett, R.A., Kirkton, S.D., Gonzalez, N.C., Wagner, H.E., Britton, S.L., Koch, L.G. and Wagner, P.D. (2009). Peripheral oxygen transport and utilization in rats following continued selective breeding for endurance running capacity. *Journal of Applied Physiology*, **106**, 1819-1825.

Harik-Khan, R.I., Wise, R.A. and Fozard, J.L. (1998). Determinants of maximal inspiratory pressure: The Baltimore longitudinal study of ageing. *American Journal of Respiratory and Critical Care Medicine*, **158**, 1459-1464.

Harms, C.A., Babcock, M.A., McClaran, S.R., Pegelow, D.F., Nickele, G.A., Nelson, W.B. and Dempsey, J.A. (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *Journal of Applied Physiology*, **82**, 1573-1583.

Harms, C.A., Wetter, T.J., McClaran, S.R., Pegelow, D.F., Nickele, G.A., Nelson, W.B., Hanson, P. and Dempsey, J.A. (1998). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *Journal of Applied Physiology*, **85**, 609-618.

Harms, C.A., Wetter, T.J., St Croix, C.M., Pegelow, D.F. and Dempsey, J.A. (2000). Effects of respiratory muscle work on exercise performance. *Journal of Applied Physiology*, **89**, 131-138.

Harridge, S.D.R., Kryger, A. And Stensgaard, A. (1999). Knee extensor strength, activation, and size in very elderly people following strength training. *Muscle Nerve*, **22**, 831-839.

Hart, N., Sylvester, K., Ward, S., Cramer, D., Moxham, J. and Polkey, M.I. (2001). Evaluation of an inspiratory muscle trainer in healthy humans. *Respiration Medicine,* **95**, 526-531.

Hashimoto, T. and Brooks, G.A. (2008). Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Medicine and Science in Sports and Exercise,* **40**, 486-494.

Hawkins, S. and Wiswell, R. (2003). Rate and mechanism of maximal oxygen consumption decline with aging: implications for exercise training. *Sports Medicine,* **33**, 877-888.

He, Z.H., Bottinelli, R., Pellegrino, M.A., Ferenczi, M.A. and Reggiani, C. (2000). ATP consumption and efficiency of human single muscle fibers with different myosin isoform composition. *Biophysical Journal*, **79**, 945-961.

Henke, K.G., Sharratt, M., Pegelow, D. and Dempsey, J.A. (1988). Regulation of end-expiratory lung volume during exercise. *Journal of Applied Physiology*, **64**, 135-146.

Hershenson, M.B., Kikuchi, Y. and Loring, S.H. (1988). Relative strengths of the chest wall muscles. *Journal of Applied Physiology*, **65**, 852-862.

Hill, J.M. (2000). Discharge of group IV phrenic afferent fibers increases during diaphragmatic fatigue. *Brain Research*, **856**, 240-244.

Hill, J.M. (2001). Increase in the discharge of muscle spindles during diaphragm fatigue. *Brain Research*, **918**, 166-170.

Holloszy, J.O. and Coyle, E.F. (1984). Adaptations of skeletal muscles to endurance exercise and their metabolic consequences. *Journal of Applied Physiology*, **56**, 831-838.

Holm, P., Sattler, A. and Fregosi, R.F. (2004). Endurance training of respiratory muscles improves cycling performance in fit young cyclists. *Biomed Central Physiology*, **4,** 1-14.

Hoppeler, H., Mathieu, O., Krauer, R., Claassen, H., Armstrong, R.B. and Weibel, E.R. (1981). Design of the mammalian respiratory system. VI Distribution of mitochondria and capillaries in various muscles. *Respiration Physiology*, **44**, 87-111.

Huang, C.H., Martin, A.D. and Davenport, P.W. (2003). Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. *Journal of Applied Physiology*, **94**, 462-468.

Hyatt, R.E. and Flath, R.E. (1966). Relationship of air flow to pressure during maximal respiratory effort in man. *Journal of Applied Physiology*, **21**, 477-482.

Ianuzzo, C.D., Spalding, M.J. and Williams, H. (1987). Exercise-induced glycogen utilization by the respiratory muscles. *Journal of Applied Physiology*, **62**, 1405-1409.

Inbar, O., Weiner, P., Azgad, Y., Rotstein, A. and Weinstein, Y. (2000). Specific inspiratory muscle training in well-trained endurance athletes. *Medicine and Science in Sports and Exercise*, **32**, 1233-1237.

Ivey, P.A. and Gaesser, GA. (1987). Postexercise muscle and liver glycogen metabolism in male and female rats. *Journal of Applied Physiology*, **62**, 1250-1254.

Jammes, Y., Buchler, B., Delpierre, S., Rasidakis, A., Grimaud, C. and Roussos, C. (1986). Phrenic afferents and their role in inspiratory control. *Journal of Applied Physiology*, **60**, 854-860.

Jammes, Y. and Balzamo, E. (1992). Changes in afferent and efferent phrenic activities with electrically induced diaphragmatic fatigue. *Journal of Applied Physiology*, **73,** 894-902.

Janse de Jonge, X.A., Boot, C.R., Thom, J.M., Ruell, P.A. and Thompson, M.W. (2001). The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. *Journal of Physiology*, **530**, 161-166.

Jeukendrup, A., Saris, W.H., Brouns, F. and Kester, A.D. (1996). A new validated endurance performance test. *Medicine and Science in Sports and Exercise*, **28**, 266-270.

Johnson, B.D., Saupe, K.W. and Depsey, J.A. (1992). Mechanical constraints on exercise hyperpnoea in endurance athletes, *Journal of Applied Physiology*, **73**, 874-886.

Johnson, B.D., Babcock, M.A., Suman, O.E. and Dempsey, J. A. (1993). Exercise-induced diaphragmatic fatigue in healthy humans. *Journal of Physiology*, **460,** 385-405.

Johnson, L.R., Rush, J.W.E., Turk, J.R., Price, E.M., and Laughlin, M.H. (2001). Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. *Journal of Applied Physiology*, **90**, 1102-1110.

Johnson, M.A., Sharpe, G.R. and McConnell, A.K. (2006). Maximal voluntary hyperpnoea increases blood lactate concentration during exercise. *European Journal of Applied Physiology*, **96**, 600-608.

Johnson, M.A., Sharpe, G.R. and Brown, P.I. (2007). Inspiratory muscle training improves cycling time trial performance and anaerobic work capacity but not critical power. *European Journal of Applied Physiology*, **101**, 761-770

Johnson, M.A., Sharpe, G.R. and Brown, P.I. (2009). Investigations of the lactate minimum test. *International Journal of Sports Medicine*, **30**, 448-454.

Johnson, P.H., Cowley, A.J. and Kinnear, W.J. (1997). Incremental threshold loading: a standard protocol and establishment of a reference range in naive normal subjects. *European Respiratory Journal*, **10**, 2868-2871.

Johnson, P.H., Cowley, A.J. and Kinnear, W.J. (1996). Evaluation of the THRESHOLD trainer for inspiratory muscle endurance training: comparison with the weighted plunger method. *European Respiratory Journal*, **9**, 2681-2684.

Jones, D.A. (1996). High-and low-frequency fatigue revisited. *Acta Physiologica Scandinavica*, **156**, 265-270.

Jones, N.L. (2008). An obsession with CO_2. *Applied Physiology, Nutrition and Metabolism*, **33**, 641-650.

Jones, N.L. and Killian, K.J. (2000). Exercise limitation in health and disease. *New England Journal of Medicine*, **343**, 632-641.

Jonville, S., Jutand, L., Similowski, T., Denjean, A. and Delpech, N. (2005). Putative protective effect of inspiratory threshold loading against exercise-induced supraspinal diaphragm fatigue. *Journal of Applied Physiology*, **98**, 991-998.

Juel, C. (2001). Current aspects of lactate exchange: lactate/H^+ transport in human skeletal muscle. *European Journal of Applied Physiology*, **86**, 12-16.

Juel, C., Holten, M.K. and Dela, F. (2004). Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. *Journal of Physiology*, **556**, 297-304.

Kabitz, H.J., Walker, D., Schwoerer, A., Sonntag, F., Walterspacher, S., Roecker, K. and Windisch, W. (2007). New physiological insights into exercise-induced diaphragmatic fatigue. *Respiratory Physiology and Neurobiology*, **158**, 88-96.

Kabitz, H.J., Walker, D., Walterspacher, S., Sonntag, F., Schwoerer, A., Roecker, K. and Windisch, W. (2008a). Independence of exercise-induced diaphragmatic fatigue from ventilatory demands. *Respiratory Physiology and Neurobiology*, **161**, 101-107.

Kabitz, H.J., Walker, D., Sonntag, F., Walterspacher, S., Kirchberger, A., Burgardt, V., Roecker, K. and Windisch, W. (2008b). Post-exercise diaphragm shielding: a novel approach to exercise-induced diaphragmatic fatigue. *Respiratory Physiology and Neurobiology*, **31**, 162, 230-237.

Kay, C. and Shephard, R.J. (1969). On muscle strength and the threshold of anaerobic work. *Internationale Zeitschrift fur Angewandte Physiologie*, **27**, 311-328.

Kayser, B., Sliwinski, P., Yan, S., Tobiasz, M. and Macklem, P.T. (1997). Respiratory effort sensation during exercise with induced expiratory-flow limitation in healthy humans. *Journal of Applied Physiology*, **83**, 936-947.

Kellerman, B.A., Martin, A.D. and Davenport, P.W. (2000). Inspiratory strengthening effect on resistive load detection and magnitude estimation. *Medicine and Science in Sports and Exercise*, **32**, 1859-1867.

Kenyon, C.M., Cala, S.J., Yan, S., Aliverti, A., Scano, G., Duranti, R., Pedotti, A. and Macklem, P.T. (1997). Rib cage mechanics during quiet breathing and exercise in humans. *Journal of Applied Physiology*, **83**, 1242-1255.

Kirkton, S.D., Howlett, R.A., Gonzalez, N.C., Giuliano, P.G., Britton, S.L., Koch, L.G., Wagner, H.E. and Wagner, P.D. (2009). Continued artificial selection for running endurance in rats is associated with improved lung function. *Journal of Applied Physiology*, **106**, 1810-1818.

Klas, J.V. and Dempsey, J.A. (1989). Voluntary versus reflex regulation of maximal exercise flow:volume loops. *American Review of Respiratory Disease,* **139**,150-156.

Knowles, J.H., Hong, S.K. and Rahn, H. (1959). Possible errors using esophageal balloon in determination of pressure-volume characteristics of the lung and thoracic cage. *Journal of Applied Physiology*, **14**, 525-530.

Kohl, J., Koller, E.A., Brandenberger, M., Cardenas, M. and Boutellier, U. (1997). Effect of exercise-induced hyperventilation on airway resistance and cycling endurance. *European Journal of Applied Physiology*, **75**, 305-311.

Koopman, R., Saris, W.H., Wagenmakers, A.J. and van Loon, L.J. (2007). Nutritional interventions to promote post-exercise muscle protein synthesis. *Sports Medicine*, **37**, 895-906.

Kowalchuk, J.M. and Scheuermann, B.W. (1995). Acid-base balance: origin of plasma [H^+] during exercise. *Canadian Journal of Applied Physiology*, **20**, 341-356.

Kowalchuk, J.M., Heigenhauser, G.J., Lindinger, M.I., Sutton, J.R. and Jones, N.L. (1988). Factors influencing hydrogen ion concentration in muscle after intense exercise. *Journal of Applied Physiology*, **65**, 2080-2089.

Kraemer, W.J., Fleck, S.J. and Evans, W.J. (1996). Strength and power training: physiological mechanisms of adaptation. *Exercise and Sport Science Reviews*, **24**, 363-397.

Lai-Fook, S.J. (2004). Pleural mechanics and fluid exchange. *Physiological Reviews,* **84**, 385-410.

Lamarra, N., Whipp, B.J., Ward, S.A. and Wasserman, K. (1987). Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *Journal of Applied Physiology*, **62**, 2003-2012.

Larson, J.L., Covey, M.K., Vitalo, C.A., Alex, C.G., Patel, M. and Kim, M.J. (1993). Maximal inspiratory pressure. Learning effect and test-retest reliability in patients with chronic obstructive pulmonary disease. *Chest*, **104**, 448-453.

LeBlanc, P., Summers, E., Inman, M.D., Jones, N.L., Campbell, E.J. and Killian, K.J. (1988). Inspiratory muscles during exercise: a problem of supply and demand. *Journal of Applied Physiology,* **64**, 2482-2489.

LeBlanc, P.J., Parolin, M.L., Jones, N.L. and Heigenhauser, G.J. (2002). Effects of respiratory alkalosis on human skeletal muscle metabolism at the onset of submaximal exercise. *Journal of Physiology,* **544**, 303-313.

Leddy, J.J., Limprasertkul, A., Patel, S., Modlich, F., Buyea, C., Pendergast, D.R. and Lundgren, C.E. (2007). Isocapnic hyperpnea training improves performance in competitive male runners. *European Journal of Applied Physiology*, **99**, 665-676.

Legrand, A., Schneider, E., Gevenois, P.A. and De Troyer, A. (2003). Respiratory effects of the scalene and sternomastoid muscles in humans. *Journal of Applied Physiology*, **94**, 1467-1472.

Leith, D.E. and Bradley, M. (1976). Ventilatory muscle strength and endurance training. *Journal of Applied Physiology*, **41**, 508-516.

Leveritt, M., Abernethy, P.J., Barry, B.K. and Logan, P.A. (1999). Concurrent strength and endurance training. A review. *Sports Medicine*, **28**, 413-427.

Lindinger, M.I., Heigenhauser, G.J., McKelvie, R.S. and Jones, N.L. (1992). Blood ion regulation during repeated maximal exercise and recovery in humans. *American Journal of Physiology, Regulatory, Integrative and Competitive Physiology*, **262**, R126-R136.

Lindinger, M.I. (1995). Origins of [H^+] changes in exercising skeletal muscle. *Canadian Journal of Applied Physiology*, **20**, 357-368.

Lindinger, M.I., McKelvie, R.S. and Heigenhauser, G.J. (1995). K^+ and Lac^- distribution in humans during and after high-intensity exercise: role in muscle fatigue attenuation? *Journal of Applied Physiology*, **78**, 765-777.

Lisboa, C., Villafranca, A., Leiva, A., Cruz, E., Pertuze, J. and Borzone, G. (1997). Inspiratory muscle training in chronic airflow limitation: effect on exercise performance. *European Respiratory Journal*, **10**, 537-542.

Loke, J., Mahler, D.A. and Virgulto, J.A. (1982). Respiratory muscle fatigue after marathon running. *Journal of Applied Physiology*, **52**, 821-824.

Lomax, M.E. and McConnell, A.K. (2003). Inspiratory muscle fatigue in swimmers after a single 200 m swim. *Journal of Sports Sciences*, **21**, 659-664.

Lotters. F., van Tol, B., Kwakkel, G. And Gosselink, R. (2002). Effects of controlled inspiratory muscle training in patients with COPD: a meta analysis. *European Respiratory Journal*, **20**, 570-576.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R.J. (1951). Protein measurements with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.

Madanmohan, M., Mahadevan, S.K., Balakrishnan, S., Gopalakrishnan, M. and Prakash, E.S. (2008). Effect of six weeks yoga training on weight loss following step test, respiratory pressures, handgrip strength and handgrip endurance in young healthy subjects. *Indian Journal of Physiology and Pharmacology*, **52**, 164-170.

Mador, M.J. and Acevedo, F.A. (1991a). Effect of inspiratory muscle fatigue on breathing pattern during inspiratory resistive loading. *Journal of Applied Physiology*, **70**, 1627-1632.

Mador, J.M. and Acevedo, F.A. (1991b). Effect of respiratory muscle fatigue on subsequent exercise performance. *Journal of Applied Physiology*, **70**, 2059-2065.

Mador, J.M, Magalang, U.J., Rodis, A. and Kufel, T.J. (1993). Diaphragmatic fatigue after exercise in healthy human subjects. *American Review of Respiratory Disease*, **148**, 1571-1575.

Mador, J.M., Rodis, A. and Diaz, J. (1996). Diaphragmatic fatigue following voluntary hyperpnea. *American Journal of Respiratory and Critical Care Medicine*, **154**, 63-67.

Man, W.D., Luo, Y.M., Mustfa, N., Rafferty, G.F., Glerant, J.C., Polkey, M.I. and Moxham, J. (2002). Postprandial effects on twitch transdiaphragmatic pressure. *European Respiratory Journal*, **20**, 577-580.

Manohar, M. (1986). Blood flow to the respiratory and limb muscles and to abdominal organs during exertion in ponies. *Journal of Physiology,* **377,** 25-35.

Manohar, M., Goetz, T.E., Holste, L.C. and Nganwa, D. (1988). Diaphragmatic O_2 and lactate extraction during submaximal and maximal exertion in ponies. *Journal of Applied Physiology*, **64**, 1203-1209.

Manohar, M. and Hassan, A.S. (1990). Diaphragm does not produce ammonia or lactate during high-intensity short-term exercise. *American Journal of Physiology, Heart and Circulatory Physiology*, **259**, H1185-H1189.

Manohar, M. and Hassan, A.S. (1991). Diaphragmatic energetics during prolonged exhaustive exercise. *American Review of Respiratory Disease*, **144**, 415-418.

Marcinik, E.J., Potts, J., Schlabach, G., Will, S., Dawson, P. and Hurley, B.F. (1991). Effects of strength training on lactate threshold and endurance performance. *Medicine and Science in Sports and Exercise*, **23**, 739-743.

Margaria, R., Edwards, H.T. and Dill, D.B. (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *American Journal of Physiology,* **106**, 689-715.

Markov, G., Spengler, C.M., Knopfli-Lenzin, C., Stuessi, C. and Boutellier, U. (2001). Respiratory muscle training increases cycling endurance without affecting cardiovascular responses to exercise. *European Journal of Applied Physiology*, **85**, 233-239.

Maharan, L.G., Bauman, P.A., Kalman, D., Skolnik, H. and Perle, S.M. (1999). Masters athletes: factors affecting performance. *Sports Medicine*, **28**, 273-285.

Martin, B.J. and Stager, J.M. (1981). Ventilatory endurance in athletes and non-athletes. *Medicine and Science in Sports and Exercise*, **13**, 21-26.

Martin, J., Aubier, M. and Engel, L.A. (1982). Effects of inspiratory loading on respiratory muscle activity during expiration. *American Review of Respiratory Disease*, **125**, 352-328.

Martin, B.J., Chen, H.I. and Kolka, M.A. (1984). Anaerobic metabolism of the respiratory muscles during exercise. *Medicine and Science in Sports and Exercise*, **16**, 82-86.

Matsunaga, S., Inashima, S., Tsuchimochi, H., Yamada, T., Hazama, T. and Wada, M. (2002). Altered sarcoplasmic reticulum function in rat diaphragm after high-intensity exercise. *Acta Physiologica Scandinavica*, **176**, 227-232.

McClaran, S.R., Harms, C. A., T.J., Pegelow, D.F. and Dempsey, J.A. (1998). Smaller lungs in woman effects exercise hyperpnoea. *Journal of Applied Physiology*, **84**, 1872-1882.

McClaran, S.R., Wetter, T.J., Pegelow, D.F. and Dempsey, J.A. (1999). Role of expiratory flow limitation in determining lung volumes and ventilation during exercise. *Journal of Applied Physiology*, **86**, 1357-1366.

McConnell, A.K. (2005). The role of inspiratory muscle function and training in the genesis of dyspnoea in asthma and COPD. *Primary Care Respiratory Journal*, **14**, 186-194.

McConnell, A.K. (2007). Lung and respiratory muscle function. In: *Sport and Exercise Physiology Testing Guidelines, the British Association of Sport and Exercise Sciences Guide*. Winter, E.M. Jones, A.M. and Davison, R.C.R. (Eds.). Routledge: Oxford, pp. 63-76.

McConnell, A.K., Caine, M.P. and Sharpe, G.R. (1997). Inspiratory muscle fatigue following running to volitional fatigue: the influence of baseline strength. *International Journal of Sports Medicine*, **18**, 169-173.

McConnell, A.K. and Copestake, A.J. (1999). Maximum static respiratory pressures in healthy elderly men and women: issues of reproducibility and interpretation. *Respiration*, **66**, 251-258.

McConnell, A.K., Romer, L.M., Volianitis, S. and Donovan, K.J. (2002). Re: Evaluation of an inspiratory muscle trainer in healthy humans (*Respiratory Medicine* 2001; 95: 526-531). *Respiratory Medicine*, **96**, 129-133.

McConnell, A.K. and Romer, L.M. (2004a). Respiratory muscle training in healthy humans: resolving the controversy. *International Journal of Sports Medicine*, **25**, 284-293.

McConnell, A.K. and Romer, L.M. (2004b). Dyspnoea in health and obstructive lung disease: the role of respiratory muscle function and training. *Sports Medicine*, **34**, 117-132.

McConnell, A.K. and Sharpe, G.R. (2005). The effect of inspiratory muscle training upon maximum lactate steady-state and blood lactate concentration. *European Journal of Applied Physiology*, **94**, 277-284.

McConnell, A.K. and Lomax, M. (2006). The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. *Journal of Physiology*, **577**, 445-457.

McKenna, M.J., Heigenhauser, G.J., McKelvie, R.S., MacDougall, J.D. and Jones, N.L. (1997). Sprint training enhances ionic regulation during intense exercise in men. *Journal of Physiology*, **15**, 687-702.

McLoughlin, P., Popham, P., Linton, R.A., Bruce, R.C. and Band, D.M. (1992). Use of arterialized venous blood sampling during incremental exercise tests. *Journal of Applied Physiology*, **73**, 937-940.

McMahon, M.E., Boutellier, U., Smith, R.M. and Spengler, C.M. (2002). Hyperpnoea training attenuates peripheral chemosensitivity and improves cycling endurance. *The Journal of Experimental Biology*, **205**, 3937-3943.

Mead, J., McIlroy, M.B., Selverstone, N.J. and Kriete, B.C. (1955). Measurement of intraesophageal pressure. *Journal of Applied Physiology*, **7**, 491-496.

Messonnier, L., Freund, H., Bourdin, M., Belli, A. and Lacour, J.R. (1997). Lactate exchange and removal abilities in rowing performance. *Medicine and Science in Sports and Exercise,* **29**, 396-401.

Messonnier, L., Freund, H., Féasson, L., Prieur, F., Castells, J., Denis, C., Linossier, M.T., Geyssant, A. and Lacour, J.R. (2001). Blood lactate exchange and removal abilities after relative high-intensity exercise: effects of training in normoxia and hypoxia. *European Journal of Applied Physiology*, **84**, 403-412.

Messonnier, L., Freund, H., Denis, C., Féasson, L. and Lacour, J.R. (2006). Effects of training on lactate kinetics parameters and their influence on short high-intensity exercise performance. *International Journal of Sports Medicine*, **27**, 60-66.

Mier, A., Brophy. C., Estenne. M., Moxham, J., Green, M. and De Troyer, A. (1985). Action of abdominal muscles on rib cage in humans. *Journal of Applied Physiology*, **58**, 1438-1443.

Milic-Emili, J. (1991). Work of breathing. In: *The Lung: Scientific Foundations.* Crystal, R.G. and West, J.B. (Eds.). Raven Press: New York, pp. 1065-1075.

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., Van der Grinten, C.P., Gustafsson, P., Jensen, R., Johnson, D.C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G. and Wanger, J. (2005). ATS/ERS Task Force. *European Respiratory Journal*, **26**, 319-338.

Miller, J.D., Pegelow, D.F., Jacques, A.J. and Dempsey, J.A. (2005). Effects of augmented respiratory muscle pressure production on locomotor limb venous return during calf contraction exercise. *Journal of Applied Physiology*, **99**, 1802-1815.

Miller, J.D., Hemauer, S.J., Smith, C.A., Stickland, M.K. and Dempsey J.A. (2006). Expiratory threshold loading impairs cardiovascular function in health and chronic heart failure during submaximal exercise. *Journal of Applied Physiology*, **101**, 213-227.

Miller, J.D., Smith, C.A., Hemauer, S.J. and Dempsey J.A. (2007). The effects of inspiratory intrathoracic pressure production on the cardiovascular response to submaximal exercise in health and chronic heart failure. *American Journal of Physiology, Heart and Circulatory Physiology*, **292**, H580-H592.

Mizuno, M. and Secher, N.H. (1989). Histochemical characteristics of human expiratory and inspiratory intercostal muscles. *Journal of Applied Physiology*, **67**, 592-598.

Morgan, D.W., Kohrt, W.M., Bates, B.J. and Skinner, J.S. (1987). Effects of respiratory muscle endurance training on ventilatory and endurance performance of moderately trained cyclists. *International Journal of Sports Medicine*, **8**, 88-93.

Mougios, V. (2006). Exercise biochemistry. Champaign, Illinois, USA: Human Kinetics.

Moussay, S., Bessot, N., Gauthier, A., Larue, J., Sesboüe, B. and Davenne, D. (2003). Diurnal variations in cycling kinematics. *Chronobiology International*, **20**, 879-892.

Nava, S., Ambrosino, N., Crotti, P., Fracchia, C. and Rampulla, C. (1993). Recruitment of some respiratory muscles during three maximal inspiratory maneuvers. *Thorax*, **48**, 702-707.

Nguyen, T., Shrager, J., Kaiser, L., Mei, L., Daood, M., Watchko, J., Rubinstein, N. and Levine, S. (2000). Developmental myosin heavy chains in the adult human diaphragm: coexpression patterns and effect of COPD. *Journal of Applied Physiology*, **88**, 1446-1456.

NHLBI workshop. Respiratory muscle fatigue: report of the respiratory muscle fatigue workshop group. *American Review of Respiratory Disease*, **142**, 474-486.

Nickerson, B.G. and Keens, T.G. (1982). Measuring ventilatory muscle endurance in humans as sustainable inspiratory pressure. *Journal of Applied Physiology*, **52**, 768-772.

Nicks, C.R., Morgan, D.W., Fuller, D.K. and Caputo, J.L. (2009). The influence of respiratory muscle training upon intermittent exercise performance. *International Journal of Sports Medicine*, **30**, 16-21.

Nielsen, O.B. and Overgaard, K. (1996). Ion gradients and contractility in skeletal muscle: the role of active Na^+, K^+ transport. *Acta Physiologica Scandinavica*, **156**, 247-256.

Ozkaplan, A., Rhodes, EC., Sheel, A.W. and Taunton, J.E. (2005). A comparison of inspiratory muscle fatigue following maximal exercise in moderately trained males and females. *European Journal of Applied Physiology*, **95**, 52-56.

Padilla, D.J., McDonough, P., Kindig, C.A., Erickson, H.H. and Poole, D.C. (2004). Ventilatory dynamics and control of blood gases after maximal exercise in the Thoroughbred horse. *Journal of Applied Physiology*, **96**, 2187-2193.

Perret, C. and Mueller, G. (2007). Impact of low-intensity isocapnic hyperpnoea on blood lactate disappearance after exhaustive arm exercise. *British Journal of Sports Medicine*, **41**, 588-591.

Perret, C., Pfeiffer, R., Boutellier, U., Wey, H.M. and Spengler, C.M. (1999). Noninvasive measurement of respiratory muscle performance after exhaustive endurance exercise. *European Respiratory Journal*, **14**, 264-269.

Perret, C., Spengler, C.M., Egger, G. and Boutellier, U. (2000). Influence of endurance exercise on respiratory muscle performance. *Medicine and Science in Sport and Exercise*, **32,** 2052-2058.

Pilegaard, H., Bangsbo, J., Richter, EA. and Juel, C. (1994). Lactate transport studied in sarcolemmal giant vesicles from human muscle biopsies: relation to training status. *Journal of Applied Physiology*, **77,** 1858-1862.

Polkey, M.I., Kyroussis, D., Hamnegard, C.H., Hughes, P.D., Rafferty, GF., Moxham, J. and Green, M. (1997). Paired phrenic nerve stimuli for the detection of diaphragm fatigue in humans. *European Respiratory Journal*, **10**, 1859-1864.

Polkey, M.I. and Moxham, J. (2004). Improvement in volitional tests of muscle function alone may not be adequate evidence that inspiratory muscle training is effective. *European Respiratory Journal*, **23**, 5-6.

Polla, B., D'Antona, G. and Reggiani, C. (2004). Respiratory muscle fibres: specialisation and plasticity. *Thorax*, **59**, 808-817.

Poole, D.C., Sexton, W.L., Farkas, G.A., Powers, S.K. and Reid, M.B. (1997). Diaphragm structure and function in health and disease. *Medicine and Science in Sport and Exercise*, **29,** 734-754.

Powers, S.K., Coombes, J. and Demirel, H. (1997). Exercise training-induced changes in respiratory muscles. *Sports Medicine*, **24**, 120-131.

Presland, J.D., Dowson, M.N. and Cairns, S.P. (2005). Changes of motor drive, cortical arousal and perceived exertion following prolonged cycling to exhaustion. *European Journal of Applied Physiology*, **95**, 42-51.

Putman, C.T., Jones, N.L. and Heigenhauser, G.J. (2003). Effects of short-term training on plasma acid-base balance during incremental exercise in man. *Journal of Physiology*, **15**, 585-603.

Quanjer, P.H., Tammeling, G.J., Cotes, J.E., Pedersen, O.F., Peslin, R. and Yernault, J.C. (1993). Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. *European Respiratory Journal*, **16**, 5-40.

Ramírez-Sarmiento, A., Orozco-Levi, M., Güell, R., Barreiro, E., Hernandez, N., Mota, S., Sangenis, M., Broquetas, J.M., Casan, P. and Gea, J. (2002). Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. *American Journal of Respiratory and Critical Care Medicine*, **166**, 1491-1497.

Rassier, D.E. and Macintosh, B.R. (2000). Coexistence of potentiation and fatigue in skeletal muscle. *Brazilian Journal of Medical and Biological Research*, **33**, 499-508.

Redline, S., Gottfried, S.B. and Altose, M.D. (1991). Effects of changes in inspiratory muscle strength on the sensation of respiratory force. *Journal of Applied Physiology*, **70**, 240-245.

Renggli, A.S., Verges, S., Notter, D.A. and Spengler, C.M. (2008). Development of respiratory muscle contractile fatigue in the course of hyperpnoea. *Respiratory Physiology and Neurobiology*, **164**, 366-372.

Revelette, W.R. and Wiley, R.L. (1987). Plasticity of the mechanism subserving inspiratory load perception. *Journal of Applied Physiology*, **62**, 1901-1906.

Riganas, C.S., Vrabas, I.S., Christoulas, K. and Mandroukas, K. (2008). Specific inspiratory muscle training does not improve performance or $\dot{V}O_2$max levels in well trained rowers. *Journal of Sports Medicine and Physical Fitness*, **48**, 285-292.

Robergs, R.A., Ghiasvand, F. and Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology, Regulatory, Integrative and Competitive Physiology*, **287**, R502-R516.

Robertson, C.H., Eschenbacher, W.L. and Johnson, R.L. (1977). Respiratory muscle blood flow distribution during expiratory resistance. *Journal of Clinical Investigation*, 60, 473-480.

Robinson, E.P. and Kjeldgaard, J.M. (1982). Improvement in ventilatory muscle function with running. *Journal of Applied Physiology*, **52**, 1400-1406.

Rochester, D.F., Arora, N.S. and Braun, N.M.T. (1981). Maximum contractile force of human diaphragm muscle, determined in vivo. *Transactions of the American Clinical and Climatological Association*, **93**, 200-208. Cited in Loring, S.h. and De Troyer, A. (1985). Actions of the human respiratory muscles. In: *The Thorax.* Roussos, C. and Macklem, P.T. (Eds.). New York: Marcel Dekker: pp 327-349.

Rodman, J.R., Henderson, K.S., Smith, C.A. and Dempsey, J.A. (2003). Cardiovascular effects of the respiratory muscle metaboreflexes in dogs: rest and exercise. *Journal of Applied Physiology*, **95**, 1159-1169.

Romer, L.M., McConnell, A.K. and Jones, D.A. (2002a). Inspiratory muscle fatigue in trained cyclists: effects of inspiratory muscle training. *Medicine and Science in Sports and Exercise*, **34**, 785-792.

Romer, L.M., McConnell, A.K. and Jones, D.A. (2002b). Effects of inspiratory muscle training upon recovery time during high intensity, repetitive sprint activity. *International Journal of Sports Medicine*, **23**, 353-360.

Romer, L.M., McConnell, A.K. and Jones, D.A. (2002c). Effects of inspiratory muscle training on time-trial performance in trained cyclists. *Journal of Sports Sciences*, **20**, 547-562.

Romer, L.M. and McConnell, A.K. (2003). Specificity and reversibility of inspiratory muscle training. *Medicine and Science in Sports and Exercise*, **35**, 237-244.

Romer, L.M., Lovering, A.T., Haverkamp, H.C., Pegelow, D.F. and Dempsey, J.A. (2006a). Effect of inspiratory muscle work on peripheral fatigue of locomotor muscles in healthy humans. *Journal of Physiology*, **571**, 425-439.

Romer, L.M., Haverkamp, H.C., Lovering, A.T., Pegelow, D.F. and Dempsey, J.A. (2006b). Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, **290**, R365-R375.

Romer, L.M., Miller, J.D., Haverkamp, H.C., Pegelow, D.F. and Dempsey, J.A. (2007a). Inspiratory muscles do not limit maximal incremental exercise performance in healthy subjects. *Respiratory Physiology and Neurobiology*, **156**, 353-361.

Romer, L.M., Haverkamp, H.C., Amann, M., Lovering, A.T., Pegelow, D.F. and Dempsey, J.A. (2007b). Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology,* **292**, R598-R606.

Ross, E., Middleton, N., Shave, R., George, K. and McConnell, A. (2008). Changes in respiratory muscle and lung function following marathon running in man. *Journal of Sports Sciences.* **26**, 1295-1301.

Rotto, D.M. and Kaufman, M.P. (1988). Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *Journal of Applied Physiology,* **64,** 2306-2313.

Sahlin, K., Harris, R.C., Nylind, B. and Hultman, E. (1976). Lactate content and pH in muscle obtained after dynamic exercise. *Pflugers Archive: European Journal of Physiology*, **28**, 143-149.

Sale, D.G., Macdougall, J.I. and Garner, S. (1990). Interaction between strength and endurance training. *Journal of Applied Physiology*, **68,** 260-270.

Sale, D.G. (2002). Postactivation potentiation: role in human performance. *Exercise and Sports Science Reviews*, **30**, 138-143.

Sarwar, R., Niclos, B.B. and Rutherford, O.M. (1996). Changes in muscle strength, relaxation rate and fatigability during the human menstrual cycle. *Journal of Physiology*, **493**, 267-272.

Scherer, T.A., Spengler, C.M., Owassapian, D., Imhof, E. And Boutellier, U. (2000). Respiratory muscle training in chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine,* **162**, 1709-1714.

Sheel, A.W. (2002). Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. *Sports Medicine*, **32**, 567-581.

Sheel, A.W., Richards, J.C., Foster, G.E. and Guenette, J.A. (2004). Sex differences in respiratory exercise physiology. *Sports Medicine*, **34**, 567-579.

Sheel, A.W., Derchak, P.A., Morgan, B.J., Pegelow, D.F., Jacques, A.J. and Dempsey, J.A. (2001). Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *Journal of Physiology*, **537**, 277-289.

Sheel, A.W., Derchak, P.A., Pegelow, D.F. and Dempsey, J.A. (2002). Threshold effects of respiratory muscle work on limb vascular resistance. *American Journal of Physiology, Heart and Circulatory Physiology*, **282**, H1732-H1738.

Siggaard-Anderson, O. and Fogh-Anderson, N. (1995). Base excess or buffer base (strong ion difference) as a measure of non-respiratory acid-base disturbance. *Acta Anaesthesiol Scandinavica*, **107**, 267-271.

Sieck, G.C. and Fournier, M. (1989). Diaphragm motor unit recruitment during ventilatory and nonventilatory behaviors. *Journal of Applied Physiology*, **66,** 2539-2545.

Similowski, T., Straus, C., Attali, V., Duguet, A. and Derenne, J.P. (1998). Cervical magnetic stimulation as a method to discriminate between diaphragm and rib cage muscle fatigue. *Journal of Applied Physiology*, **84,** 1692-1700.

Sinoway, L.I., Rea, R.F., Mosher, T.J., Smith, M.B. and Mark, A.L. (1992). Hydrogen ion concentration is not the sole determinant of muscle metaboreceptor responses in humans. *Journal of Clinical Investigation*, **89**, 1875-1884.

Sinoway, L.I., Hill, J.M., Pickar, J.G. and Kaufman, M.P. (1993). Effects of contraction and lactic acid on the discharge of group III muscle afferents in cats. *Journal of Neurophysiology*, **69**, 1053-1059.

Sinoway, L.I., Shenberger, J., Leaman, G., Zelis, R., Gray, K., Baily, R. and Leuenberger, U. (1996). Forearm training attenuates sympathetic responses to prolonged rhythmic forearm exercise. *Journal of Applied Physiology*, **81**, 1778-1784.

Sirker, A.A., Rhodes, A., Grounds, R.M. and Bennett, E.D. (2002). Acid-base physiology: the 'traditional' and the 'modern' approaches. *Anaesthesia*, **57**, 348-356.

Siri, W.E. (1961). Body composition from fluid spaces and density. In: *Techniques for measuring body composition*. Brozek, J. and Henschel, A. (Eds.). National Academy of Sciences: Washington DC, pp. 223-44.

Sliwiński, P., Yan, S., Gauthier, A.P. and Macklem, P.T. (1996). Influence of global inspiratory muscle fatigue on breathing during exercise. *Journal of Applied Physiology,* **80**, 1270-1280.

Smeltzer, S.C. and Lavietes, M.H. (1999). Reliability of maximal respiratory pressures in multiple sclerosis. *Chest*, **115**, 1546-1552.

Sonetti, D.A., Wetter, T.J., Pegelow, D.F. and Dempsey, J.A. (2001). Effects of respiratory muscle training versus placebo on endurance exercise performance. *Respiration Physiology*, **127**, 185-199.

Spengler, C.M., Laube, S.M., Roos, M. and Boutellier, U. (1996). The effect of breathing pattern during respiratory training on cycling endurance. In: *The Physiology and Pathophysiology of Exercise Tolerance.* Steinacker, J.M. and Ward, S.A. (Eds.). Plenum Press: New York, pp. 315-319.

Spengler, C.M., Roos, M., Laube, S.M. and Boutellier, U. (1999). Decreased exercise blood lactate concentrations after respiratory endurance training in humans. *European Journal of Applied Physiology*, **79**, 299-305.

Spengler, C.M., Knöpfli-Lenzin, C., Birchler, K., Trapletti, A. and Boutellier, U. (2000). Breathing pattern and exercise endurance time after exhausting cycling or breathing. *European Journal of Applied Physiology*, **81**, 368-374.

Staempfli, H.R. and Constable, P.D. (2003). Experimental determination of net protein charge and A(tot) and K(a) of nonvolatile buffers in human plasma. *Journal of Applied Physiology*, **95**, 620-630.

St Croix, C., Morgan, B.J., Wetter, T.J. and Dempsey, J.A. (2000). Fatiguing inspiratory muscle work causes reflex sympathetic activation in humans. *Journal of Physiology*, **529**, 493-504.

Steier, J., Kaul, S., Seymour, J., Jolley, C., Rafferty, G., Man, W., Luo, Y.M., Roughton, M., Polkey, M.I. and Moxham, J. (2007). The value of multiple tests of respiratory muscle strength. *Thorax*, **62**, 975-980.

Steinacker, J.M., Both, M. and Whipp, B.J. (1993). Pulmonary mechanics and entrainment of respiration and stroke rate during rowing [abstract]. *International Journal of Sports Medicine,* **14 suppl.:** S15-S19

Stewart, P.A. (1983). Modern quantitative acid-base chemistry. *Canadian Journal of Physiology and Pharmacology*, **61**, 1444-1461.

Stone, R.J. and Stone, J.A. (2000). *Atlas of Skeletal Muscles*. 3rd ed. McGraw-Hill: London.

Stringer, W.W. and Wasserman, K. (2005). Statement on exercise: American College of Chest Physicians/American Thoracic Society--exercise for fun or profit? *Chest*, **127**, 1072-1073.

Stuessi, C., Spengler, C.M., Knopfli-Lenzin, C., Markov, G. and Boutellier, U. (2001). Respiratory muscle endurance training in humans increases cycling endurance without affecting blood gas concentrations. *European Journal of Applied Physiology*, **84**, 582-586.

Suzuki, S., Sato, M. and Okubo, T. (1995). Expiratory muscle training and sensation of respiratory effort during exercise in normal subjects. *Thorax*, **50**, 366-370.

Taylor, B.J., How, S.C. and Romer, L.M. (2006). Exercise-induced abdominal muscle fatigue in healthy humans. *Journal of Applied Physiology*, **100**, 1554-1562.

Taylor, B.J. and Romer, L.M. (2008). Effect of expiratory muscle fatigue on exercise tolerance and locomotor muscle fatigue in healthy humans. *Journal of Applied Physiology*, **104**, 1442-1451.

Taylor, B.J. and Romer, L.M. (2009). Effect of expiratory resistive loading on inspiratory and expiratory muscle fatigue. *Respiratory Physiology and Neurobiology*, **166**, 164-174.

Taylor, B.J. and Romer, L.M. (*In Press*). Effect of expiratory muscle fatigue on operational lungs during high-intensity in healthy humans. *Proceedings of the European Respiratory Society*, Vienna, Austria (September 2009).

Tegtbur, U., Busse, M.W. and Braumann, K.M. (1993). Estimation of an individual equilibrium between lactate production and catabolism during exercise. *Medicine and Science in Sports and Exercise*, **25**, 620-627.

Thomas, C., Sirvent, P., Perrey, S., Raynaud, E. and Mercier, J. (2004). Relationships between maximal muscle oxidative capacity and blood lactate removal after supramaximal exercise and fatigue indexes in humans. *Journal of Applied Physiology*, **97**, 2132-2138.

Tipton, K.D. and Wolfe, R.R. (2004). Protein and amino acids for athletes. *Journal of Sports Sciences*, **22**, 65-79.

Tong, T.K., Fu, F.H., Chung, P.K., Eston, R., Lu, K., Quach, B., Nie, J. and So, R. (2008). The effect of inspiratory muscle training on high-intensity, intermittent running performance to exhaustion. *Applied Physiology, Nutrition and Metabolism*, **33**, 671-681.

Tzelepis, G.E., Vega, D.L., Cohen, M.E., Fulambarker, A.M., Patel, K.K. and McCool, F.D. (1994). Pressure-flow specificity of inspiratory muscle training. *Journal of Applied Physiology*, **77**, 795-801.

Verges, S., Schulz, C., Perret, C. and Spengler, C.M. (2006). Impaired abdominal muscle contractility after high-intensity exhaustive exercise assessed by magnetic stimulation. *Muscle Nerve*, **34**, 423-430.

Verges, S., Sager, Y., Erni, C. and Spengler, C.M. (2007a). Expiratory muscle fatigue impairs exercise performance. *European Journal of Applied Physiology*, **101**, 225-232.

Verges, S., Lenherr, O., Haner, A.C., Schulz, C. and Spengler, C.M. (2007b) Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. *American Journal of Physiology, Regulatory, Integrative and Comparative Physiology*, **292**, R1246-R1253.

Verin, E., Ross, E., Demoule, A., Hopkinson, N., Nickol, A., Fauroux, B., Moxham, J., Similowski, T. and Polkey, M.I. (2004). Effects of exhaustive incremental treadmill exercise on diaphragm and quadriceps motor potentials evoked by transcranial magnetic stimulation. *Journal of Applied Physiology*, **96**, 253-259.

Vincken, W., Ghezzo, H. and Cosio, M.G. (1987). Maximal static respiratory pressures in adults: normal values and their relationship to determinants of respiratory function. *Bulletin Europeen de Physiopathologie Respiratoire*, **23**, 435-439.

Vogiatzis, I., Athanasopoulos, D., Boushel, R., Guenette, J.A., Koskolou, M., Vasilopoulou, M., Wagner, H., Roussos, C., Wagner, P.D. and Zakynthinos, S. (2008). Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. *Journal of Physiology*, **586**, 5575-5587.

Volianitis, S., McConnell, A.K., Koutedakis, Y., McNaughton, L., Backx, K. and Jones, D.A. (2001). Inspiratory muscle training improves rowing performance. *Medicine and Science in Sports and Exercise*, **33**, 803-809.

Wagner, P.D. (2005). Why doesn't exercise grow the lungs when other factors do? *Exercise and Sport Science Reviews*, **33,** 3-8.

Walls, J., Maskrey, M., Wood-Baker, R. and Stedman, W. (2002). Exercise-induced oxyhaemoglobin desaturation, ventilatory limitation and lung diffusing capacity in women during and after exercise. *European Journal of Applied Physiology,* **87**, 145-152.

Walsh, M.L. (2000). Whole body fatigue and critical power: a physiological interpretation. *Sports Medicine*, **29**, 153-166.

Ward, M.E., Ward, J.W. and Macklem, P.T. (1992). Analysis of human chest wall motion using a two-compartment rib cage model. *Journal of Applied Physiol*ogy, **72**, 1338-1347.

Watsford, M. and Murphy, A. (2008). The effects of respiratory muscle training on exercise in elderly women. *Journal of Aging and Physical Activity,* **16**, 245-260

Watt, M.J., Howlett, K.F., Febbraio, M.A., Spriet, L.L. and Hargreaves, M. (2001). Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. *Journal of Physiol*ogy, **534**, 269-278.

Weiner, P., Magadle, R., Beckerman, M., Weiner, M., Berar-Yanay, N. (2003). Comparison of specific expiratory, inspiratory, and combined muscle training programs in COPD. *Chest*, **124**, 1357-1364.

Wells, G.D., Plyley, M., Thomas, S., Goodman, L. and Duffin, J. (2005). Effects of concurrent inspiratory and expiratory muscle training on respiratory and exercise performance in competitive swimmers. *European Journal of Applied Physiology,* **94,** 527-540.

West, J.B. (2000). *Respiratory Physiology the essentials*. 6th ed. Lippincott, Williams and Wilkins: USA.

Westerblad, H., Bruton, J.D. and Lännergren, J. (1997). The effects of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *Journal of Physiology*, **500**, 193-204.

Wetter, T.J., Harms, C.A., Nelson, W.B., Pegelow, D.F. and Dempsey, J.A. (1999) Influence of respiratory muscle work on $\dot{V}O_2$ and leg blood flow during submaximal exercise. *Journal of Applied Physiol*ogy, **87**, 643-651.

Wetter, T.J. and Dempsey, J.A. (2000). Pulmonary system and endurance exercise. In: *Endurance in Sport*. Shephard, R.J. and Åstrand P.-O. (Eds.). London: Blackwell science: pp. 52-67.

Williams, J.S., Wongsathikun, J., Boon, S.M. and Acevedo, E.O. (2002). Inspiratory muscle training fails to improve endurance capacity in athletes. *Medicine and Science in Sports and Exercise*, **34**, 1194-1198.

Wilson, S.H., Cooke, N.T., Edwards, R.H.T. and Spiro, S.G. (1984). Predicted normal values for maximal respiratory pressures in Caucasian adults and children. *Thorax*, **39**, 535-538.

Wilson, T.A., Legrand, A., Gevenois, P.A. and De Troyer, A. (2001). Respiratory effects of the external and internal intercostal muscles in humans. *Journal of Physiology*, **530**, 319-330.

Winkler, G., Zifko, U., Nader, A., Frank, W., Zwick, H., Toifl, K. and Wanke, T. (2000). Dose-dependent effects of inspiratory muscle training in neuromuscular disorders. *Muscle Nerve*, **23**, 1257-1260.

Witt, J.D., Guenette, J.A., Rupert, J.L., McKenzie, D.C. and Sheel, A.W. (2007) Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. *Journal of Physiol*ogy, **584**, 1019-1028.

Wolfe, R.R. (2000). Protein supplements and exercise. *American Journal of Clinical Nutrition*, **72**, S 551- S 557.

Wolfe, R.R. (2001). Effects of amino acid intake on anabolic processes. *Canadian Journal of Applied Physiology*, **26**, S220-S227.

Wragg, S., Hamnegard, C., Road, J., Kyroussis, D., Moran, J., Green, M. and Moxham, J. (1994). Potentiation of diaphragmatic twitch after voluntary contraction in normal subjects. *Thorax*., **49**, 1234-1237.

Xu, F. and Rhodes, E.C. (1999). Oxygen uptake kinetics during exercise. *Sports Medicine*, **27**, 313-327.

Yan, S., Gauthier, A.P., Similowski, T., Faltus, R., Macklem, P.T. and Bellemare, F. (1993). Force-frequency relationships of in vivo human and in vitro rat diaphragm using paired stimuli. *European Respiratory Journal,* **6**, 211-218.

APPENDICES

APPENDIX 1

Inspiratory muscle training diary example.

APPENDIX 2

Brown, P.I., Sharpe, G.R. and Johnson, M.A. (2008). Inspiratory muscle training reduces blood lactate concentration during volitional hyperpnoea. *European Journal of Applied Physiology*, **104**, 111-117.

APPENDIX 3

BASES conference poster, Brunel University, UK (September 2009):

Brown, P.I., Johnson, M.A. and Sharpe, G.R. (2009). Determinants of inspiratory muscle strength. *Journal of Sports Sciences,* S123-S124.

APPENDIX 4

Johnson, M.A., Sharpe, G.R. and **Brown, P.I.** (2009). Investigations of the lactate minimum test. *International Journal of Sports Medicine*, **3**, 448-454.

APPENDIX 5

Johnson, M.A., Sharpe, G.R. and **Brown, P.I.** (2007). Inspiratory muscle training improves cycling time-trial performance and anaerobic work capacity but not critical power. *European Journal of Applied Physiology*, **101**, 761-770.

APPENDIX 1

Appendix 1. Inspiratory muscle training diary

NAME :	WEEK NUMBER :	WEEK STARTING :

MONDAY		**TUESDAY**		**WEDNESDAY**		**THURSDAY**	
SESSION 1	SESSION 2	SESSION 1	SESSION 2	SESSION 1	SESSION 2	SESSION 1	SESSION 2
OWN TRAINING		OWN TRAINING		OWN TRAINING		OWN TRAINING	
FRIDAY		**SATURDAY**		**SUNDAY**		NOTES:	
SESSION 1	SESSION 2	SESSION 1	SESSION 2	SESSION 1	SESSION 2	Tick box when complete	
OWN TRAINING		OWN TRAINING		OWN TRAINING		(2 X 30 BREATHS) (25 – 30 SHOULD BE HARD) OTHER COMMENTS :	

Problems / Questions: tel: 07976 822248, peter.brown@ntu.ac.uk

APPENDIX 2

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ORIGINAL ARTICLE

Inspiratory muscle training reduces blood lactate concentration during volitional hyperpnoea

Peter I. Brown · Graham R. Sharpe · Michael A. Johnson

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Abstract Although reduced blood lactate concentrations ($[lac^-]_B$) have been observed during whole-body exercise following inspiratory muscle training (IMT), it remains unknown whether the inspiratory muscles are the source of at least part of this reduction. To investigate this, we tested the hypothesis that IMT would attenuate the increase in $[lac^-]_B$ caused by mimicking, at rest, the breathing pattern observed during high-intensity exercise. Twenty-two physically active males were matched for 85% maximal exercise minute ventilation $(\dot{V}_E \text{ max})$ and divided equally into an IMT or a control group. Prior to and following a 6 week intervention, participants performed 10 min of volitional hyperpnoea at the breathing pattern commensurate with 85% $\dot{V}_E$ max . The IMT group performed 6 weeks of pressure-threshold IMT; the control group performed no IMT. Maximal inspiratory mouth pressure increased (mean ± SD) 31 ± 22% following IMT and was unchanged in the control group. Prior to the intervention in the control group, $[lac^-]_B$ increased from 0.76 ± 0.24 mmol L^{-1} at rest to 1.50 ± 0.60 mmol L^{-1} ($P < 0.05$) following 10 min volitional hyperpnoea. In the IMT group, $[lac^-]_B$ increased from 0.85 ± 0.40 mmol L^{-1} at rest to 2.02 ± 0.85 mmol L^{-1} following 10 min volitional hyperpnoea ($P < 0.05$). After 6 weeks, increases in $[lac^-]_B$ during volitional hyperpnoea were unchanged in the control group. Conversely, following IMT the increase in $[lac^-]_B$ during volitional hyperpnoea was reduced by 17 ± 37% and 25 ± 34% following 8 and 10 min, respectively ($P < 0.05$). In conclusion, increases in $[lac^-]_B$ during volitional hyperpnoea at 85% $\dot{V}_E$ max were attenuated following IMT. These findings suggest that the inspiratory muscles were the source of at least part of this reduction, and provide a possible explanation for some of the IMT-mediated reductions in $[lac^-]_B$, often observed during whole-body exercise.

Keywords Respiratory muscle training · Diaphragm · Intercostal muscles · Blood lactate concentration · Hyperventilation

Introduction

Specific respiratory muscle training (RMT) can be performed using voluntary isocapnic hyperpnoea (VIH), flow-resistive loading, or pressure-threshold loading; with the exception of VIH, these are commonly referred to as inspiratory muscle training (IMT). Ventilatory endurance is enhanced with all three techniques, whereas IMT also increases diaphragm thickness (Downey et al. 2007; Enright et al. 2006) and the maximal strength, shortening velocity and power of the inspiratory muscles (for a full review see McConnell and Romer 2004). Furthermore, well controlled studies have shown improvements in endurance exercise performance following both IMT (Gething et al. 2004; Griffiths and McConnell 2007; Johnson et al. 2007; Romer et al. 2002a; Volianitis et al. 2001) and VIH (Leddy et al. 2007).

The mechanisms underlying such performance improvements remain speculative but may include reduced perception of effort (Downey et al. 2007; Gething et al. 2004; Griffiths and McConnell 2007; Romer et al. 2002a; Verges et al. 2007; Volianitis et al. 2001) and possibly

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reductions in both diaphragm fatigue (Verges et al. 2007) and an associated metaboreflex that attenuates limb blood flow (McConnell and Lomax 2006; Witt et al. 2007). The notion that genuine physiological adaptation explains, in part, RMT-mediated improvements in endurance exercise performance is further supported by the frequently observed reduction in blood lactate concentration ($[lac^-]_B$) during whole-body exercise following both IMT (Griffiths and McConnell 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Volianitis et al. 2001) and VIH (Leddy et al. 2007; Spengler et al. 1999). Furthermore, correlations have been reported between reductions in $[lac^-]_B$ and performance improvements following RMT (Romer et al. 2002b; Spengler et al. 1999), with up to 52% of the variation in performance being attributed to the reduced $[lac^-]_B$ (Romer et al. 2002b).

The mechanism(s) by which RMT reduces $[lac^-]_B$ remains equivocal. An RMT-mediated change in minute ventilation $(\dot{V}_E)$, which may conceivably alter both the work of breathing and acid base balance, is an unlikely mechanism since reductions in $[lac^-]_B$ following RMT have been observed irrespective of whether $\dot{V}_E$ is lower (Leddy et al. 2007), unchanged (McConnell and Sharpe 2005; Spengler et al. 1999; Volianitis et al. 2001), or increased (Kohl et al. 1997). The concept that RMT-mediated respiratory muscle adaptations explain, in part, that the reductions observed in $[lac^-]_B$ remains contentious: the small size of these muscles and observations, that loading and unloading of the respiratory muscles during exercise fails to influence systemic $[lac^-]_B$, argue against this premise (Wetter and Dempsey 2000). However, volitional hyperpnoea increases $[lac^-]_B$ both at rest (Martin et al. 1984; Verges et al. 2007) and during exercise (Johnson et al. 2006) suggesting that the respiratory muscles are capable of net lactate release. Furthermore, VIH appears to attenuate such net release during volitional hyperpnoea (Verges et al. 2007). However, this study did not rigorously control isocapnia that is essential for the interpretation of changes in $[lac^-]_B$. Also, the use of a breathing challenge based on maximum voluntary ventilation (MVV) limits external validity as both the breathing pattern and work of breathing are unreflective of that seen during exercise (Coast et al. 1993). Since many of the muscle adaptations associated with endurance-orientated training (i.e. VIH) are different from those associated with strength-orientated training (i.e. IMT), it also remains uncertain whether IMT would reduce $[lac^-]_B$ during volitional hyperpnoea.

Therefore, to investigate this issue further the present study examined the hypothesis that 6 weeks of IMT would attenuate the increase in $[lac^-]_B$ caused by mimicking, at rest, the breathing pattern observed during high-intensity endurance exercise.

Methods

Subjects

Following approval from Nottingham Trent University's ethics committee, 22 non-smoking, recreationally active males provided written informed consent to participate in the study. Throughout the study, subjects were instructed to adhere to their usual training regimen and not to engage in strenuous exercise the day before test days, during which subjects refrained from ingesting caffeine and arrived at the laboratory 2 h post-prandial. Descriptive characteristics of the subjects are presented in Table 1.

Experimental procedure

Baseline pulmonary function and maximal inspiratory mouth pressure (MIP) were measured during the first laboratory visit. On subsequent visits separated by at least 48 h, subjects performed a maximal incremental cycling test, and two 10 min isocapnic volitional hyperpnoea tests (the first being a familiarisation test). The volitional hyperpnoea tests were performed at the $\dot{V}_E$, tidal volume (V_T), breathing frequency (f_R) and duty cycle (T_I/T_{TOT})

Table 1 Descriptive characteristics of the subjects (mean ± SD)

	Control ($n = 11$)	IMT ($n = 11$)
Age (years)	28.5 ± 4.1	22.4 ± 4.5*
Body mass (kg)	75.5 ± 5.6	78.6 ± 9.7
Height (cm)	176.9 ± 7.4	181.6 ± 7.6
FVC (L)	5.32 ± 0.55 (104 ± 8)	5.67 ± 0.92 (106 ± 12)
FEV_1 (L)	4.28 ± 0.62 (99 ± 11)	4.93 ± 0.67 (109 ± 11)
FEV_1/FVC (%)	80.3 ± 7.1 (96 ± 9)	87.7 ± 8.3 (103 ± 9)*
MVV_{10} (L min^{-1})	176.3 ± 15.0 (102.3 ± 10.9)	173.4 ± 53.7 (122.4 ± 30.3))
MIP (cmH_2O)	163 ± 19 (113 ± 4)	147 ± 27 (119 ± 5)
$\dot{V}O_2$ max (L min^{-1})	3.75 ± 0.55	3.77 ± 0.75
$\dot{W}$max (W)	353 ± 44	362 ± 38

FVC forced vital capacity; FEV_1 forced expiratory volume in 1 s; MVV_{10} maximum voluntary ventilation in 10 s. Values in parenthesis represent the percent of predicted values (Quanjer et al. 1993; Wilson et al. 1984)

* Between group differences; $P < 0.05$

associated with 85% maximal exercise $\dot{V}_E(\dot{V}_E\,\text{max})$. During volitional hyperpnoea tests, blood samples were taken every 2 min from 0 to 10 min, inclusive, and respiratory variables were measured breath by breath and averaged over 2 min intervals. Subjects were subsequently matched for 85% $\dot{V}_E$ max and divided into an IMT group ($n = 11$) or a control (no IMT) group ($n = 11$). Not more than a week, following a 6 week intervention MIP was measured and at least 48 h following this, subjects repeated the volitional hyperpnoea test. Each subject completed a 24 h diet record prior to the criterion pre-intervention volitional hyperpnoea test and this was then replicated during the 24 h prior to the post-intervention volitional hyperpnoea test.

Pulmonary function, maximal inspiratory pressure, and respiratory measurements

Pulmonary function was assessed using a pneumotachograph (ZAN 600USB, Nspire Health, Oberthulba, Germany), calibrated using a 3-L syringe. Each measurement was repeated three times and the highest recorded value was used for subsequent analysis (Quanjer et al. 1993). A hand-held mouth pressure metre (Ferraris Respiratory Europe, Hertford, UK) measured MIP as an index of global inspiratory muscle strength. The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure during inspiratory efforts. Manoeuvres were performed in an upright standing posture, were initiated from residual volume, and sustained for at least 1 s. Repeat measurements separated by 30 s were taken until three values within 5 cmH_2O of each other were produced (McConnell 2007). The highest recorded value was used for subsequent analysis. Throughout the maximal exercise test and volitional hyperpnoea, subjects wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) connected to a pneumotachograph and respiratory variables were measured breath by breath (ZAN 600USB, Nspire Health, Oberthulba, Germany). During volitional hyperpnoea tests, a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) and a 1.5 m length of corrugated tubing was attached distally to the pneumotachograph allowing additional CO_2 to be added to the inspirate.

Blood sampling and analysis

Arterialised venous blood was sampled from a dorsal hand vein via an indwelling cannula (Forster et al. 1972; McLoughlin et al. 1992). Arterialisation was ensured by immersing the hand in water at ~40°C for 10 min prior to cannulation and by warming the hand during volitional hyperpnoea tests using an infrared lamp. Blood samples were drawn into a 2 ml pre-heparinised syringe (PICO 50, Radiometer, Copenhagen, Denmark) and analysed immediately for blood gases (ABL520, Radiometer, Copenhagen, Denmark), including the partial pressure of carbon dioxide (PCO_2) and pH, and $[lac^-]_B$ (Biosen C_line Sport, EKF Diagnostics, Barleben, Germany). Plasma bicarbonate concentration ($[HCO_3^-]$) was calculated from PCO_2 and pH values using the Henderson Hasselbalch equation:

$$\text{pH} = \text{p}K + \log\frac{[\text{HCO}_3^-]}{0.03 \times PCO_2}$$

$[HCO_3^-]$ was then subsequently incorporated into the Siggaard–Anderson equation to calculate base excess of the extracellular fluid (BE_{ECF}) (Siggaard-Anderson and Fogh-Anderson 1995):

$$BE_{ECF} = 0.93 \times \left([\text{HCO}_3^-] - 24.4 + 14.83 \times (\text{pH} - 7.40)\right)$$

Maximal exercise test

Subjects performed a maximal incremental cycling test on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Cycling began at 0 W and power was subsequently increased by 10 W every 15 s in order to result in exercise intolerance within ~10 min. This rapid incremental protocol was selected to maximise $\dot{V}_E$ at the cessation of the test and therefore reflect intense endurance exercise. The power at which exercise intolerance ensued defined maximal power output $(\dot{W}\text{max})$, and the highest oxygen uptake, $(\dot{V}O_2)$ and $\dot{V}_E$, recorded in any 30 s period defined $\dot{V}O_2$max and $\dot{V}_E$ max, respectively.

Volitional hyperpnoea

Volitional hyperpnoea was performed whilst seated on the cycle ergometer in an body position identical to that adopted during the maximal exercise test. Subjects were instructed to increase $\dot{V}_E$ and f_R in a square wave manner to a level commensurate with 85% $\dot{V}_E$ max, which during pilot work was shown to represent the maximum square wave response that could be maintained for 10 min. An audio metronome paced f_R and real-time visual feedback of $\dot{V}_E$ was provided throughout the test. The prescribed breathing pattern ($\dot{V}_E$, V_T, f_R and T_I/T_{TOT}) during volitional hyperpnoea was identical pre- and post-intervention and was chosen to provide a breathing challenge reflective of the work of breathing associated with exercise hyperpnoea. This methodology is deemed superior to an arbitrary %MVV as it more closely reflects the work of breathing during whole-body exercise: for a given $\dot{V}_E$ greater than approximately 60 L min^{-1} the work of breathing of exercise hyperpnoea can be overestimated by as much as 25%

when a spontaneous breathing pattern is adopted during volitional hyperpnoea (Coast et al. 1993). Isocapnia was maintained during volitional hyperpnoea by adding CO_2 into the inspiratory circuit in order to maintain resting PCO_2.

Intervention

IMT was performed using an inspiratory pressure-threshold device (POWERbreathe®, Gaiam, UK). The IMT group performed 30 dynamic inspiratory efforts twice daily for 6 weeks against a pressure-threshold load of ~50% MIP. Thereafter, subjects periodically increased the load to a level that would permit them to only just complete 30 manoeuvres. Each inspiratory manoeuvre was initiated from residual volume and subjects strove to maximise V_T. This protocol is known to be effective in eliciting an adaptive response (Johnson et al. 2007; McConnell and Lomax 2006; McConnell and Sharpe 2005; Romer et al. 2002a, b; Volianitis et al. 2001). Subjects completed a training diary to record IMT adherence and habitual training, which the control group also recorded. The control group did not perform sham IMT since the duration of the volitional hyperpnoea test and breathing pattern employed was identical pre- and post-intervention, thus responses would not be influenced by either motivation or expectation.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS, Chicago, Illinois, USA). Within group changes over time during volitional hyperpnoea were determined using one-way ANOVA for repeated measures and Tukey's HSD post hoc analysis. Within and between group interaction effects were determined using two-way ANOVA for repeated measures. Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at $P \leq 0.05$. Results are presented as mean ± SD.

Results

Pulmonary function and maximal inspiratory pressure

Baseline pulmonary function and MIP were all within normal limits (Table 1). The IMT group demonstrated excellent training compliance (91% adherence) and subjects' habitual training remained unchanged in both IMT and control groups. MIP increased from 147 ± 27 to 189 ± 27 cmH_2O (+31 ± 22%) following IMT ($P < 0.01$). No change was observed in the control group (pre- vs. post-: 163 ± 19 vs. 166 ± 20 cmH_2O).

Responses to volitional hyperpnoea

Group mean values for ventilatory and acid base responses to 10 min volitional hyperpnoea, pre- and post-intervention are shown in Table 2. Before and after the intervention, $\dot{V}_E$, V_T, f_R, T_I/T_{TOT} and measures of acid base balance were not different between groups and remained unchanged over time during volitional hyperpnoea. The mean $\dot{V}_E$ during volitional hyperpnoea represented 72 ± 8 and 81 ± 19% of MVV_{10} in control and IMT groups, respectively. PCO_2 was maintained at resting levels throughout volitional hyperpnoea, prior to and following the intervention and was not different between groups (Fig. 1).

Prior to the intervention, significant increases in $[lac^-]_B$ above rest were observed following 10 min of volitional hyperpnoea in IMT and control groups ($P < 0.05$) (Fig. 2) and such changes were not different between the groups. Following the intervention, the $[lac^-]_B$ response to

Table 2 Mean (±SD) ventilatory and acid-base responses to 10 min volitional hyperpnoea, pre- and post-intervention

	Control ($n = 11$)		IMT ($n = 11$)	
	Pre	Post	Pre	Post
$\dot{V}_E$ (L min^{-1})	127.1 ± 2.3	128.7 ± 2.4	132.9 ± 9.6	136.8 ± 3.2
V_T (L)	2.62 ± 0.04	2.64 ± 0.07	2.60 ± 0.03	2.66 ± 0.06
f_R (breaths min^{-1})	50 ± 0	50 ± 0	52 ± 0	52 ± 0
T_I/T_{TOT}	0.44 ± 0.00	0.44 ± 0.00	0.52 ± 0.00	0.49 ± 0.00
pH	7.392 ± 0.031	7.406 ± 0.024	7.397 ± 0.023	7.395 ± 0.014
$[H^+]$ (nmol L^{-1})	40.6 ± 2.9	39.4 ± 2.2	40.2 ± 2.2	40.3 ± 1.0
$[HCO_3^-]$ (mmol L^{-1})	26.0 ± 0.9	26.9 ± 2.5	26.5 ± 1.4	27.0 ± 1.3
BE_{ECF} (mEq L^{-1})	1.38 ± 0.91	1.72 ± 2.04	1.52 ± 1.11	2.35 ± 1.23

$\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, respiratory frequency; T_I/T_{TOT}, duty cycle; $[H^+]$, hydrogen ion concentration; $[HCO_3^-]$, plasma bicarbonate concentration; BE_{ECF}, base excess of the extracellular fluid

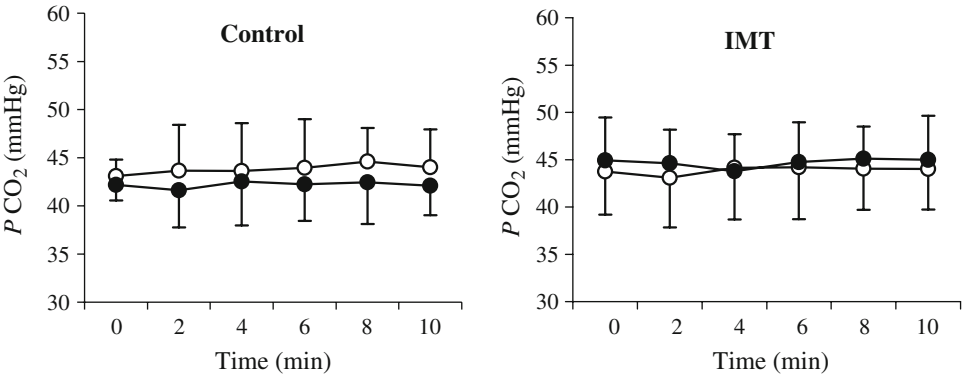

Fig. 1 Partial pressure of carbon dioxide in arterialised venous blood (PCO_2) during volitional hyperpnoea, pre- (*open circle*) and post-intervention (*filled circle*) in control and IMT groups

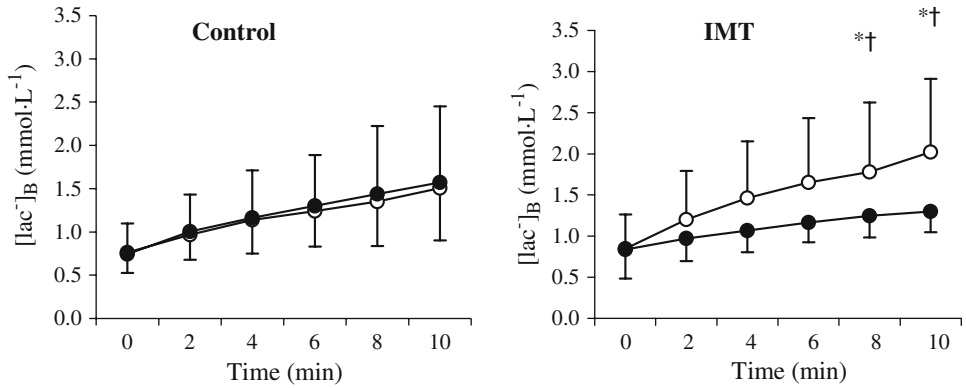

Fig. 2 Blood lactate concentration ($[lac^-]_B$) during volitional hyperpnoea, pre- (*open circle*) and post-intervention (*filled circle*) in control and IMT groups. *Significant difference from pre-IMT ($P < 0.05$). †Significant interaction effect ($P < 0.05$)

volitional hyperpnoea was unchanged in the control group. Conversely, $[lac^-]_B$ during volitional hyperpnoea was reduced following IMT with 17 ± 37 and 25 ± 34% reductions being observed at 8 and 10 min, respectively ($P < 0.05$). These reductions exceeded changes observed in the control group ($P < 0.05$).

Correlations amongst variables

Prior to the intervention, increases in $[lac^-]_B$ during volitional hyperpnoea did not correlate with any measure of pulmonary function, MIP, endurance training status $\left(\dot{V}O_2\,\text{max}, \dot{W}\,\text{max}\right)$, or ventilatory responses to volitional hyperpnoea. Increases in $[lac^-]_B$ during volitional hyperpnoea did not correlate with absolute $\dot{V}_E$ nor when expressed as %MVV. The attenuated increase in $[lac^-]_B$ during volitional hyperpnoea after IMT was not correlated with increases in MIP. However, baseline MIP was negatively correlated with relative IMT-induced increases in MIP ($r = -0.70$, $P < 0.05$).

Discussion

The main finding of this study was that, 10 min of volitional hyperpnoea approximately doubled resting $[lac^-]_B$, and that 6 weeks of pressure-threshold IMT attenuated this increase by 25%. These findings strongly support the notion that the respiratory muscles are capable of increasing $[lac^-]_B$ and are the first to show that this can be attenuated through specific IMT. This observation may help to explain some of the IMT-mediated reductions in $[lac^-]_B$, previously observed during whole-body exercise.

We report an increased $[lac^-]_B$ of 0.96 ± 0.58 mmol L^{-1} ($n = 22$; range: 0.20–2.50 mmol L^{-1}) from rest during 10 min of intense volitional hyperpnoea at 85% $\dot{V}_E$ max (130.7 ± 19.7 L min^{-1}, 77 ± 15% MVV_{10}; $n = 22$). Comparable increases in $[lac^-]_B$ have been reported whilst breathing at similar (72% MVV, Martin et al. 1984; 70% MVV, Verges et al. 2007), but not at lower (62% MVV, Spengler et al. 2000), relative intensities. Therefore it is apparent that when $\dot{V}_E$ surpasses a certain level, the respiratory muscles are capable of net lactate release. However, the potential for respiratory alkalosis to elevate $[lac^-]_B$ is well documented (Davies et al. 1986; LeBlanc et al. 2002). Consequently, we were careful to maintain, with considerable accuracy, resting PCO_2 throughout the 10 min of volitional hyperpnoea (see Fig. 1). Other measures of acid base status also remained unchanged from rest during volitional hyperpnoea in both the groups, pre- and post-intervention. We are thus confident that the increase in $[lac^-]_B$ during volitional hyperpnoea was a consequence of increased lactate efflux from the respiratory muscles rather than respiratory alkalosis.

The attenuated increase in $[lac^-]_B$ during volitional hyperpnoea following IMT is similar to that observed in healthy subjects performing an exhaustive respiratory endurance test at ~70% MVV following VIH training, although this reduction did not exceed that of a control group (Verges et al. 2007). Given the aforementioned importance of maintaining isocapnia, it is also unfortunate that end-tidal CO_2 and/or PCO_2 was not controlled during the respiratory endurance test. Furthermore, subjects were prescribed a pre-determined arbitrary breathing pattern which has previously received criticism for failing to accurately represent the work of breathing during exercise hyperpnoea (Coast et al. 1993). Notwithstanding this, VIH- and IMT-mediated reductions in $[lac^-]_B$ observed during volitional hyperpnoea are similar to the reductions often observed during submaximal, whole-body exercise (Griffiths and McConnell 2007; Leddy et al. 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999; Volianitis et al. 2001); however, whether these observations during volitional hyperpnoea and exercise share a common mechanistic explanation is unclear.

RMT-mediated reductions in $[lac^-]_B$, occur (e.g. see Leddy et al. 2007; McConnell and Sharpe 2005; Spengler et al. 1999; Volianitis et al. 2001) when net lactate production from the respiratory muscles is probably negligible given the relatively low $\dot{V}_E$ and minimal activation of less efficient accessory muscles (Martin et al. 1984; Johnson et al. 2006). Hence, under such conditions it seems more likely that reductions in $[lac^-]_B$ result from increased uptake and metabolism of lactate by the trained respiratory muscles (Griffiths and McConnell 2007; Spengler et al. 1999) rather than a decrease in net lactate release. Conversely, during high-intensity exercise where $\dot{V}_E$ relative to MVV, approaches/exceeds levels achieved in the breathing challenge of this study (e.g. see Edwards and Cooke 2004; Kohl et al. 1997; Spengler et al. 1999), it is possible that RMT-mediated respiratory muscle adaptation contributes to lowering $[lac^-]_B$ through affecting both lactate clearance by and efflux from the trained respiratory muscles.

The plasticity of the inspiratory muscles has been well documented (McConnell and Romer 2004; Powers et al. 1997). It is thus attractive to suggest that changes in inspiratory muscle morphology may explain, in part, the attenuated hyperpnoea-mediated increase in $[lac^-]_B$ following IMT. An approximate 10% increase in diaphragm thickness (Downey et al. 2007; Enright et al. 2006), and a 21% increase in the size of type II muscle fibres in the external intercostal muscles (Ramírez-Sarmiento et al. 2002), has been reported following 6 and 5 weeks of IMT, respectively. Increasing inspiratory muscle fibre cross-sectional area and subsequent strength decreases the relative intensity for a given absolute work load, which may reduce/delay fast twitch fibre recruitment and thus lactate production (Marcinik et al. 1991). A decrease in the relative workload per muscle fibre may also decrease blood flow occlusion, which may influence lactate production and/or clearance (Marcinik et al. 1991).

Increased muscle monocarboxylate transport (MCT) protein content, which facilitates inter- and intra-cellular lactate shuttling in sarcolemmal and mitochondrial membranes, respectively (Brooks et al. 1999; Dubouchaud et al. 2000), has been reported following endurance (Baker et al. 1998; Burgomaster et al. 2007) and strength (Juel et al. 2004) based training regimens. It is thus possible (cf. McConnell and Sharpe 2005) that similar adaptations would occur in the respiratory muscles following both IMT (strength-orientated) and VIH (endurance-orientated) training and may explain, in part, the decrease in $[lac^-]_B$ observed during whole-body exercise and volitional hyperpnoea following these dissimilar training stimuli.

Finally, the attenuated $[lac^-]_B$ response to volitional hyperpnoea following IMT (and VIH training) may also reside in a training-induced increase in the oxidative capacity of the inspiratory muscles. In support of this notion, Ramírez-Sarmiento et al. (2002) reported a 38% increase in the number of type I muscle fibres in the external intercostals following 5 weeks IMT. Moderate intensity, high repetition strength training, similar to the IMT protocol used in the present study, can increase oxidative enzyme activity (Costill et al. 1979; Sale et al. 1990) thereby reducing net lactate production (Holloszy and Coyle 1984). Since similar oxidative adaptations would be expected to occur following VIH (endurance-orientated) training (Holloszy and Coyle 1984), this also offers an attractive explanation for the decrease in $[lac^-]_B$ observed during whole body exercise (Griffiths and McConnell 2007; Kohl et al. 1997; Leddy et al. 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Spengler et al. 1999; Volianitis et al. 2001) and volitional hyperpnoea (present study; Verges et al. 2007).

Conclusions

In summary, the present study provides novel evidence that increases in $[lac^-]_B$ when mimicking the breathing pattern observed during heavy exercise can be attenuated following IMT. These data suggest that the inspiratory muscles were the source of at least part of this reduction, and provide a possible explanation for at least some of the IMT-mediated reductions in $[lac^-]_B$, previously observed during whole-body exercise. The precise mechanisms that underpin these changes remain unknown, but an IMT-mediated increase in the oxidative and/or lactate transport capacity of the inspiratory muscles is an attractive possibility that merits further investigation.

References

Baker SK, McCullagh KJ, Bonen A (1998) Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. J Appl Physiol 84:987–994

Brooks GA, Brown M, Butz CE et al (1999) Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. J Appl Physiol 87:1713–1718

Burgomaster KA, Cermak NM, Phillips SM et al (2007) Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. Am J Physiol Regul Integr Comp Physiol 292:R1970–R1976

Coast JR, Rasmussen SA, Krause KM et al (1993) Ventilatory work and oxygen consumption during exercise and hyperventilation. J Appl Physiol 74:793–798

Costill DL, Coyle EF, Fink WF et al (1979) Adaptations in skeletal muscle following strength training. J Appl Physiol 46:96–99

Davies SF, Iber C, Keene SA et al (1986) Effect of respiratory alkalosis during exercise on blood lactate. J Appl Physiol 61:948–952

Downey AE, Chenoweth LM, Townsend DK et al (2007) Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. Respir Physiol Neurobiol 156:137–146

Dubouchaud H, Butterfield GE, Wolfel EE et al (2000) Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. Am J Physiol Endocrinol Metab 278:E571–E579

Edwards AM, Cooke CB (2004) Oxygen uptake kinetics and maximal aerobic power are unaffected by inspiratory muscle training in healthy subjects where time to exhaustion is extended. Eur J Appl Physiol 93:139–144

Enright SJ, Unnithan VB, Heward C et al (2006) Effect of high-intensity inspiratory muscle training on lung volumes, diaphragm thickness, and exercise capacity in subjects who are healthy. Phys Ther 86:345–454

Forster HV, Dempsey JA, Thomson J et al (1972) Estimation of arterial PO_2, PCO_2, pH, and lactate from arterialized venous blood. J Appl Physiol 32:134–137

Gething AD, Williams M, Davies B (2004) Inspiratory resistive loading improves cycling capacity: a placebo controlled trial. Br J Sports Med 38:730–736

Griffiths LA, McConnell AK (2007) The influence of inspiratory and expiratory muscle training upon rowing performance. Eur J Appl Physiol 99:457–466

Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscles to endurance exercise and their metabolic consequences. J Appl Physiol 56:831–838

Johnson MA, Sharpe GR, McConnell AK (2006) Maximal voluntary hyperpnoea increases blood lactate concentration during exercise. Eur J Appl Physiol 96:600–608

Johnson MA, Sharpe GR, Brown PI (2007) Inspiratory muscle training improves cycling time trial performance and anaerobic work capacity but not critical power. Eur J Appl Physiol 101:761–770

Juel C, Holten MK, Dela F (2004) Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. J Physiol 556:297–304

Kohl J, Koller EA, Brandenberger M et al (1997) Effect of exercise-induced hyperventilation on airway resistance and cycling endurance. Eur J Appl Physiol 75:305–311

LeBlanc PJ, Parolin ML, Jones NL et al (2002) Effects of respiratory alkalosis on human skeletal muscle metabolism at the onset of submaximal exercise. J Physiol 544:303–313

Leddy JJ, Limprasertkul A, Patel S et al (2007) Isocapnic hyperpnea training improves performance in competitive male runners. Eur J Appl Physiol 99:556–676

Marcinik EJ, Potts J, Schlabach G et al (1991) Effects of strength training on lactate threshold and endurance performance. Med Sci Sports Exerc 23:739–743

Martin BJ, Chen HI, Kolka MA (1984) Anaerobic metabolism of the respiratory muscles during exercise. Med Sci Sports Exerc 16:82–86

McConnell AK (2007) Lung and respiratory muscle function. In: Winter EM, Jones AM, Davison RCR et al. (eds) Sport and exercise physiology testing guidelines, the British Association of Sport and Exercise Sciences Guide. Routledge, Oxford, UK

McConnell AK, Lomax M (2006) The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. J Physiol 577:445–457

McConnell AK, Romer LM (2004) Respiratory muscle training in healthy humans: resolving the controversy. Int J Sports Med 25:284–293

McConnell AK, Sharpe GR (2005) The effect of inspiratory muscle training upon maximum lactate steady-state and blood lactate concentration. Eur J Appl Physiol 94:277–284

McLoughlin P, Popham P, Linton RA et al (1992) Use of arterialized venous blood sampling during incremental exercise tests. J Appl Physiol 73:937–940

Powers SK, Coombes J, Demirel H (1997) Exercise training-induced changes in respiratory muscles. Sports Med 24:120–131

Quanjer PH, Tammeling GJ, Cotes JE et al (1993) Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European respiratory society. Eur Respir J 16:5–40

Ramírez-Sarmiento A, Orozco-Levi M, Güell R et al (2002) Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. Am J Respir Crit Care Med 166:1491–1497

Romer LM, McConnell AK, Jones DA (2002a) Effects of inspiratory muscle training on time-trial performance in trained cyclists. J Sports Sci 20:547–562

Romer LM, McConnell AK, Jones DA (2002b) Effects of inspiratory muscle training upon recovery time during high-intensity, repetitive sprint activity. Int J Sports Med 23:353–360

Sale DG, Macdougall JI, Garner S (1990) Interaction between strength and endurance training. J Appl Physiol 68:260–270

Siggaard-Anderson O, Fogh-Anderson N (1995) Base excess or buffer base (strong ion difference) as a measure of non-respiratory acid-base disturbance. Acta Anaesthesiol Scand 107:267–271

Spengler CM, Roos M, Laube SM et al (1999) Decreased exercise blood lactate concentrations after respiratory endurance training in humans. Eur J Appl Physiol 79:299–305

Spengler CM, Knöpfli-Lenzin C, Birchler K et al (2000) Breathing pattern and exercise endurance time after exhausting cycling or breathing. Eur J Appl Physiol 81:368–374

Verges S, Lenherr O, Haner AC et al (2007) Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. Am J Physiol Regul Integr Comp Physiol 292:R1246–R1253

Volianitis S, McConnell AK, Koutedakis Y et al (2001) Inspiratory muscle training improves rowing performance. Med Sci Sports Exerc 33:803–809

Wetter TJ, Dempsey JA (2000) Pulmonary system and endurance exercise. In: Shephard RJ, Astrand P-O (eds) Endurance in Sport. Blackwell Science, London, UK

Wilson SH, Cooke NT, Edwards RHT et al (1984) Predicted normal values for maximal respiratory pressures in Caucasian adults and children. Thorax 39:535–538

Witt JD, Guenette JA, Rupert JL et al (2007) Inspiratory muscle training attenuates the human respiratory muscle metaboreflex. J Physiol 584:1019–1028

APPENDIX 3

Determinants of Inspiratory Muscle Strength

Peter I. Brown[1, 2], Michael A. Johnson[2] & Graham R. Sharpe[2]

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INTRODUCTION

- Maximal inspiratory mouth pressure (MIP) reflects global inspiratory muscle strength
- MIP varies widely amongst individuals
- It has been suggested that MIP is determined by the strength of the chest wall inspiratory muscles relative to the diaphragm
- MIP is also used to monitor changes in MIP during specific inspiratory muscle training (IMT)
- The increase in MIP following IMT is also highly variable
- It is unknown whether the baseline MIP or chest wall muscle activation affects the increases in MIP observed after IMT

AIM:

" TO INVESTIGATE THE DETERMINANTS OF MIP PRIOR TO AND FOLLOWING IMT "

METHODS

- **Experiment 1 (*n* = 38)**
- MIP measured pre and post 4wk control and intervention (IMT)

- **Experiment 2 (*n* = 20)**
- Control and IMT group
- MIP measured pre and post 4wk intervention (IMT or no IMT)
- Intrathoracic pressures measured during MIP using balloon catheters inserted into the oesophagus and stomach (Figure 1).
- Relative chest wall and diaphragm recruitment: ratio *P*oe/*P*di

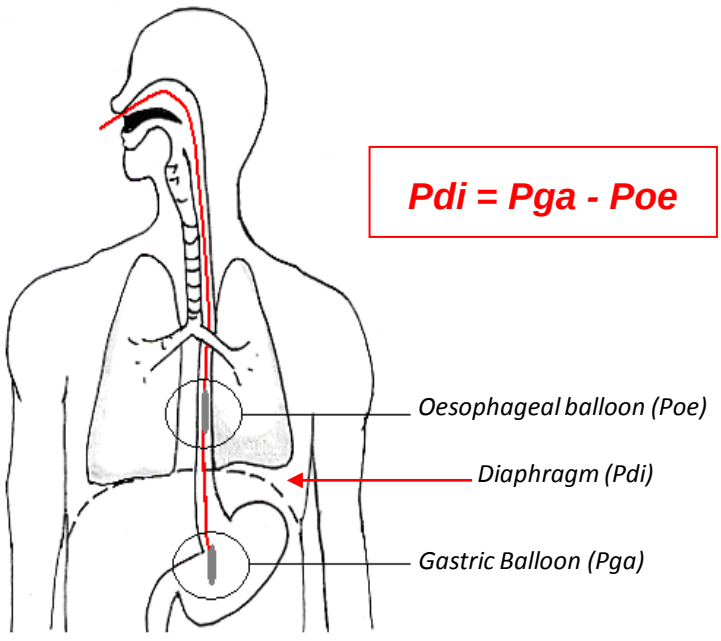

Figure 1. Placement of intrathoracic balloon catheters

RESULTS

- IMT-induced ΔMIP Exp. 1 = 13 ± 13%, Exp. 2 = 11 ± 15% (*P*<0.05)
- Figure 2 shows how baseline MIP effects IMT-induced changes in MIP

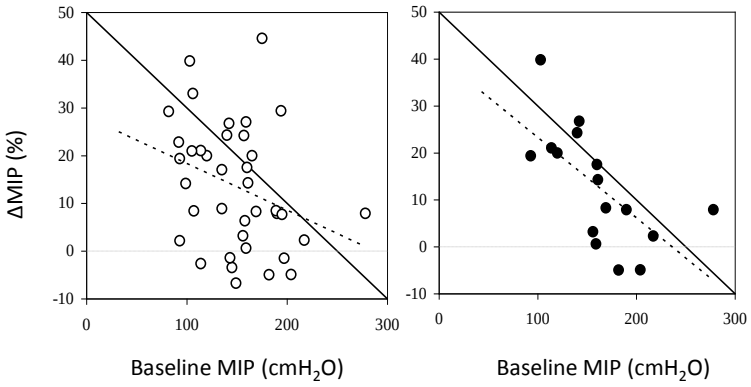

Figure 2. Left: *n*=38, effects of baseline MIP on IMT-induced ΔMIP (*P*=0.055). Right: *n*=16 where compliance >93% (*P*=0.007).

- Figure 3 shows *P*oe/*P*di prior to and following the intervention

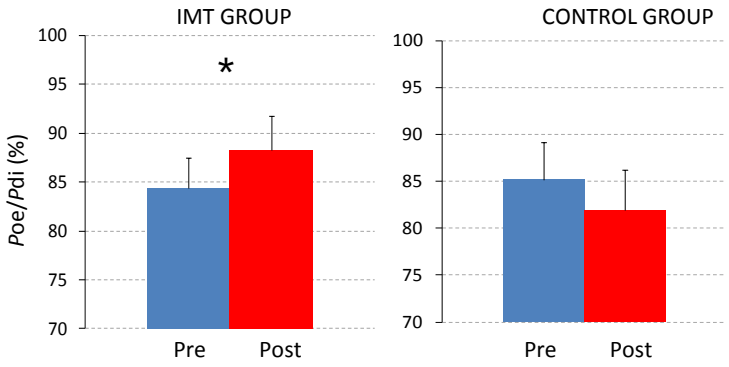

Figure 3. Relative chest wall muscle recruitment (*P*oe/*P*di) during MIP effort. *, significantly different to pre-IMT (*P*<0.05)

- Baseline MIP was correlated with baseline *P*oe/*P*di (*r*=0.582, *P*=0.014)
- Following IMT, the relative ΔMIP was correlated with Δ*P*oe/*P*di (*r*=0.719, *P*=0.044)

Summary and Conclusions

- Increases in MIP following IMT are dependent upon baseline MIP
- Largest increase in MIP occurred in those with lowest *P*oe/*P*di
- Increases in chest wall inspiratory muscles strength permits the diaphragm to contract more forcefully which may increase MIP

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APPENDIX 4

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ORIGINAL ARTICLE

Inspiratory muscle training improves cycling time-trial performance and anaerobic work capacity but not critical power

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Abstract We examined whether inspiratory muscle training (IMT) improved cycling time-trial performance and changed the relationship between limit work (W_{lim}) and limit time (T_{lim}), which is described by the parameters critical power (CP) and anaerobic work capacity (AWC). Eighteen male cyclists were assigned to either a pressure-threshold IMT or sham hypoxic-training placebo (PLC) group. Prior to and following a 6 week intervention subjects completed a 25-km cycling time-trial and three constant-power tests to establish the W_{lim}–T_{lim} relationship. Constant-power tests were prescribed to elicit exercise intolerance within 3–10 (Ex1), 10–20 (Ex2), and 20–30 (Ex3) min. Maximal inspiratory mouth pressure increased by (mean ± SD) 17.1 ± 12.2% following IMT ($P < 0.01$) and was accompanied by a 2.66 ± 2.51% improvement in 25-km time-trial performance ($P < 0.05$); there were no changes following PLC. Constant-power cycling endurance was unchanged following PLC, as was CP (pre vs. post: 249 ± 32 vs. 250 ± 32 W) and AWC (30.7 ± 12.7 vs. 30.1 ± 12.5 kJ). Following IMT Ex1 and Ex3 cycling endurance improved by 18.3 ± 15.1 and 15.3 ± 19.1% ($P < 0.05$), respectively, CP was unchanged (264 ± 62 vs. 263 ± 61 W), but AWC increased from 24.8 ± 5.6 to 29.0 ± 8.4 kJ ($P < 0.05$). In conclusion, these data provide novel evidence that improvements in constant-power and cycling time-trial performance following IMT in cyclists may be explained, in part, by an increase in AWC.

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Keywords Respiratory muscle training · Exercise performance · Ergogenic · Critical power · Anaerobic work capacity

Introduction

There is now little doubt that the structure and function of the respiratory muscles can be modified through specific training. Respiratory muscle training (RMT) can be performed using voluntary isocapnic hyperpnoea (VIH), flow-resistive loading, and pressure-threshold loading, otherwise commonly referred to as inspiratory muscle training (IMT). All three techniques increase breathing endurance whilst IMT increases diaphragm thickness (Downey et al. 2007), and maximal inspiratory muscle strength, endurance, shortening velocity and power output (for a full review see McConnell and Romer 2004).

Whether RMT has an ergogenic effect remains somewhat more controversial (for reviews see McConnell and Romer 2004; Sheel 2002). Comparisons of the literature are complicated by the potentially variable outcomes with each RMT technique (or concurrent IMT-VIH training (Sonetti et al. 2001)) and by inter-study differences in RMT protocols and durations. In addition, the mode, intensity, duration and type (time-trial or constant-power) of exercise performance evaluation test has differed considerably between studies, which also hinders comparison and interpretation. A further consideration is that some of the literature has been characterised by weak experimental design (McConnell and Romer 2004). A brief synopsis is that VIH training improved 4-mile running time-trial performance (Leddy et al. 2007) and constant-power cycling endurance at moderate (70–85% maximum power or maximal oxygen uptake, $\dot{W}_{max}$ and $\dot{V}O_{2\,max}$, respectively)

(Boutellier et al. 1992; Markov et al. 2001; McMahon et al. 2002), but not high (90–95% $\dot{W}_{max}/\dot{V}O_{2\,max}$) (Fairbarn et al. 1991; Morgan et al. 1987), exercise intensities. Accordingly, it is suggested that the efficacy of RMT is more apparent when the performance evaluation test is submaximal and prolonged, with performance gains becoming less discernable, although not eradicated, at higher exercise intensities (Leddy et al. 2007; McConnell and Romer 2004). IMT improved constant-power cycling endurance at both high $\left(100\%\ \dot{V}O_{2\,max}\right)$ (Edwards and Cooke 2004) and moderate (75–80% $\dot{W}_{max}/\dot{V}O_{2\,max}$)) exercise intensities (Gething et al. 2004; Guenette et al. 2006; Sonetti et al. 2001), although others failed to observe improved cycling and running endurance at 85% $\dot{V}O_2$ max (Downey et al. 2007; Williams et al. 2002). Improvements of 1.0–4.6% have been observed for 6 min rowing and 20- and 40-km cycling time-trials following IMT in trained athletes (Griffiths and McConnell 2007; Romer et al. 2002a; Volianitis et al. 2001), whereas a 1.8% improvement in 8-km cycling time-trial performance following concurrent IMT and VIH training in trained cyclists failed to surpass that observed in a sham training placebo group (Sonetti et al. 2001). The lack of consensus is thus apparent but may be explained by the principle of training specificity: RMT ergogenicity may be dependent upon the nature of the performance evaluation test and the underlying mechanism(s) of action (McConnell and Romer 2004).

The mechanism(s) by which RMT may improve exercise tolerance has yet to be fully revealed although it has been argued that constant-power and time-trial exercise performances are predominantly governed by the parameters critical power (CP) and anaerobic work capacity (AWC) (Brandon 1995; Bulbulian et al. 1986; Fernández-García et al. 2000; Fukuba and Whipp 1999; Smith et al. 1999). Therefore, if RMT elicits a genuine ergogenic effect one might expect one or both of these parameters to be affected. CP and AWC can be derived from a series of exhaustive constant-power exercise tests in which exercise duration (T_{lim}) is measured (Hill 1993). The power-T_{lim} relationship is hyperbolic but can be linearised by plotting total work performed (W_{lim}) against T_{lim}, with the resulting gradient and y-intercept representing CP and AWC, respectively (Hill 1993; Monod and Scherrer 1965). Theoretically, CP is an inherent component of the aerobic energy supply system that characterises the highest exercise intensity at which a steady-state can be maintained in $\dot{V}O_2$, blood lactate concentration, and blood acid–base balance (Hill et al. 2002; Poole et al. 1988). Conversely, although the physiological mechanisms of AWC are less clear, it may represent a constant, but finite, energy store that can be utilised when exercise intensity exceeds CP (Morton 2006). The magnitude of AWC may also be determined by fatigue-inducing metabolite accumulation (Fukuba et al. 2003), and/or the capacity to resist the adverse consequences of metabolic perturbations in heavy exercise (Jenkins and Quigley 1993).

The aim of this study was to examine whether IMT improves cycling time-trial performance and results in a corresponding increase in CP and/or AWC. Therefore, we examined the effects of 6 weeks IMT upon 25-km cycling time-trial performance and the W_{lim}–T_{lim} relationship in competitive cyclists.

Methods

Subjects

Following approval from Nottingham Trent University's ethics committee, 18 non-smoking, competitive male cyclists provided written informed consent to participate in the study. Throughout the study subjects were instructed to adhere to their usual training regimen and not to partake in strenuous exercise the day before test days, during which subjects abstained from ingesting caffeine and arrived at the laboratory at least 2 h post-prandial. For each participant, tests were performed at a similar time of day. Descriptive characteristics of the subjects are presented in Table 1.

Experimental design

Subjects were initially familiarised with the test procedures and subsequently performed pulmonary and inspiratory muscle function tests. Subjects were then randomly, and equally, assigned to either a pressure-threshold IMT group or a sham hypoxic training placebo group. Prior to the intervention subjects completed a simulated 25-km cycling time-trial test and three constant-power cycling tests to establish the W_{lim}–T_{lim} relationship. Both groups then completed the prescribed training regimen for 6 weeks, after which the battery of exercise tests was repeated in random order, starting at least 48 h after the final training session. All tests were completed on separate days and separated by at least 48 h.

Pulmonary function and maximal inspiratory pressure

Pulmonary function was assessed using a pneumotachograph spirometer (Compact II, Vitalograph, Buckinghamshire, UK) previously calibrated using a 1 l syringe. Each test was repeated three times and the highest recorded value was used for subsequent analysis (Cotes 1993). A

Table 1 Baseline anthropometric characteristics, pulmonary function, and maximal inspiratory pressure of IMT and placebo subjects (mean ± SD)

Parameter	IMT (n = 9)	Placebo (n = 9)
Age (years)	31.6 ± 7.5	29.9 ± 8.9
Height (cm)	180.6 ± 4.7	177.5 ± 7.8
Body mass (kg)	75.5 ± 6.2	73.8 ± 7.9
FVC (l)	5.97 ± 0.56 (117 ± 11)	5.34 ± 0.83 (110 ± 14)
FEV_1 (l)	4.90 ± 0.74 (119 ± 16)	4.56 ± 0.61 (111 ± 10)
FEV_1/FVC (%)	82.6 ± 7.9 (104 ± 9)	86 ± 8.7 (105 ± 11)
PEF ($l\ s^{-1}$)	10.2 ± 2.2 (98 ± 21)	10.2 ± 1.0 (99 ± 13)
MVV_{12} ($l\ min^{-1}$)	188.4 ± 43.7 (124 ± 27)	166.7 ± 27.3 (110 ± 19)
MIP (cmH_2O)	150 ± 29 (137 ± 26)	153 ± 32 (137 ± 23)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; MVV_{12}, maximal voluntary ventilation in 12 s; MIP, maximal inspiratory mouth pressure. Values in parenthesis represent the percent of predicted value (Cotes 1993; Wilson et al. 1984)

hand-held mouth pressure meter (P.K. Morgan, Kent, UK) measured maximal inspiratory mouth pressure (MIP) as an index of global inspiratory muscle strength. The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure during inspiratory efforts. Manoeuvres were performed in an upright standing posture, were initiated from residual volume, and were sustained for at least 1 s. Repeat measurements separated by 30–60 s were taken until consistent values within 5 cm H_2O of each other were produced (Volianitis et al. 2001). The highest recorded value was then used for subsequent analysis.

25-km time-trial performance

Subjects performed a 25-km cycling time-trial on their own racing bicycle, which was mounted on an air-braked ergometry system (Kingcycle, High Wycombe, Buckinghamshire, UK). Use of this system has been described previously (Palmer et al. 1996). Subjects performed a 2 min warm-up at a self-selected intensity and began the test from a rolling start. Subjects were instructed to complete the 25-km as quickly as possible and the only feedback provided during exercise was the elapsed distance. Heart rate was recorded using short-range telemetry (Accurex Plus, Polar, Kempele, Finland). During exercise subjects wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) connected to a two-way, non-rebreathing valve (model 2730, Hans Rudolph), and expired air was collected in Douglas bags at 5-, 10-, 15-, and 20-km intervals. Concentrations of oxygen and carbon dioxide were determined by sampling through paramagnetic and infrared transducers, respectively (Series 1440, Servomex, Crowborough, UK), which were calibrated using certified gases (BOC gases, Guilford, UK). Sample volume was determined using a dry gas meter (Harvard, Edenbridge, UK). Minute ventilation ($\dot{V}_E$) is presented at BTPS, whereas $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$) are presented at STPD. Upon completion of the test, subjects performed a 3 min cool-down at a self-selected intensity. MIP was measured prior to exercise, following the 3 min cool-down, and 15 min thereafter.

W_{lim}–T_{lim} relationship

The W_{lim}–T_{lim} relationship was determined using three separate, square-wave constant-power tests performed to the limit of volitional tolerance on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Power outputs were chosen to elicit exercise intolerance within each of the following time domains: 3–10 (Ex1), 10–20 (Ex2), and 20–30 (Ex3) min. Subjects adopted a spontaneous cycling cadence in order to maximise T_{lim} (Hill 1993), although in practice the experienced cyclists participating in this study adopted their usual working cadence (approximately 80–100 rpm) and scarcely deviated from this throughout the duration of the tests. Exercise was terminated when cycling cadence could not be maintained above 60 rpm. A 3 min cool-down at 60 W was performed following exercise. MIP was assessed prior to exercise, following the 3 min cool-down, and 15 min thereafter. For each subject W_{lim} was plotted against T_{lim} and the slope and y-intercept of this relationship were taken to represent CP and AWC, respectively (e.g. see Bishop and Jenkins 1996; Monod and Scherrer 1965).

Training protocols

IMT was performed using an inspiratory pressure-threshold device (POWERbreathe®, Gaiam, UK). The IMT group performed 30 dynamic inspiratory efforts twice daily for

6 weeks against a pressure-threshold load of ~50% MIP. Thereafter, subjects periodically increased the load to a level that would permit them to only just complete 30 manoeuvres. Subjects initiated each inspiratory effort from residual volume and strove to maximise tidal volume. To avoid hyperventilation and therefore hypocapnia due to the increased tidal volume, subjects adopted a reduced breathing frequency. This IMT protocol is known to be effective in eliciting an adaptive response (Griffiths and McConnell 2007; McConnell and Lomax 2006; McConnell and Sharpe 2005; Romer et al. 2002a, 2002b; Volianitis et al. 2001). The placebo group used a sham hypoxic trainer for 15 min, 5 days week^{-1} (Sonetti et al. 2001). The placebo device was identical to that used by the IMT group, except that the resistance spring was removed and the lower chamber was loosely packed with aquarium gravel, which was promoted to the subjects as being oxygen absorbent, thus reducing the oxygen content of inspired air and mimicking altitude exposure. Subjects were instructed to breathe normally through the device and not increase their normal breathing effort. MIP was assessed every 2 weeks during the intervention, at which time the "oxygen absorbent" gravel in the placebo device was also replaced. All subjects completed a training diary throughout the study to record training adherence and whole-body training sessions.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Pre- and post-intervention results and group interactions were compared using one-way or two-way ANOVA for repeated measures and Tukey's HSD post-hoc analysis. Pearson product-moment correlation coefficients were calculated to assess the relationship between selected variables. Statistical significance was set at $P < 0.05$. Values are presented as mean ± SD.

Results

Each subject's habitual training regimen remained unchanged during the intervention. Subjects' training diaries demonstrated excellent training compliance for both IMT (95% adherence) and placebo (97% adherence) groups.

Pulmonary function and maximal inspiratory pressure

Baseline pulmonary function and MIP were all within normal limits and were not different between IMT and placebo groups (Table 1). Throughout the intervention MIP remained unchanged in the placebo group. Conversely, relative to baseline, MIP increased following 2, 4 and 6 weeks of IMT by 8.4 ± 9.1% ($P < 0.05$), 10.6 ± 8.3% ($P < 0.01$), and 17.1 ± 12.2% ($P < 0.01$), respectively (Fig. 1).

Time-trial performance and physiological responses

Group mean changes in time-trial performance are shown in Fig. 2. Time taken to cycle 25-km decreased by 2.66 ± 2.51% following IMT (pre vs. post:- 36.29 ± 3.64 vs. 35.33 ± 3.70 min, $P < 0.05$) (average power output: 274 ± 66 vs. 290 ± 71 W), whereas no change was observed following placebo (35.72 ± 1.97 vs. 35.98 ± 2.12 min, $P = 0.51$) (average power output: 275 ± 37 vs. 271 ± 42 W). Changes in time-trial performance were also different between groups, as indicated by a significant group × time interaction effect ($P < 0.05$). There was a consistent improvement in 5-km split times following IMT with significant group × time interaction effects being observed at 15-, 20-, and 25-km (Fig. 2). Figure 3 shows individual changes in time-trial performance for IMT and placebo subjects. The regression line of pre- vs. post-intervention time-trial performance for the placebo group is similar to the line of identity indicating no change in time-trial performance, whereas that for the IMT group was below, but parallel to, the line of identity. This reflects the improvement in time-trial performance following IMT and also indicates that the improvement was not related to baseline time-trial performance. With the exception of a significant increase in $\dot{V}CO_2$ at 20-km following IMT (3.17 ± 0.73 vs. 3.34 ± 0.86 l min^{-1}), physiological responses to time-trial exercise were not changed at equal

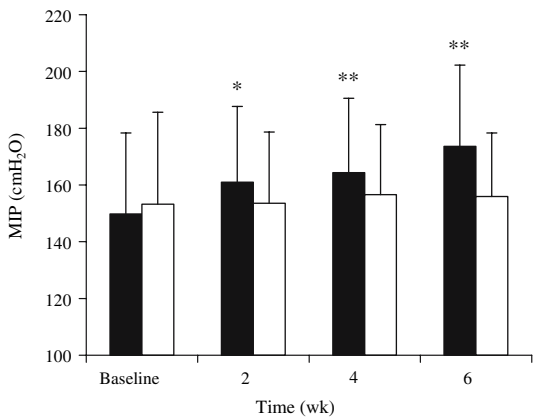

Fig. 1 Maximal inspiratory mouth pressure (MIP) at baseline and after 2, 4, and 6 weeks of IMT (*filled bars*) and placebo (*open bars*) (mean ± SD). *Significantly different from baseline ($P < 0.05$). ** Significantly different from baseline ($P < 0.01$)

distances following IMT or placebo. Prior to the intervention the average $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, and heart rate during time-trial exercise in IMT and placebo groups were 126.6 ± 39.9 and 116.8 ± 22.0 l min^{-1}, 3.65 ± 0.86 and 3.51 ± 0.36 l min^{-1}, 3.47 ± 0.82 and 3.33 ± 0.34 l min^{-1}, 0.95 ± 0.03 and 0.95 ± 0.05, and 176 ± 11 and 176 ± 11 beats min^{-1}, respectively. These responses remained unchanged following IMT and placebo. HR measured upon completion of time-trial exercise was also unchanged following IMT (189 ± 15 vs. 187 ± 12 beats min^{-1}) and placebo (189 ± 12 vs. 188 ± 12 beats min^{-1}).

W_{lim}–T_{lim} relationship

Power outputs for Ex1, Ex2, and Ex3 in IMT and placebo groups were 333 ± 74, 286 ± 63, and 281 ± 62 W, and 320 ± 37, 288 ± 28, and 272 ± 29 W, respectively. Constant-power cycling endurance times are shown in Fig. 4. Ex1, Ex2, and Ex3 cycling endurance times were unchanged following placebo. Conversely, although Ex2 endurance time was unchanged following IMT ($P = 0.11$), endurance times for Ex1 and Ex3 increased by 18.3 ± 15.1 and 15.3 ± 19.1%, respectively ($P < 0.05$), although group × time interaction effects were not significant ($P = 0.08$ and 0.11 for Ex1 and Ex3, respectively).

Changes in CP and AWC are shown in Fig. 5. The relationship between work and cycling endurance time was well described by the W_{lim}–T_{lim} model in both IMT (R^2 = 1.000 ± 0.000 vs. 1.000 ± 0.000) and placebo (R^2 = 0.999 ± 0.002 vs. 0.999 ± 0.002) groups. There was no change in CP following either IMT or placebo, and no change in AWC following placebo. Conversely, AWC increased following IMT ($P < 0.05$), although a group × time interaction effect was not observed.

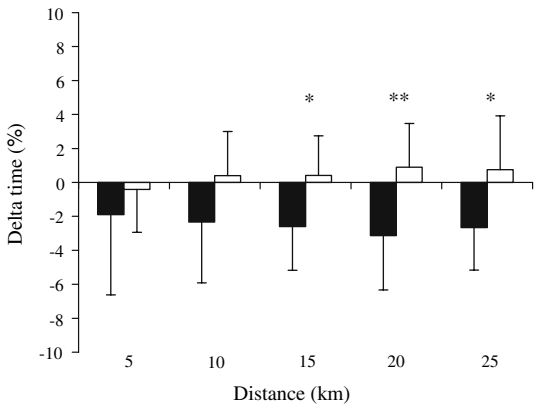

Fig. 2 Relative changes in time-trial performance in IMT (filled bars) and placebo (*open bars*) groups (mean ± SD). *Significant interaction effect ($P < 0.05$). **Significant interaction effect ($P < 0.01$)

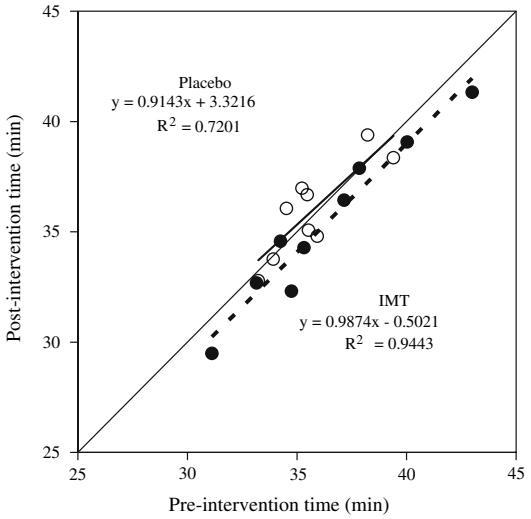

Fig. 3 Pre- and post-intervention time-trial duration for IMT (*filled circle*) and placebo (*open circle*) subjects. Regression lines for placebo (*solid line*) and IMT (*dashed line*) groups are shown, as is the line of identity

Exercise-induced changes in maximal inspiratory pressure

Prior to the intervention MIP measured 3 min after time-trial exercise was reduced relative to baseline by 11.8 ± 9.6 and 10.4 ± 7.2% in IMT and placebo groups, respectively ($P < 0.01$). Following 15 min recovery there was, relative to baseline, a non-significant 6.6 ± 8.8% reduction in MIP in the IMT group, and a 8.2 ± 5.6% reduction ($P < 0.05$) in the placebo group. Prior to the intervention no decreases in MIP were observed following constant-power exercise except following Ex2 in the IMT group where 7.7 ± 6.0 and 5.9 ± 5.1% decreases ($P < 0.05$) in MIP were observed 3 and 15 min post-exercise, respectively. When expressed relative to pre-exercise MIP, exercise-induced decrements in MIP were unchanged following IMT and placebo.

Correlations among variables

Relative improvements in cycling time-trial performance following IMT were not correlated with relative IMT-induced changes in MIP or AWC. Increased MIP following IMT was also not correlated with changes in Ex1 and Ex2 endurance times, or increases in AWC. Conversely, although the relative increase in AWC following IMT was

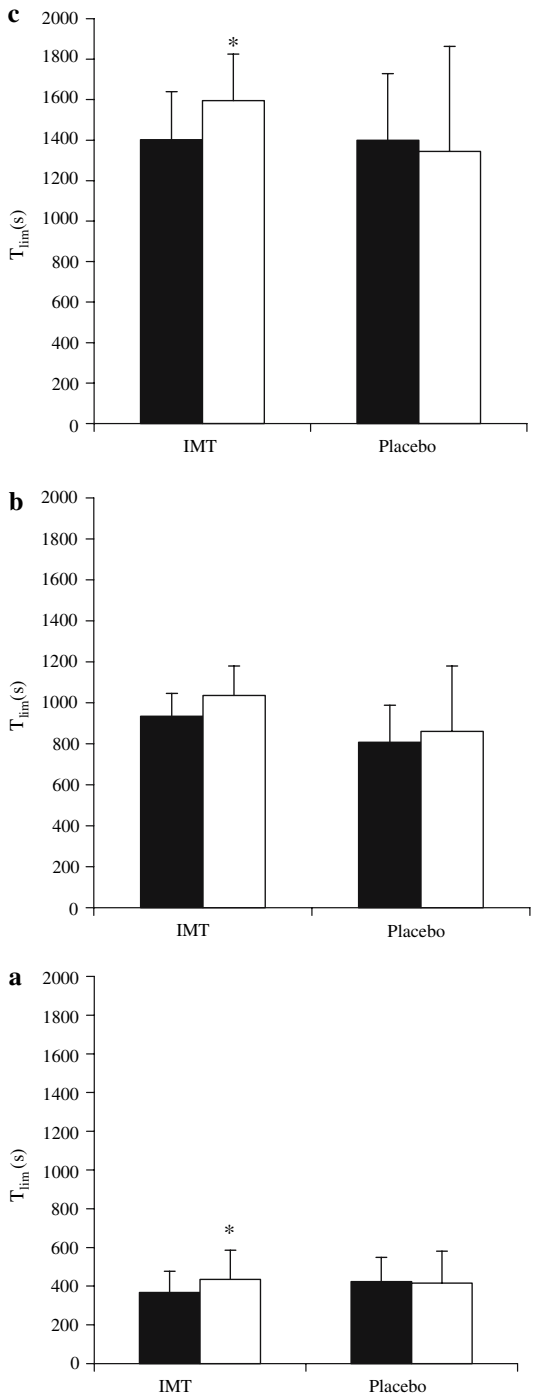

Fig. 4 Ex1 (**a**), Ex2 (**b**), and Ex3 (**c**) cycling endurance pre- (*filled bars*) and post- (*open bars*) IMT and placebo (mean ± SD). *Significantly different from pre-IMT ($P < 0.05$)

not correlated with changes in Ex2 ($r = 0.14$) or Ex3 ($r = -0.42$) endurance times, it was correlated with the relative improvement in Ex1 endurance time ($r = 0.73$, $P < 0.05$). Pre- and post-intervention values for CP and time-trial performance were also significantly correlated in IMT ($r = -0.89$ and -0.93, $P < 0.01$) and placebo ($r = -0.69$ and -0.75, $P < 0.05$) groups separately, and when pooled together ($r = -0.82$ and -0.89, $P < 0.01$).

Discussion

Main findings

The present study examined the effects of 6 weeks IMT upon 25-km cycling time-trial performance and the W_{lim}–T_{lim} relationship in competitive cyclists. The main finding was that IMT improved cycling time-trial performance to a greater extent than that observed in a sham training placebo group, and that this was accompanied by an increase in AWC.

Inspiratory muscle strength

The 17.1% increase in MIP after 6 weeks IMT is consistent with previous IMT studies (Downey et al. 2007; Edwards and Cooke 2004; Griffiths and McConnell 2007; Hanel and Secher 1991; Inbar et al. 2000; McConnell and Sharpe 2005; Wells et al. 2005), but less than the 30–50% increase reported by others (Gething et al. 2004; Guenette et al. 2006; Huang et al. 2003; Leith and Bradley 1976; Romer and McConnell 2003; Volianitis et al. 2001; Williams et al. 2002). Established training principles appear to apply to IMT (Romer and McConnell 2003), thus these discrepancies may be related, in part, to inter-study differences in IMT mode, intensity, and duration. It is also striking that baseline MIP was high in our subjects (137% predicted, Wilson et al. 1984). The scale of physiological adaptation within a system is dependent upon its baseline status (Åstrand et al. 2003), thus compared to previous studies a smaller window for adaptation may have existed in our subjects. Despite this we observed a rapid increase in MIP of 8.4% following just 2 weeks of IMT. This surprisingly swift pattern of change has been reported elsewhere with ~14 and ~28% increases in MIP observed after 1 and 2 weeks of IMT, respectively (Downey et al. 2007; Huang et al. 2003). Such rapid training-induced increases in limb muscle strength are usually ascribed to neural adaptations, with structural alterations becoming evident after about 6–8 weeks of training (Kraemer et al. 1996). However, the signalling pathways that elicit structural alterations in inspiratory muscles might arise earlier than anticipated because of the greater training frequency with IMT (up to 14 sessions week^{-1}) (Kraemer et al. 1996). In support, diaphragm thickness and type II fibre size in the external intercostals increased by 8–12 and 21%, respectively, following 4–5 weeks of IMT (Downey et al. 2007; Ramírez-Sarmiento et al. 2002).

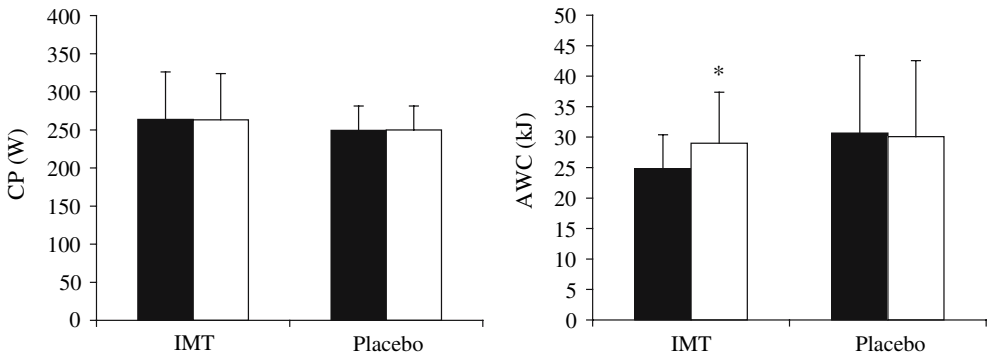

Fig. 5 Critical power (CP) and anaerobic work capacity (AWC) pre- (*filled bars*) and post- (*open bars*) IMT and placebo (mean ± SD). *Significantly different from pre-IMT ($P < 0.05$)

Increased MIP per se is, however, unlikely to explain IMT-induced improvements in exercise performance, since these variables do not correlate (present study; Griffiths and McConnell 2007; Guenette et al. 2006). Rather, IMT-induced alterations to the structure and function of the inspiratory muscles probably elicit systemic and perceptual repercussions that are beneficial to performance (McConnell and Romer 2004).

Time-trial performance and physiological responses

In trained cyclists using the Kingcycle variation in cycling time-trial performance lasting ~30 min is 1.1% (Palmer et al. 1996). The 2.66% improvement in 25-km cycling time-trial performance observed following IMT exceeds half of the variation between individual performances and can thus be considered worthwhile (Hopkins et al. 1999). Similar improvements in 20- and 40-km cycling (3.8–4.6%) (Romer et al. 2002a), and 6-min rowing (1–3.5%) (Griffiths and McConnell 2007; Volianitis et al. 2001), time-trial performances have been reported in trained athletes following IMT, whereas Leddy et al. (2007) observed a 4% improvement in 4-mile running time following VIH training. The use of an identical sham hypoxic trainer to that used by Sonetti et al. (2001), which anecdotal evidence suggests fully deceived our subjects, contests the notion that placebo effects underpin the efficacy of RMT. Sonetti et al. (2001) argue that the sham hypoxic trainer is superior to the placebo regimens used in other IMT studies, which typically involve training with negligible resistance. Our findings thus suggest a genuine ergogenic effect of IMT on time-trial performance in cyclists. The absence of an ergogenic effect reported by Sonetti et al. (2001) may be because their concurrent IMT-VIH training regimen provided conflicting training stimuli, as evidenced by the modest 8% increase in MIP.

Following IMT physiological responses ($\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, and heart rate) to time-trial exercise were unchanged despite an increased power output. This is consistent with previous studies showing reduced metabolic and heart rate responses during constant-power exercise following pressure-threshold IMT (Downey et al. 2007; Gething et al. 2004; Griffiths and McConnell 2007; Romer et al. 2002a). The improvement in time-trial performance following IMT may therefore be linked to an ability to sustain a higher intensity of exercise for the same metabolic and cardiovascular demand.

W_{lim}–T_{lim} relationship

The IMT-induced improvement in cycling time-trial performance was not associated with an increase in CP. This finding is supported by those of McConnell and Sharpe (2005) who observed no change in maximal lactate steady-state cycling power following a similar IMT regimen. Whilst maximal lactate steady-state and CP share similar definitions intra-subject comparisons suggest that CP occurs at a slightly higher power output (Pringle and Jones 2002). Notwithstanding this it appears that adaptations resulting from IMT do not lead to an increase in maximum sustainable power output. It appears more likely that IMT-mediated increases in time-trial performance are linked, in part, to the observed 12% increase in AWC. Unlike CP, $\dot{V}O_{2\,max}$, and ventilatory/lactate threshold, AWC is not correlated with time-trial performance (Bulbulian et al. 1986; Smith et al. 1999). Furthermore, we found no correlation between IMT-induced increases in AWC and improvements in 25-km cycling time-trial performance. Conversely, IMT-mediated increases in AWC were correlated with increases in Ex1 cycling endurance. This is perhaps unsurprising given that AWC is related to the ability to perform high-intensity exercise with a large anaerobic component (Hill 1993; Jenkins and Quigley 1993). However, although considered a limiting factor (Brandon 1995; Bulbulian et al. 1986; Fernández-García

et al. 2000; Fukuba and Whipp 1999) the contribution of AWC to more prolonged endurance exercise performance is difficult to assess (Brandon 1995). This is reflected by the lack of correlation between IMT-induced increases in AWC and Ex2 and Ex3 cycling endurance. During cycling time-trial exercise lasting ~30 min power output fluctuates above and below CP, thus AWC is utilised periodically (Tucker et al. 2006). Presumably therefore, the IMT-mediated increase in AWC would have allowed the cyclists to perform a greater volume of work above CP, although the 4.2 kJ increase in AWC alone cannot explain the 16 W increase in mean time-trial cycling power output. Other adaptations, for example perceptual changes, may have influenced the intrinsic system control mechanisms that regulate power output (c.f. Tucker et al. 2006) thereby reducing the volume of work performed below CP and increasing the time spent exercising at, or close to CP.

Our baseline AWC was broadly similar to that reported by others (Bishop and Jenkins 1996), although these values underestimate the true AWC because the W_{lim}–T_{lim} model does not account for the temporal lag in oxidative ATP synthesis at the onset of square-wave exercise (Morton 2006). Previous studies have also shown that strength and high-intensity interval training increase AWC independently of CP (Bishop and Jenkins 1996; Jenkins and Quigley 1993). AWC is thought to reflect finite intramuscular energy stores comprising a phosphagen pool, an anaerobic glycolytic component, and an oxygen store (Morton 2006). Increases in AWC with high-intensity training have thus been attributed to upregulation of phosphofructokinase activity and/or increased buffering capacity within the locomotor muscles (Jenkins and Quigley 1993). It is highly unlikely that IMT would stimulate such adaptations (within the locomotor muscles), thus other mechanisms must explain the increase in AWC. An attractive hypothesis resides in the potential effects of exercise-induced diaphragm fatigue on exercise performance. High-intensity exercise causes diaphragm fatigue, which may reduce limb vascular conductance via a metaboreflex and exacerbate locomotor muscle fatigue (for a review see Dempsey et al. 2006). Since IMT attenuates this metaboreflex (McConnell and Lomax 2006), the increase in AWC may be explained, in part, by improved perfusion of, and oxygen transport to, locomotor muscles and a subsequent reduction in the accumulation of fatigue-inducing intramuscular metabolites. Unfortunately, exercise-induced changes in MIP before and after IMT did not support this hypothesis. However, inferences regarding peripheral diaphragm fatigue cannot be made from MIP measures, which are unable to discriminate either fatigue in different inspiratory muscles, or peripheral and central components of fatigue. Therefore, our data do not exclude the possibility that exercise-induced diaphragm fatigue was attenuated following IMT, as was recently reported following VIH training (Verges et al. 2007). It is also possible that following IMT the same absolute decrease in MIP imparts a smaller influence on limb muscle endurance (McConnell and Lomax 2006).

Ventilatory work during heavy endurance exercise may contribute to the accumulation of metabolites (Johnson et al. 2006) that exacerbate respiratory and locomotor muscle fatigue. One of the most consistent observations following both IMT (Edwards and Cooke 2004; Griffiths and McConnell 2007; McConnell and Sharpe 2005; Romer et al. 2002b; Volianitis et al. 2001) and VIH training (Kohl et al. 1997; Leddy et al. 2007; Spengler et al. 1999; Verges et al. 2007) is a reduction in blood lactate concentration. Although the origin of this reduction remains unknown, it may reflect favourable changes in acid-base balance and/or a delay in the accumulation of fatigue-inducing metabolites, which may also partly explain the increase in AWC following IMT.

It is well known that even after exhaustive exercise locomotor muscle function is preserved and intramuscular glycogen stores are only partially depleted (Morton 2006). It follows that exercise tolerance (and presumably the capacity to deplete AWC) is not exclusively determined by physiological factors; it is likely that perceived effort and feelings of discomfort also play a significant role. Indeed, perhaps the most consistent feature of humans at the limit of exercise tolerance is the reporting of maximal ratings of perceived exertion (Noakes 2004). IMT attenuates ratings of limb discomfort and dyspnoea during exercise (McConnell and Romer 2004) and this may provide a mechanism by which AWC can be more fully exploited.

In summary, IMT-induced improvements in 25-km cycling time-trial performance in competitive cyclists are not explained by an increase in CP, but might be explained, in part, by an increase in AWC. Mechanisms underpinning an IMT-mediated increase in AWC remain unknown, but might be partly related to a reduction/delay in the accumulation of fatigue-inducing metabolites. That IMT resulted in comparable improvements in Ex1 (+18%) and Ex3 (+15%) cycling endurance disputes the notion that the efficacy of RMT is inversely proportional to the intensity of the performance evaluation test (Leddy et al. 2007; McConnell and Romer 2004).

References

Åstrand P-O, Rodahl K, Dahl HA, Stromme SB (2003) Textbook of work physiology: physiological bases of exercise, 4th edn. Human Kinetics, Leeds, pp 313–368

Bishop D, Jenkins DG (1996) The influence of resistance training on the critical power function and time to fatigue at critical power. Aust J Sci Med Sport 28:101–105

Boutellier U, Buchel R, Kundert A, Spengler C (1992) The respiratory system as an exercise limiting factor in normal trained subjects. Eur J Appl Physiol 65:347–353

Brandon LJ (1995) Physiological factors associated with middle distance running performance. Sports Med 19:268–277

Bulbulian R, Wilcox AR, Darabos BL (1986) Anaerobic contribution to distance running performance of trained cross-country athletes. Med Sci Sports Exerc 18:107–113

Cotes JE (1993) Lung function: assessment and application in medicine, 5th edn. Blackwell Scientific Publications, London, pp 496–497

Dempsey JA, Romer L, Rodman J, Miller J, Smith C (2006) Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol 151:242–250

Downey AE, Chenoweth LM, Townsend DK, Ranum JD, Ferguson CS, Harms CA (2007) Effects of inspiratory muscle training on exercise responses in normoxia and hypoxia. Respir Physiol Neurobiol 156:137–146

Edwards AM, Cooke CB (2004) Oxygen uptake kinetics and maximal aerobic power are unaffected by inspiratory muscle training in healthy subjects where time to exhaustion is extended. Eur J Appl Physiol 93:139–144

Fairbarn MS, Coutts KC, Pardy RL, McKenzie DC (1991) Improved respiratory muscle endurance of highly trained cyclists and the effects on maximal exercise performance. Int J Sports Med 12:66–70

Fernández-García B, Pérez-Landaluce J, Rodríguez-Alonso M, Terrados N (2000) Intensity of exercise during road race pro-cycling competition. Med Sci Sports Exerc 32:1002–1006

Fukuba Y, Whipp BJ (1999) A metabolic limit on the ability to make up for lost time in endurance events. J Appl Physiol 87:853–861

Fukuba Y, Miura A, Endo M, Kan A, Yanagawa K, Whipp BJ (2003) The curvature constant parameter of the power-duration curve for varied-power exercise. Med Sci Sports Exerc 35:1413–1418

Gething AD, Williams M, Davies B (2004) Inspiratory resistive loading improves cycling capacity: a placebo controlled trial. Br J Sports Med 38:730–736

Griffiths LA, McConnell AK (2007) The influence of inspiratory and expiratory muscle training upon rowing performance. Eur J Appl Physiol 99:457–466

Guenette JA, Martens AM, Lee AL, Tyler GD, Richards JC, Foster GE, Warburton DER, Sheel AW (2006) Variable effects of respiratory muscle training on cycle exercise performance in men and women. Appl Physiol Nutr Metab 31:159–166

Hanel B, Secher NH (1991) Maximal oxygen uptake and work capacity after inspiratory muscle training: a controlled study. J Sports Sci 9:43–52

Hill DW (1993) The critical power concept. Sports Med 16:237–254

Hill DW, Poole DC, Smith JC (2002) The relationship between power and the time to achieve $\dot{V}O_{2\,max}$. Med Sci Sports Exerc 34:709–714

Hopkins WG, Hawley JA, Burke LM (1999) Design and analysis of research on sport performance enhancement. Sports Med 31:472–485

Huang CH, Martin AD, Davenport PW (2003) Effect of inspiratory muscle strength training on inspiratory motor drive and RREP early peak components. J Appl Physiol 94:462–468

Inbar O, Weiner P, Azgad Y, Rotstein A, Weinstein Y (2000) Specific inspiratory muscle training in well-trained endurance athletes. Med Sci Sports Exerc 32:1233–1237

Jenkins DG, Quigley BM (1993) The influence of high-intensity exercise training on the W_{lim}–T_{lim} relationship. Med Sci Sports Exerc 25:275–282

Johnson MA, Sharpe GR, McConnell AK (2006) Maximal voluntary hyperpnoea increases blood lactate concentration during exercise. Eur J Appl Physiol 96:600–608

Kohl J, Koller EA, Brandenberger M, Cardenas M, Boutellier U (1997) Effect of exercise-induced hyperventilation on airway resistance and cycling endurance. Eur J Appl Physiol 75:305–311

Kraemer WJ, Fleck SJ, Evans WJ (1996) Strength and power training: physiological mechanisms of adaptation. Exerc Sport Sci Rev 24:363–397

Leddy JJ, Limprasertkul A, Patel S, Modlich F, Buyea C, Pendergast DR, Lundgren CE (2007) Isocapnic hyperpnea training improves performance in competitive male runners. Eur J Appl Physiol 99:556–676

Leith DE, Bradley M (1976) Ventilatory muscle strength and endurance training. J Appl Physiol 41:508–516

Markov G, Spengler CM, Knopfli-Lenzin C, Stuessi C, Boutellier U (2001) Respiratory muscle training increases cycling endurance without affecting cardiovascular responses to exercise. Eur J Appl Physiol 85:233–239

McConnell AK, Lomax M (2006) The influence of inspiratory muscle work history and specific inspiratory muscle training upon human limb muscle fatigue. J Physiol 577:445–457

McConnell AK, Romer LM (2004) Respiratory muscle training in healthy humans: resolving the controversy. Int J Sports Med 25:284–293

McConnell AK, Sharpe GR (2005) The effect of inspiratory muscle training upon maximum lactate steady-state and blood lactate concentration. Eur J Appl Physiol 94:277–284

McMahon ME, Boutellier U, Smith RM, Spengler CM (2002) Hyperpnoea training attenuates peripheral chemosensitivity and improves cycling endurance. J Exp Biol 205:3937–3943

Monod H, Scherrer J (1965) The work capacity of a synergic muscular group. Ergonomics 8:329–338

Morgan DW, Kohrt WM, Bates BJ, Skinner JS (1987) Effects of respiratory muscle endurance training on ventilatory and endurance performance of moderately trained cyclists. Int J Sports Med 8:88–93

Morton RH (2006) The critical power and related whole-body bioenergetic models. Eur J Appl Physiol 96:339–354

Noakes TD (2004) Linear relationship between the perception of effort and the duration of constant load exercise that remains. J Appl Physiol 96:1571–1572

Palmer GS, Dennis SC, Noakes TD, Hawley JA (1996) Assessment of the reproducibility of performance testing on an air-braked cycle ergometer. Int J Sports Med 17:293–298

Poole DC, Ward SA, Gardner GW, Whipp BJ (1988) Metabolic and respiratory profile of the upper limit for prolonged exercise in man. Ergonomics 31:1265–1279

Pringle JS, Jones AM (2002) Maximal lactate steady state, critical power and EMG during cycling. Eur J Appl Physiol 88:214–226

Ramírez-Sarmiento A, Orozco-Levi M, Güell R, Barreiro E, Hernandez N, Mota S, Sangenis M, Broquetas JM, Casan P, Gea J (2002) Inspiratory muscle training in patients with chronic obstructive pulmonary disease: structural adaptation and physiologic outcomes. Am J Respir Crit Care Med 166:1491–1497

Romer LM, McConnell AK (2003) Specificity and reversibility of inspiratory muscle training. Med Sci Sports Exerc 35:237–244

Romer LM, McConnell AK, Jones DA (2002a) Effects of inspiratory muscle training on time-trial performance in trained cyclists. J Sports Sci 20:547–562

Romer LM, McConnell AK, Jones DA (2002b) Effects of inspiratory muscle training upon recovery time during high-intensity, repetitive sprint activity. Int J Sports Med 23:353–360

Sheel AW (2002) Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. Sports Med 32:567–581

Smith JC, Dangelmaier BS, Hill DW (1999) Critical power is related to cycling time trial performance. Int J Sports Med 20:374–378

Sonetti DA, Wetter TJ, Pegelow DF, Dempsey JA (2001) Effects of respiratory muscle training versus placebo on endurance exercise performance. Respir Physiol 127:185–199

Spengler CM, Roos M, Laube SM, Boutellier U (1999) Decreased exercise blood lactate concentrations after respiratory endurance training in humans. Eur J Appl Physiol 79:299–305

Tucker R, Bester A, Lambert EV, Noakes TD, Vaughan CL, St Clair Gibson A (2006) Non-random fluctuations in power output during self-paced exercise. Br J Sports Med 40:912–917

Verges S, Lenherr O, Haner AC, Schulz C, Spengler CM (2007) Increased fatigue resistance of respiratory muscles during exercise after respiratory muscle endurance training. Am J Physiol Regul Integr Comp Physiol 292:1246–1253

Volianitis S, McConnell AK, Koutedakis Y, McNaughton L, Backx K, Jones DA (2001) Inspiratory muscle training improves rowing performance. Med Sci Sports Exerc 33:803–809

Wells GD, Plyley M, Thomas S, Goodman L, Duffin J (2005) Effects of concurrent inspiratory and expiratory muscle training on respiratory and exercise performance in competitive swimmers. Eur J Appl Physiol 94:527–540

Williams JS, Wongsathikun J, Boon SM, Acevedo EO (2002) Inspiratory muscle training fails to improve endurance capacity in athletes. Med Sci Sports Exerc 34:1194–1198

Wilson SH, Cooke NT, Edwards RHT, Spiro SG (1984) Predicted normal values for maximal respiratory pressures in caucasian adults and children. Thorax 39:535–538

APPENDIX 5

Investigations of the Lactate Minimum Test

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Key words
- lactate kinetics
- test protocol
- prior exercise
- constant power

Abstract

We evaluated: the agreement between lactate minimum and maximal lactate steady state (MLSS) cycling powers (study 1); whether rates of change of blood lactate concentration during the lactate minimum test reflect that of constant power exercise (study 2); whether the lactate minimum power is influenced by the muscle groups used to elevate blood lactate concentration (study 3). Study 1: 32 subjects performed a lactate minimum test comprising a lactate elevation phase, recovery phase, and incremental phase (five 4 min stages); MLSS was subsequently determined. Study 2: 8 subjects performed a lactate minimum test and five 22 min constant power tests at the incremental phase exercise intensities. Study 3: 10 subjects performed two identical lactate minimum tests, except during the second test the lactate elevation phase comprised arm-cranking. Lactate minimum and MLSS powers demonstrated good agreement (mean bias ± 95% limits of agreement: 2 ± 22 W). Rates of change of blood lactate concentration during each incremental phase stage and corresponding constant power test did not correlate. Lactate minimum power was lowered when arm-cranking was used during the lactate elevation phase (157 ± 29 vs. 168 ± 21 W; $p < 0.05$). The lactate elevation phase modifies blood lactate concentration responses during the incremental phase, thus good agreement between lactate minimum and MLSS powers seems fortuitous.

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Bibliography
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Introduction

Tegtbur et al. [28] proposed the lactate minimum test as a method to predict maximal lactate steady state (MLSS). The test comprises three consecutive exercise phases: a lactate elevation phase, a short recovery phase, and an incremental phase in which with increasing intensity blood lactate concentration ($[lac^-]_B$) decreases (net lactate clearance) to a nadir (the lactate minimum) and then increases (net lactate appearance). The $[lac^-]_B$ is determined by rates of lactate release into the interstitium or circulation, and consumption by adjacent or remote lactate-consuming oxidative muscle fibres or organs (the lactate shuttle [9]). The lactate minimum test has considerable value since MLSS is an important physiological determinant of endurance exercise performance [17,21,24]. Collectively, with the exception of one conflicting report [17], the literature suggests good agreement between lactate minimum and MLSS intensities [3,21,24,28] and the test has been recommended over other methods of MLSS prediction [27].

The ability to determine MLSS in one session has been used to advocate the use of the lactate minimum test [27]. However, Tegtbur et al. [28], and others thereafter [3,17], used prior knowledge of subjects' training status to determine the incremental phase exercise intensities, whilst others [13,21,23,24,26] have used pre-tests (time-trials and maximal incremental tests) to achieve the same end. Thus the test would be improved if the incremental phase exercise intensities could be resolved within the same test. Smith et al. [26] found that lactate minimum cycling power was independent of whether the lactate elevation phase comprised a maximal incremental ramp test or sprint exercise. Thus, modifying the original protocol of Tegtbur et al. [28] by replacing the short, high-intensity exercise of the lactate elevation phase with incremental exercise ought not to affect lactate minimum test validity but should also allow concurrent determination of maximal oxygen uptake. Therefore, in study 1 the aim was

to evaluate the agreement between lactate minimum and MLSS cycling powers using a modified lactate minimum protocol in which the incremental phase intensities are based upon a maximal incremental ramp test performed in the lactate elevation phase.

Another feature used to promote the lactate minimum test is its "sound theoretical basis" [21]. This refers to the observations of Davis and Gass [11] on which Tegtbur et al. [28] based their protocol. Davis and Gass [11] introduced the innovation of examining $\Delta[lac^-]_B/\Delta t$ (where t=time) during incremental exercise commencing with hyperlactaemia and proposed that such changes have "predictive value for steady-state work". However, this central premise remains unexplored and therefore in study 2 the aim was to examine whether $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase reflects $\Delta[lac^-]_B/\Delta t$ measured during constant power exercise (after the initial transient change that occurs over the first 12 min of exercise [16]) at intensities identical to those performed during the incremental phase.

Prior exercise using the same muscle groups profoundly affects physiological responses to subsequent exercise, including reduced lactate production and release from the "primed" muscles [5,10,15]. Thus it seems likely that the lactate elevation phase will affect subsequent physiological responses and therefore the outcome of the test. To explore this issue the aim of study 3 was to examine whether $\Delta[lac^-]_B/\Delta t$ and the lactate minimum cycling power during the incremental phase are influenced by the muscle groups used (leg cycling vs. arm-cranking) during the lactate elevation phase.

Methods

Participants, equipment and measurements

Following local ethics committee approval, 50 non-smoking, recreationally active male subjects provided written informed consent to participate in the study. Subjects refrained from strenuous exercise during the 24 h preceding an exercise test. On test days subjects abstained from alcohol and caffeine and reported to the laboratory at least 2 h post-prandial. Successive tests were separated by at least 48 h, but no more than 1 week, and were performed at a similar time of day.

Exercise was performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) and also, during study 3, an electromagnetically-braked arm-cranking ergometer (Angio, Lode, Groningen, The Netherlands). The same self-selected cycling cadence was used throughout all tests. Arterialised venous blood samples were taken from a heated dorsal hand vein via an indwelling cannula [22] and analysed for $[lac^-]_B$ (P-GM7 MicroStat, Analox Instruments, London, UK). During the lactate elevation phase of study 1, subjects wore a facemask (model 7940, Hans Rudolph, Kansas City, Missouri) and respiratory variables were measured breath-by-breath (Pulmolab EX670, Ferraris Respiratory Europe, Hertford, UK).

Study 1 – agreement between lactate minimum and MLSS powers

Subjects (n=32; age 29.9±6.9 years, height 179.1±6.9 cm, body mass 79.5±10.3 kg) initially performed a lactate minimum test comprising 3 consecutive phases: (I) lactate elevation phase comprising maximal, incremental exercise; (II) 8 min recovery phase at 60 W; and (III) incremental phase comprising five consecutive 4 min stages at intensities of 45, 50, 55, 60, and 65% of the maximum power ($\dot{W}max$) achieved during the lactate elevation phase. Changes in intensity during the incremental phase were based upon the original protocol of Tegtbur et al. [28] and pilot work was used to determine a range of intensities that would encompass the lactate minimum power in all subjects. Like previous studies [13,17,24] the incremental phase comprised a fixed number of submaximal exercise stages rather than a maximal incremental exercise test, and the use of 5 stages was based upon the work of Jones and Doust [17]. The stage duration of 4 min was based upon the work of Tegtbur et al. [28]. Blood samples were taken from 11 subjects at the start of the incremental phase and every minute thereafter, and $\Delta[lac^-]_B/\Delta t$ was taken as the gradient of a linear regression of $[lac^-]_B$ against time during each stage. Blood samples were taken from the remaining subjects at the end of each incremental phase stage. During the lactate elevation phase power increased every 15 s by a constant amount (8–10 W, depending upon the subject's training history) chosen so that exercise intolerance (cadence <60 revs·min^{-1}) occurred in ~10 min. The final power defined $\dot{W}max$ and the highest oxygen uptake recorded over any 30 s period defined maximal oxygen uptake.

Maximal lactate steady state was resolved using 30 min constant power tests preceded by 3 min of cycling at 50% of the prescribed power. The first test was performed at lactate minimum power, and for subsequent tests power was adjusted by ±2.5% until MLSS was verified. Blood samples were taken every 2 min from 16–30 min, inclusive, and MLSS power was defined as the highest power at which a positive gradient of a linear regression fitted through the plot of $[lac^-]_B$ against time was not observed [16].

Study 2 – comparison of the $[lac^-]_B$ response to the lactate minimum test and constant power cycling

Subjects (n=8; age 23.4±5.2 years, height 180.4±6.4 cm, body mass 79.9±5.5 kg) initially performed a lactate minimum test (see study 1) with blood samples being taken at the start of the incremental phase and every minute thereafter. Subsequently, subjects performed, in random order and on different days, five 22 min cycling tests with blood samples being taken every 2 min from 0–22 min, inclusive. Cycling powers in each of the five tests corresponded to those used in the incremental phase, except that the initial 12 min of each test was always performed at 60% $\dot{W}max$ to elevate $[lac^-]_B$. The magnitude of $[lac^-]_B$ is known to influence $\Delta[lac^-]_B/\Delta t$ [11,26], thus it was essential that $[lac^-]_B$ at the start of the lactate minimum incremental phase and corresponding constant power were closely matched; note also that pilot work showed that $[lac^-]_B$ remained unchanged from rest during square-wave exercise at 45 and 50% $\dot{W}max$. The initial 12 min allowed for the rapid, transient increase in $\Delta[lac^-]_B/\Delta t$ from rest [16] and the total test duration (22 min) was chosen as pilot work showed that this corresponded to the limit of exercise tolerance when cycling at 65% $\dot{W}max$. During constant power exercise $\Delta[lac^-]_B/\Delta t$ was taken as the gradient of a linear regression of $[lac^-]_B$ against time (14–22 min, inclusive). Our analysis of $\Delta[lac^-]_B/\Delta t$ was therefore performed over an 8 min period: this was the maximum possible time allowed by our subjects' ability to tolerate cycling at 65% $\dot{W}max$ (22 min) and the need to allow the initial transient increase in $\Delta[lac^-]_B/\Delta t$ during the initial 12 min of exercise [16].

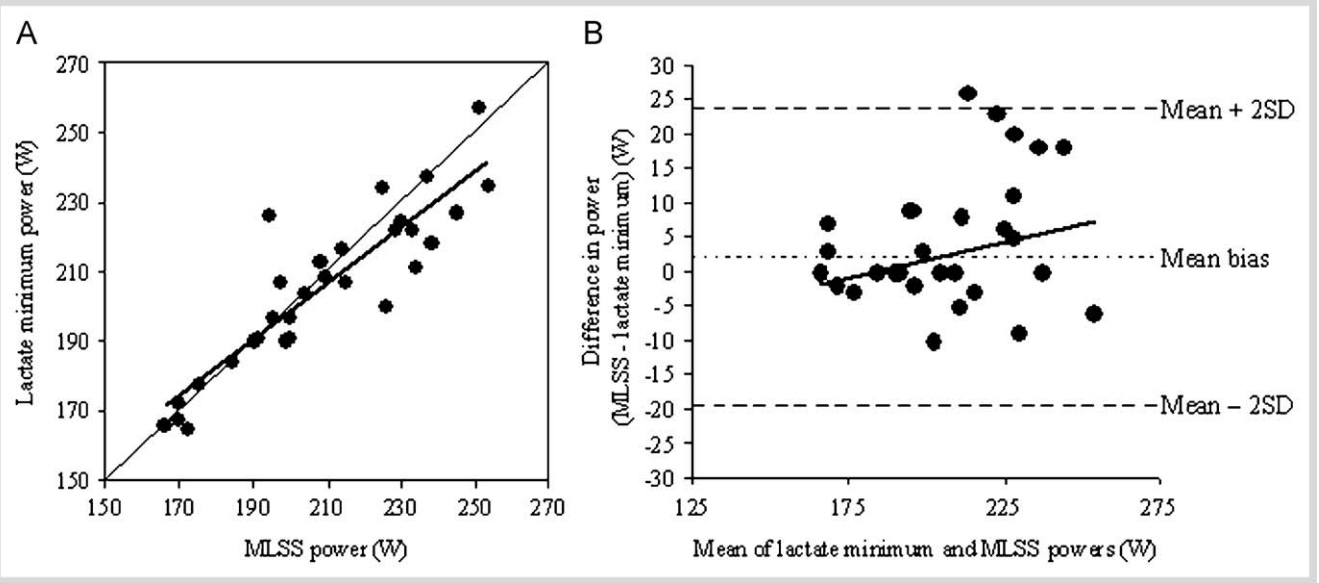

Fig. 1 **A:** Relationship between lactate minimum and MLSS powers, showing the line of identity. **B:** Agreement between lactate minimum and MLSS powers, showing the mean bias and 95% limits of agreement. Individual data are shown.

Study 3 – effect of muscle groups used during the lactate elevation phase

Subjects (n = 10; age 23.5 ± 5.5 years, height 178.8 ± 6.9 cm, body mass 78.0 ± 7.5 kg) initially performed a lactate minimum test (see study 1, hereafter termed LM_{LEG}). On a separate day, subjects repeated LM_{LEG}, except that the lactate elevation phase comprised maximal incremental arm-cranking exercise (hereafter termed LM_{ARM}). The centre of the arm-crank shaft was aligned to shoulder level and subjects were seated so that the elbow was slightly flexed when the hand was most distal. Following 15 s unloaded exercise, power was increased every 15 s by either 4 or 5 W up to the limit of exercise tolerance (cadence < 40 revs·min^{-1}). Subjects then transferred to the adjacently-positioned cycle ergometer and, following the recovery phase (8 min at 60 W), repeated the incremental phase (using identical cycling powers) performed in LM_{LEG}. Thus, LM_{LEG} always preceded LM_{ARM}. Blood samples were taken at the start of the incremental phase and every minute thereafter.

Data analyses

Data analyses were performed using SPSS (version 15). The lactate minimum power was determined from the zero gradient tangent to a cubic spline function fitting the $[lac^-]_B$ (measured at the end of each stage) vs. power data. Data were analysed using repeated measures ANOVA and paired t-tests where appropriate. Agreement between variables was assessed using a Bland-Altman plot [7], along with the calculated bias ± 95% limits of agreement. Pearson product-moment correlation coefficients (r) were determined to assess the relationship between variables. Results are reported as mean ± SD unless otherwise stated. Statistical significance was set at $p < 0.05$.

Results

The $[lac^-]_B$ profile during the incremental phase was well described by the cubic spline function ($r^2 = 0.94$ and 0.98 in study 1 and 2 respectively, and in study 3, $r^2 = 0.95$ and 0.99 for LM_{LEG} and LM_{ARM}, respectively). Also, in each study lactate minimum power was correlated with $\dot{W}max$ (r = 0.97 and 0.98 in study 1 and 2, respectively, and in study 3, r = 0.93 for LM_{LEG}) ($p < 0.01$).

Study 1 – agreement between lactate minimum and MLSS powers

The $\dot{W}max$ and maximal oxygen uptake were 379 ± 42 W and 3.99 ± 0.58 L·min^{-1}, respectively. The lactate minimum power (205 ± 22 W; 54.2 ± 1.5% $\dot{W}max$) was not different from MLSS power (208 ± 25 W; 54.9 ± 3.6% $\dot{W}max$), with which it was correlated (r = 0.89, $p < 0.01$) (**Fig. 1A**). Although the gradient of the regression line in **Fig. 1A** (0.804) is different from 1 ($p < 0.05$), this does not imply a lack of agreement between the two measurements [1, 7]. Indeed, **Fig. 1B**, which shows the difference between lactate minimum and MLSS powers against their mean (Bland-Altman plot) [7], along with the bias ± 95% limits of agreement (2 ± 22 W) for the comparison between the two variables, indicates good agreement between lactate minimum and MLSS powers (for further commentary see Discussion). Furthermore, the gradient of the regression line in **Fig. 1B** (0.105) is not different from zero ($p = 0.245$), which indicates uniformity of systematic error across the range of measurements studied [1]. The difference between lactate minimum and MLSS powers was correlated with MLSS power (r = 0.42, $p < 0.05$).

Where $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase was determined (n = 11), the power at which $\Delta[lac^-]_B/\Delta t = 0$ (220 ± 21 W; 58.5 ± 5.7% $\dot{W}max$; $r^2 = 0.90 \pm 0.12$) was greater ($p < 0.01$) than lactate minimum (203 ± 22 W; 53.7 ± 1.4% $\dot{W}max$) and MLSS powers (208 ± 26 W; 54.7 ± 2.7% $\dot{W}max$). The bias ± 95% limits of agreement for the comparison of the power at which $\Delta[lac^-]_B/\Delta t = 0$ and MLSS power was 12 ± 43 W.

Study 2 – comparison of $\Delta[lac^-]_B/\Delta t$ during the lactate minimum test and constant power cycling

The $\dot{W}max$ and lactate minimum power were 363 ± 42 W and 193 ± 24 W (52.8 ± 1.2% $\dot{W}max$), respectively. During constant power exercise at 50% $\dot{W}max$, the $[lac^-]_B$ at 14 min

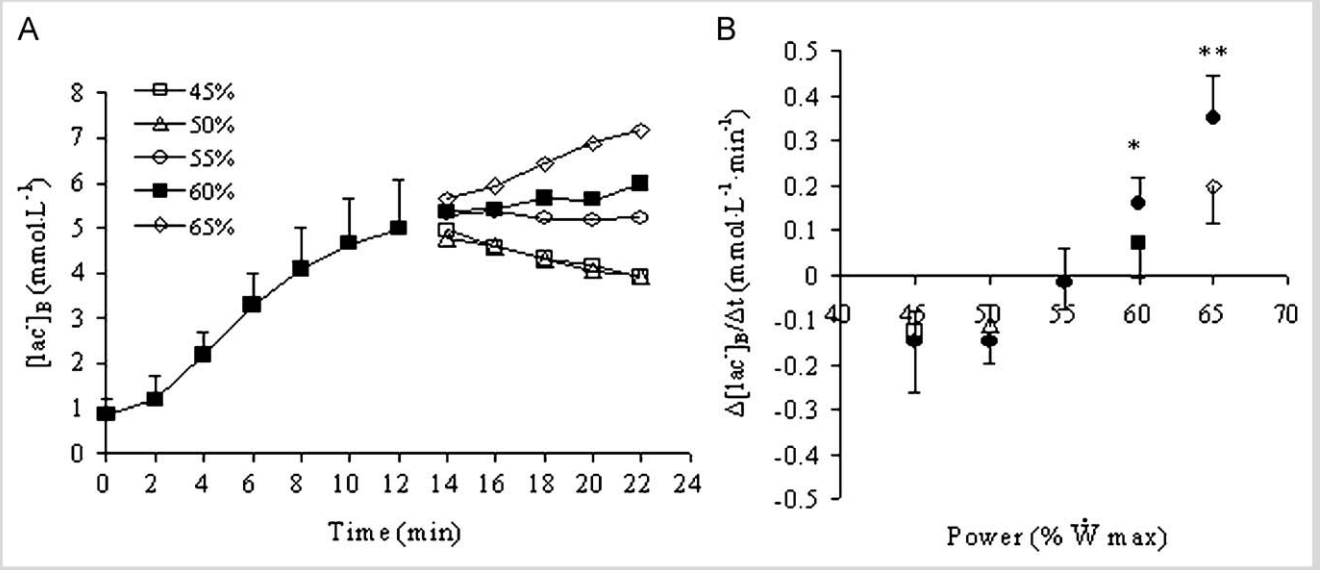

Fig. 2 **A:** Blood lactate concentration ($[lac^-]_B$) during constant power exercise. **B:** $\Delta[lac^-]_B/\Delta t$ during constant power exercise (see panel 'A' for symbols) and each stage of the lactate minimum incremental phase (●). Note that $\Delta[lac^-]_B/\Delta t$ for constant power exercise reflects that measured over 14–22 min in 'A'. Values are mean ± SD, except in 'A' where for clarity error bars are shown only for the initial 12 min of exercise. Difference between trials, $^*p<0.05$, $^{**}p<0.01$.

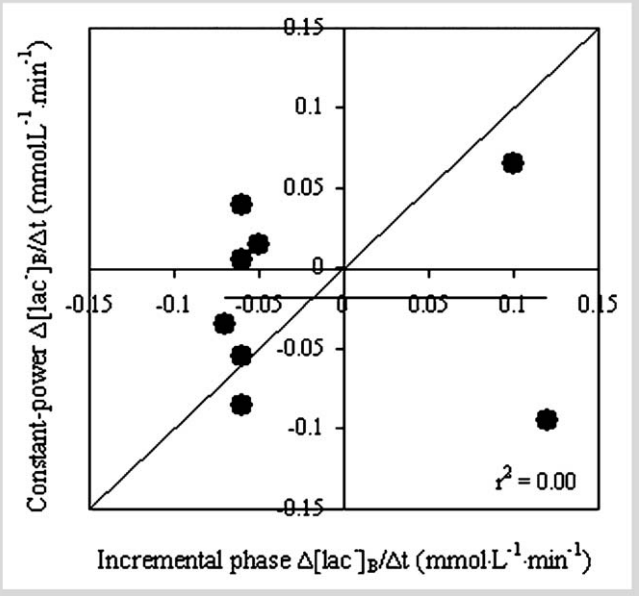

Fig. 3 Individual rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during exercise at 55% Ẇ max during the lactate minimum incremental phase and constant power exercise. Line of identity is shown.

$(4.8 \pm 1.0\,mmol \cdot L^{-1})$ (see ○ **Fig. 2A**) was lower than that measured at the commencement of 50% Ẇmax during the incremental phase $(5.5 \pm 1.2\,mmol \cdot L^{-1})$. There were no such differences at the other 4 intensities.

Compared to constant power exercise, $\Delta[lac^-]_B/\Delta t$ was greater during the incremental phase at 60% ($p<0.05$) and 65% Ẇmax ($p<0.01$) (○ **Fig. 2B**). The power at which $\Delta[lac^-]_B/\Delta t=0$ during the incremental phase (195 ± 24 W; 53.7 ± 1.9% Ẇmax; $r^2=0.84\pm0.10$) was not different from that determined in constant power exercise (200 ± 28 W; 55.1 ± 3.3% Ẇmax; $r^2=0.91\pm0.06$). However, $\Delta[lac^-]_B/\Delta t$ during each incremental phase stage and corresponding constant power test did not correlate (at each % Ẇmax, r values were: 45% = 0.07, 50% = 0.00, 55% = −0.03, 60% = 0.14, and 65% = 0.63) (e.g., ○ **Fig. 3**).

Study 3 – effect of muscle groups used during the lactate elevation phase

The duration of the lactate elevation phase during LM_{ARM} (9.13 ± 1.62 min) was not different from that in LM_{LEG} (9.49 ± 0.74 min), although Ẇmax was expectedly lower during LM_{ARM} (161 ± 44 vs. 317 ± 44 W) ($p<0.01$). All subjects demonstrated a U-shaped $[lac^-]_B$ vs. power profile during the incremental phase of LM_{LEG} and the lactate minimum power was 168 ± 21 W (53.3 ± 2.9% Ẇmax). Conversely, a clear U-shaped profile was not observed in LM_{ARM} (○ **Fig. 4A**), and in one subject a lactate minimum power could not be determined because $[lac^-]_B$ increased linearly ($r^2=1$). In the remaining subjects lactate minimum power during LM_{ARM} (157 ± 29 W) was lower than that in LM_{LEG} ($p<0.05$). The $[lac^-]_B$ at the end of each stage of the incremental phase was not different between LM_{LEG} and LM_{ARM}, although at 60% Ẇmax there was a trend ($p=0.055$) for $[lac^-]_B$ to be higher during LM_{ARM}.

The power at which $\Delta[lac^-]_B/\Delta t=0$ in LM_{LEG} (175 ± 24 W; 55.4 ± 2.6% Ẇmax; $r^2=0.85\pm0.13$) was greater than that in LM_{ARM} (157 ± 31 W; 49.4 ± 6.3% Ẇmax; $r^2=0.88\pm0.12$) (○ **Fig. 4B**). There was a trial × stage interaction effect for $\Delta[lac^-]_B/\Delta t$ ($p<0.01$), and differences were observed between LM_{LEG} and LM_{ARM} at 50% ($p<0.01$) and 60% ($p<0.05$) Ẇmax.

Discussion

The main findings of the present study were threefold: (I) there was good agreement between lactate minimum and MLSS powers using the modified lactate minimum protocol; (II) $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase did not reflect $\Delta[lac^-]_B/\Delta t$ during constant power exercise; and (III) the $[lac^-]_B$

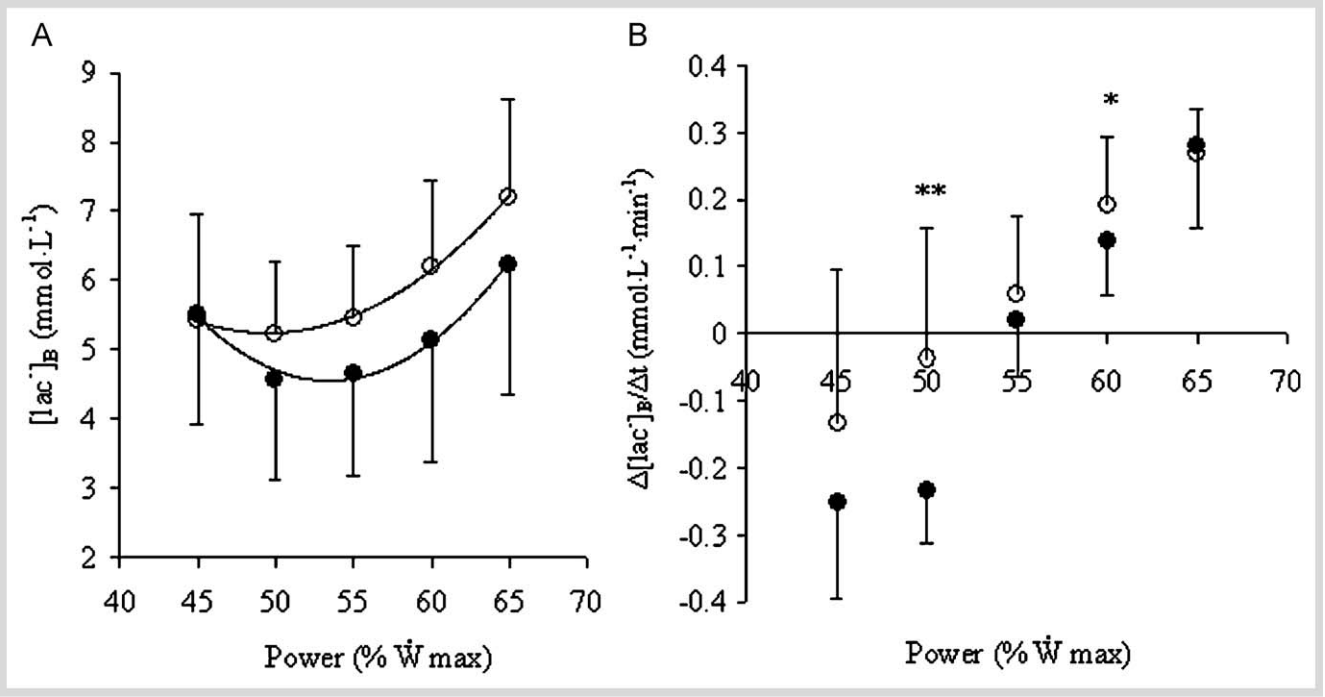

Fig. 4 **A:** Blood lactate concentration ($[lac^-]_B$) at the end of each lactate minimum incremental phase stage. **B:** $\Delta[lac^-]_B/\Delta t$ during each lactate minimum incremental phase stage. LM_{LEG} (●), LM_{ARM} (○). Values are mean ± SD. Difference between trials, $^*p<0.05$, $^{**}p<0.01$.

profile of the incremental phase was dependent upon whether the lactate elevation phase was performed using the same or different muscle groups.

Given the resolution with which MLSS is typically determined (10–20 W) [27], the mean difference and 95% limits of agreement (2 ± 22 W) for the comparison between lactate minimum and MLSS powers indicates that our modified lactate minimum test provides an acceptable estimate of MLSS power. These findings support other work showing the lactate minimum to be a valid predictor of MLSS [21,24], although others report conflicting evidence [17]. Note also that statistical power analysis revealed that, in our study, the minimum detectable difference (based upon our sample size (n = 32), an alpha level of 0.05, and power of 0.8) between lactate minimum and MLSS powers was 6 W. This corresponds closely to our MLSS resolution and therefore may be considered the minimum difference of practical significance [27]. In addition to estimating MLSS, our lactate minimum protocol also allows determination of maximal oxygen uptake and does not require detailed knowledge of subjects' training status. This is an improvement on previously described lactate minimum protocols in which prescription of incremental phase exercise intensities has required either separate testing or familiarity with subjects' training status (see Introduction). Furthermore, determining both maximal oxygen uptake and MLSS provides a more complete assessment of training status and training programme effectiveness. Our lactate minimum protocol was also well tolerated by subjects of varied training status (MLSS range: 166–253 W), which allayed our initial concerns that combining a maximal incremental test with the incremental phase may be too demanding for less-trained subjects.

Absolute $[lac^-]_B$ values and "thresholds" in ventilatory, pulmonary gas exchange, and $[lac^-]_B$ responses to incremental exercise have also been used to predict MLSS, although the validity of such protocols is poorly documented. It is well recognised, however, that the large inter-individual variation in $[lac^-]_B$ at MLSS (3–10 mmol·L^{-1} [21],) precludes MLSS prediction from absolute $[lac^-]_B$ values [2,17,27]. Conversely, the "lactate turnpoint" may provide a good estimate of MLSS [2,18], although Smith and Jones [25] report significant over- or under-estimation in individual subjects. The validity of the individual anaerobic threshold test is also variable, providing close estimates of MLSS in cycling [29] and running [4], but overestimating MLSS in rowing [6]. The respiratory compensation point often overestimates MLSS [12,20], whereas Laplaud et al. [20] suggest that MLSS corresponds to the intensity during incremental exercise at which the respiratory exchange ratio = 1.00. However, the lack of resolution in MLSS determination in this study (5% Ẇmax, ~16 W) could have masked significant disagreement between the predicted and the "true" MLSS. When viewed collectively, no single test can accurately determine MLSS, and comparison of tests is complicated by inter-study differences in exercise modalities and protocols, participants, and MLSS determination methods. Unlike many of the aforementioned protocols, however, the lactate minimum test provides a reliable, objective MLSS estimate [21,26] that is insensitive to changes in muscle glycogen stores [28]. It is therefore an attractive option amongst a plethora of single-test methods to predict MLSS. However, the level of exertion required during the lactate minimum test renders the protocol impractical for clinical populations [27]; threshold determinations from submaximal exercise are more appropriate under these circumstances.

We avoided basing our MLSS criterion on absolute increases in $[lac^-]_B$ over time (see MacIntosh et al. [21] for a discussion) since this results in dissimilar relative physiological stress due to the large inter-individual variation in $[lac^-]_B$ at MLSS (3–10 mmol·L^{-1} [21],). We also used relatively small step changes at an intensity of ±2.5% (~4–6 W), which allows greater resolution of MLSS than those studies using increments of 4–5% or ~10–20 W (reviewed in Svedahl and MacIntosh [27]).

Lactate minimum and MLSS agreement decreased as these cycling powers increased. Variability in MLSS prediction accuracy has been reported previously [3,21], and though the reason(s) for this remains unclear, MacIntosh et al. [21] suggest that prior training-related fatigue may affect the outcome of the lactate minimum test. Although our subjects refrained from exercise for at least 24 h prior to each test it remains possible that residual effects of the increased training volume associated with higher MLSS powers adversely affected the MLSS prediction.

The findings of study 2 show that $\Delta[lac^-]_B/\Delta t$ during the incremental phase and constant power exercise did not correlate. To our knowledge, we are the first to report such data and they challenge the proposition of Davis and Gass [11] that $\Delta[lac^-]_B/\Delta t$ during incremental exercise commencing with hyperlactaemia has "predictive value for steady state work". Since the magnitude of $[lac^-]_B$ partly determines $\Delta[lac^-]_B/\Delta t$ [11,26], we strived to match $[lac^-]_B$ at the start of each incremental phase stage and corresponding constant power. This was achieved at all powers except 50% $\dot{W}$max and we feel that this difference is unlikely to explain the absence of a correlation for $\Delta[lac^-]_B/\Delta t$ between the two conditions. Thus, our findings not only challenge the theoretical underpinning that has been used to promote the lactate minimum test [21], but they also suggest that the dynamics of the lactate shuttle during the incremental phase differ from that in constant power exercise at equivalent intensities. Furthermore, lactate minimum and MLSS powers have different mathematical definitions: $\Delta[lac^-]_B/\Delta P=0$ (where P=cycling power) and $\Delta[lac^-]_B/\Delta t=0$, respectively, and the cycling powers corresponding to these solutions were different (see study 1). Also, paradoxically the agreement with MLSS power was stronger for $\Delta[lac^-]_B/\Delta P=0$ (lactate minimum) compared to $\Delta[lac^-]_B/\Delta t=0$. These findings support previous conjecture [17] that good agreement between lactate minimum and MLSS powers partly reflects a fortuitous artefact of the protocol design.

The findings of study 3 are the first to show that the lactate minimum test is strongly influenced by the muscle groups used during the lactate elevation phase. The LM_{ARM} protocol precluded a U-shaped $[lac^-]_B$ profile during the incremental phase, which resulted in consistently greater values for $\Delta[lac^-]_B/\Delta t$, and lowered the lactate minimum power compared to LM_{LEG}. These findings support previous observations of increased $\Delta[lac^-]_B/\Delta t$ during cycling exercise preceded by heavy exercise using different compared to the same muscle groups [8,14]. Since $\Delta[lac^-]_B/\Delta t$ depends upon the magnitude of $[lac^-]_B$ [11,26] we ensured that this was equal at the beginning of the incremental phase of LM_{ARM} and LM_{LEG}, and thus attribute the different responses to the effects of using the same or different muscle groups in the lactate elevation phase. The influence of the lactate elevation phase may partially explain why $\Delta[lac^-]_B/\Delta t$ during the incremental phase and constant power exercise failed to correlate (study 2). The lactate elevation phase may well "prime" the working muscles prior to the incremental phase, thus reducing metabolic inertia (i.e., the delay in oxidative metabolism at the onset of exercise [15]). Such priming only occurs when prior exercise involves the same muscle groups [8,14,19] and is predominantly manifest early in the subsequent exercise bout [10]. This is consistent with the data shown in **Fig. 3**, where differences in $\Delta[lac^-]_B/\Delta t$ between LM_{LEG} and LM_{ARM} were manifest during the initial stages of the incremental phase. Therefore, the lactate elevation phase of LM_{LEG}, unlike that of LM_{ARM}, probably increased the aerobic contribution to the energy demand during the initial stages of the incremental phase, thus resulting in less lactate accumulation and greater lactate oxidation [5,10,15]. This is supported by Simões et al. [24] who compared responses to the incremental phase with and without prior exercise and found elevated oxygen uptake and reduced respiratory exchange ratio in the former condition.

In summary, our modified lactate minimum test protocol provides valid measures of both maximal oxygen uptake and MLSS. However, $\Delta[lac^-]_B/\Delta t$ during the incremental phase fails to reflect that of constant power exercise, possibly because the dynamics of the lactate shuttle, and therefore the $[lac^-]_B$ response, are modified by the high-intensity exercise performed in the lactate elevation phase. Consequently, good agreement between lactate minimum and MLSS powers seems fortuitous and requires a physiological explanation different from that based on the proposition of Davis and Gass [11].

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References

1 *Atkinson G, Davison RCR, Nevill AM.* Performance characteristics of gas analysis systems: what we know and what we need to know. Int J Sports Med 2005; 26: 2–10
2 *Aunola S, Rusko H.* Does anaerobic threshold correlate with maximal lactate steady state? J Sports Sci 1992; 10: 309–323
3 *Bacon L, Kern M.* Evaluating a test protocol for predicting maximum lactate steady state. J Sports Med Phys Fitness 1999; 39: 300–308
4 *Baldari C, Guidetti L.* A simple method for individual anaerobic threshold as predictor of max lactate steady state. Med Sci Sports Exerc 2000; 32: 1798–1802
5 *Bangsbo J, Krustrup P, González-Alonso J, Saltin B.* ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. Am J Physiol Endocrinol Metab 2001; 280: 956–964
6 *Beneke R.* Anaerobic threshold, individual anaerobic threshold, and maximal lactate steady state in rowing. Med Sci Sports Exerc 1995; 27: 863–867
7 *Bland JM, Altman DG.* Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1: 307–310
8 *Bohnert B, Ward SA, Whipp BJ.* Effects of prior arm exercise on pulmonary gas exchange kinetics during high-intensity leg exercise in humans. Exp Physiol 1998; 83: 557–570
9 *Brooks GA.* Intra- and extra-cellular lactate shuttles. Med Sci Sports Exerc 2000; 32: 790–799
10 *Campbell-O'Sullivan SP, Constantin-Teodosiu D, Peirce N, Greenhaff PL.* Low intensity exercise in humans accelerates mitochondrial ATP production and pulmonary oxygen kinetics during subsequent more intense exercise. J Physiol 2002; 538: 931–939
11 *Davis HA, Gass GC.* Blood lactate concentrations during incremental work before and after maximum exercise. Br J Sports Med 1979; 13: 165–169
12 *Dekerle J, Baron B, Dupont L, Vanvelcenaher J, Pelayo P.* Maximal lactate steady state, respiratory compensation threshold and critical power. Eur J Appl Physiol 2003; 89: 281–288
13 *Denadai BS, Higino WP.* Effect of the passive recovery period on the lactate minimum speed in sprinters and endurance runners. J Sci Med Sport 2004; 7: 488–496
14 *Fukuba Y, Hayashi N, Koga S, Yoshida Y.* VO_2 kinetics in heavy exercise is not altered by prior exercise with a different muscle group. J Appl Physiol 2002; 92: 2467–2474
15 *Gurd BJ, Peters SJ, Heigenhauser GJF, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM.* Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. J Physiol 2006; 577: 985–996

16 *Johnson MA*, *Sharpe GR*, *MacConnell AK*. Maximal voluntary hyperpnoea increases blood lactate concentration during exercise. Eur J Appl Physiol 2006; 96: 600–608
17 *Jones AM*, *Doust JH*. The validity of the lactate minimum test for determination of the maximal lactate steady state. Med Sci Sports Exerc 1998; 30: 1304–1313
18 *Kilding AE*, *Jones AM*. Validity of a single-visit protocol to estimate the maximum lactate steady state. Med Sci Sports Exerc 2005; 37: 1734–1740
19 *Koppo K*, *Jones AM*, *Bouckaert J*. Effect of prior heavy arm and leg exercise on VO_2 kinetics during heavy leg exercise. Eur J Appl Physiol 2003; 88: 593–600
20 *Laplaud D*, *Guinot M*, *Favre-Juvin A*, *Flore P*. Maximal lactate steady state determination with a single incremental test exercise. Eur J Appl Physiol 2006; 96: 446–452
21 *MacIntosh BR*, *Esau S*, *Svedahl K*. The lactate minimum test for cycling: estimation of the maximal lactate steady state. Can J Appl Physiol 2002; 27: 232–249
22 *MacLoughlin P*, *Popham P*, *Linton RAF*, *Bruce RCH*, *Band DM*. Use of arterialised venous blood sampling during incremental exercise tests. J Appl Physiol 1992; 73: 937–940
23 *Ribeiro L*, *Balikian P*, *Malachias P*, *Baldissera V*. Stage length, spline function and lactate minimum swimming speed. J Sports Med Phys Fitness 2003; 43: 312–318
24 *Simões HG*, *Denadai BS*, *Baldissera V*, *Campbell CSG*, *Hill DW*. Relationships and significance of lactate minimum, critical velocity, heart rate deflection and 3000 m track-tests for running. J Sports Med Phys Fitness 2005; 45: 441–451
25 *Smith CGM*, *Jones AM*. The relationship between critical velocity, maximal lactate steady-state velocity and lactate turnpoint velocity in runners. Eur J Appl Physiol 2001; 85: 19–26
26 *Smith MF*, *Balmer J*, *Coleman DA*, *Bird SR*, *Davison RCR*. Method of lactate elevation does not affect the determination of the lactate minimum. Med Sci Sports Exerc 2002; 34: 1744–1749
27 *Svedahl K*, *MacIntosh BR*. Anaerobic threshold: the concept and methods of measurement. Can J Appl Physiol 2003; 28: 299–323
28 *Tegtbur U*, *Busse MW*, *Braumann KM*. Estimation of an individual equilibrium between lactate production and catabolism during exercise. Med Sci Sports Exerc 1993; 25: 620–627
29 *Urhausen A*, *Coen B*, *Weiler B*, *Kindermann W*. Individual anaerobic threshold and maximum lactate steady state. Int J Sports Med 1993; 14: 134–139