THE RESPIRATORY MUSCLES: RESPONSES TO TRAINING AND HEAVY ENDURANCE EXERCISE

MICHAEL A. JOHNSON

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ABSTRACT

Mechanisms that underlie the ergogenic effects of pressure-threshold inspiratory muscle training (IMT) remain unclear, and whether the respiratory muscles contribute to the blood lactate concentration ($[lac^-]_B$) of heavy endurance exercise has yet to be evaluated using a rigorous experimental design. Accordingly, this thesis evaluated: (I) the effects of IMT upon endurance exercise performance; (II) the mechanisms by which IMT improves performance; and (III) the contribution of respiratory muscle work to the $[lac^-]_B$ of heavy endurance exercise. Additionally, as an essential methodological precursor to these primary empirical studies, this thesis also evaluated the theoretical validity and protocol dependency of the lactate minimum test.

The lactate minimum test underestimated maximal lactate steady state (MLSS) power by, on average, 12 W. Temporal changes in $[lac^-]_B$ during the lactate minimum test did not reflect changes observed during constant power exercise, thus suggesting a flaw in the theoretical basis of the test. Furthermore, the lactate minimum power and temporal changes in $[lac^-]_B$ were dependent upon several facets of the test protocol, including the muscle groups used to elevate $[lac^-]_B$, the starting power of the incremental phase, and whether intra-stage rest intervals were included during the incremental phase.

25 km cycling time-trial performance improved 3.0% following 6 weeks IMT in cyclists, and performance enhancements exceeded changes observed in a sham hypoxic training placebo group. However, although enhanced time-trial performance was accompanied by a reduced $[lac^-]_B$ and perceptual response to exercise, it was not explained by a (measurable) increase in maximal sustainable power output, as defined by the critical power function of the hyperbolic relationship between power and time to exercise intolerance.

A 1.0 mmol·l^{-1} (+25%) increase in $[lac^-]_B$ was observed when isocapnic volitional hyperpnoea at a level commensurate with maximal exercise minute ventilation ($\dot{V}_E$ max) was superimposed on exercise at MLSS. These findings are the first to suggest that the respiratory muscles of humans make a significant contribution to the $[lac^-]_B$ of heavy endurance exercise.

A 0.77 mmol·l^{-1} increase in $[lac^-]_B$ was observed when $\dot{V}_E$ max and the associated breathing pattern were mimicked under isocapnic resting conditions, thus suggesting that at least part of the increase in $[lac^-]_B$ when volitional hyperpnoea is superimposed on exercise results from lactate efflux from respiratory muscles.

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ABBREVIATIONS USED IN THIS THESIS

ATP = adenosine triphosphate

ATPase = adenosine triphosphatase

ATPS = ambient temperature and pressure, saturated with water vapour

BE_{ECF} = base excess of the extracellular fluid

BTPS = body temperature and pressure, saturated with water vapour

$C(a\text{-}vDO_2)$ = arteriovenous oxygen content difference

CO_2 = carbon dioxide

d = day

EIAH = exercise-induced arterial hypoxaemia

FEV_1 = forced expiratory volume in 1 s

F_ICO_2 = fractional concentration of carbon dioxide in inspired air

F_IO_2 = fractional concentration of oxygen in inspired air

f_R = respiratory frequency

FRC = functional residual capacity

FVC = forced vital capacity

h = hour(s)

$[H^+]$ = hydrogen ion concentration

$[HCO_3^-]$ = bicarbonate concentration

HR = heart rate

Hz = hertz – equal to one cycle per second

kJ = kilojoule(s)

km = kilometre

K_M = Michaelis-Menton constant

$[lac^-]_B$ = blood lactate concentration

MEP = maximal expiratory mouth pressure

min = minute(s)

MIP = maximal inspiratory mouth pressure

MLSS = maximal lactate steady state

MSNA = muscle sympathetic nerve activity

MVV = maximal voluntary ventilation (subscripted number following abbreviation denotes duration (s) of MVV manoeuvre

O_2 = oxygen

$P(A\text{-}a)O_2$ = alveolar-to-arterial oxygen difference

Pab = abdominal pressure

$PACO_2$ = partial pressure of carbon dioxide in alveolus

$PaCO_2$ = partial pressure of carbon dioxide in arterial/arterialised venous blood

PAO_2 = partial pressure of oxygen in alveolus

PaO_2 = partial pressure of oxygen in arterial/arterialised venous blood

PCO_2 = partial pressure of carbon dioxide

Pdi = transdiaphragmatic pressure

PEF = peak expiratory flow rate

pH = negative logarithm (base 10) of the hydrogen ion concentration

PIF = peak inspiratory flow rate

PO_2 = partial pressure of oxygen

Poes = oesophageal pressure

Ppl = pleural pressure

$\dot{Q}$ = cardiac output per unit time

RER = respiratory exchange ratio

RMT = respiratory muscle training

RPE = rating of perceived exertion

RV = residual volume

s = second

SaO_2 = oxygen saturation of arterial/arterialised venous blood

STPD = standard temperature (0°C) and pressure (760 mmHg), dry

SV = stroke volume

T_I/T_{tot} = inspiratory time/total breath time (duty cycle)

TLC = total lung capacity

T_{lim} = limit time (time to volitional exercise intolerance)

$\dot{V}_A$ = alveolar ventilation per minute

VC = vital capacity

$\dot{V}CO_2$ = rate of carbon dioxide production

$\dot{V}_E$ = volume of expired air per minute (minute ventilation)

$\dot{V}O_2$ = rate of oxygen consumption

$\dot{V}O_2$ peak = peak rate of oxygen consumption

V_T = tidal volume

W = watt(s)

wk = week

W_{lim} = limit work (maximum work accomplished at exercise intolerance)

$\dot{W}$ max = maximal aerobic power output

STATISTICAL NOTATIONS

ANOVA = analysis of variance

CV = coefficient of variation

n = number of observations

SEE = standard error of the estimate

SEM = standard error of the mean

SD = standard deviation

r = Pearson's product moment correlation coefficient

P = probability

CHAPTER 1

GENERAL INTRODUCTION

1.1 THE PULMONARY SYSTEM: GENERAL OVERVIEW

The pulmonary system is comprised of two primary components: (I) a gas-exchange organ, represented by the lungs; and (II) a pump unit that ventilates the lungs, represented by the respiratory muscles and the chest wall. The primary function of the pulmonary system is to maintain homeostasis of arterial blood gases and arterial pH through supplying oxygen to the body and facilitating the removal of carbon dioxide. In healthy humans performing whole-body exercise pulmonary gas exchange is accomplished with great precision, even though the transit time of blood perfusing the pulmonary capillaries declines due to an increase in cardiac output. For example, during moderate exercise $PaCO_2$ is maintained close to resting levels (approximately 40 mmHg), whereas during heavy and maximal exercise, despite the PCO_2 of mixed venous blood increasing to >80 mmHg, compensatory hyperventilation may actually reduce $PaCO_2$ to as low as 30-35 mmHg (Dempsey et al. 1984; Wasserman et al. 1967). Similarly, despite heavy endurance exercise causing a marked decline in the PO_2 of mixed venous blood, healthy individuals maintain SaO_2 close to the resting value of approximately 98%, and only in trained endurance athletes is an exercise-induced arterial hypoxaemia (i.e. a drop in PaO_2 >10 mmHg or a drop in SaO_2 to below 95%) occasionally observed (Dempsey and Wagner 1999; Prefaut et al. 2000). The homeostatic control of blood gases thus requires precise regulation of $\dot{V}_A$ in order to maintain PAO_2 and $PACO_2$ within narrow limits. To accord with these demands, respiratory muscle activity is highly coordinated with neural and sensory systems of ventilatory control (Wetter and Dempsey 2000), and during exercise the mechanical action of the respiratory muscles appears to be coordinated in such a manner as to minimise the work of breathing (Aliverti et al. 1997).

Respiratory muscle work can be partitioned into 2 categories: (I) elastic work, which involves changing anatomical structures associated with the thorax; and (II) resistive

work, which is required to overcome resistance to airflow in the airways (Sheel et al. 2004). During high-intensity exercise, $\dot{V}_E$ may exceed 200 $l \cdot min^{-1}$ in endurance athletes (Secher 1993). An exercise hyperpnoea of this magnitude requires marked changes in intrathoracic pressure and flow rate, which are accomplished through increasing respiratory muscle activity (Johnson et al. 1992). Although healthy respiratory muscles possess adequate capacity to meet such ventilatory requirements (Johnson et al. 1993), there is a substantial physiologic cost in terms of respiratory muscle perfusion and oxygen consumption (Aaron et al. 1992; Harms et al. 1998b).

Whether the healthy pulmonary system limits oxygen transport and utilisation and carbon dioxide transport and elimination during endurance exercise was an issue of great debate around 20 years ago (Bye et al. 1983; Dempsey 1986), primarily because of a lack of empirical evidence that provided answers to untested hypotheses. However, based on the results of more recent studies that have examined, primarily, cardio-pulmonary interactions, lung mechanics, pulmonary gas exchange, respiratory muscle recruitment patterns and fatigue, and responses of humans to specific respiratory muscle training, new perspectives have emerged. The inference derived from these studies is that the healthy pulmonary system may indeed limit endurance exercise performance (Sheel et al. 2004; Wetter and Dempsey 2000). Furthermore, although poorly understood, dyspnoea is becoming increasingly recognised as an additional factor that may constrain human exercise performance (Harms et al. 2000). However, although our understanding of pulmonary function during exercise has advanced considerably in recent years, many questions remain unanswered. In particular, the effects of respiratory muscle training on endurance exercise performance and the mechanism(s) by which performance may improve remain obscure, and whether human respiratory muscles contribute to the $[lac^-]_B$ of heavy endurance exercise warrants a more comprehensive exploration.

1.2 FUNCTIONAL ANATOMY OF THE RIB CAGE AND RESPIRATORY MUSCLES

A primary focus of this thesis was to evaluate the implications of respiratory muscle work during endurance exercise. It is thus appropriate to commence this chapter with an outline of: (I) the structures moved through respiratory muscle activity (i.e. the ribs), and (II) the functional anatomy of the respiratory muscles.

1.2.1 RIB CAGE

The thoracic vertebrae, the bony ribs, costal cartilages, and the sternum constitute the rib cage, and their arrangements determine respiratory displacements of the chest wall. Rib movement occurs around a fixed axis made from articulations with the vertebral bodies and transverse processes (Figure 1.1). The upper ribs have an axis almost parallel to the frontal plane of the body, thus during inspiration they move cranially and ventrally, the "pump-handle" movement. Conversely, because the lower ribs have an axis oriented dorsally, these ribs move laterally and cranially during inspiration, the "bucket-handle" movement. Finally, floating ribs lack costotransverse joints, thus they demonstrate great mobility around a wide range of axes. During inspiration, they flare open and backwards, the "calliper" movement (Osmond 1985).

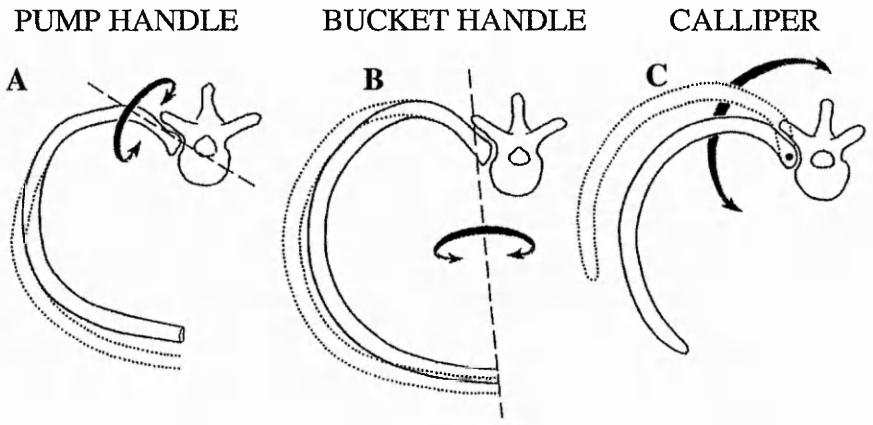

Figure 1.1 Axes and directions of movement of upper ribs (A), lower ribs (B), and lowest ribs (C). (From Osmond 1985).

1.2.2 DIAPHRAGM

The diaphragm is the primary inspiratory muscle in healthy humans and is anatomically described as a thin musculo-fibrous septum that partitions the thorax and abdomen (Figure 1.2). It consists of 3 main segments: (I) a non-contractile central tendon; (II) the costal diaphragm muscle; and (III) the crural diaphragm muscle (Poole et al. 1997). Because the costal and crural segments differ in embryological origin, nerve root innervation, mechanical action on the rib cage, and muscle fibre type, the diaphragm is essentially comprised of two discrete muscular portions (Figure 1.3) (Poole et al. 1997). The "zone of apposition" describes the region where the costal fibres, from their insertions, run cranially and appose the inner aspect of the lower rib cage. The diaphragm is innervated by the phrenic nerves, which are supplied by cervical nerve roots C3-C5 (De Troyer and Estenne 1988).

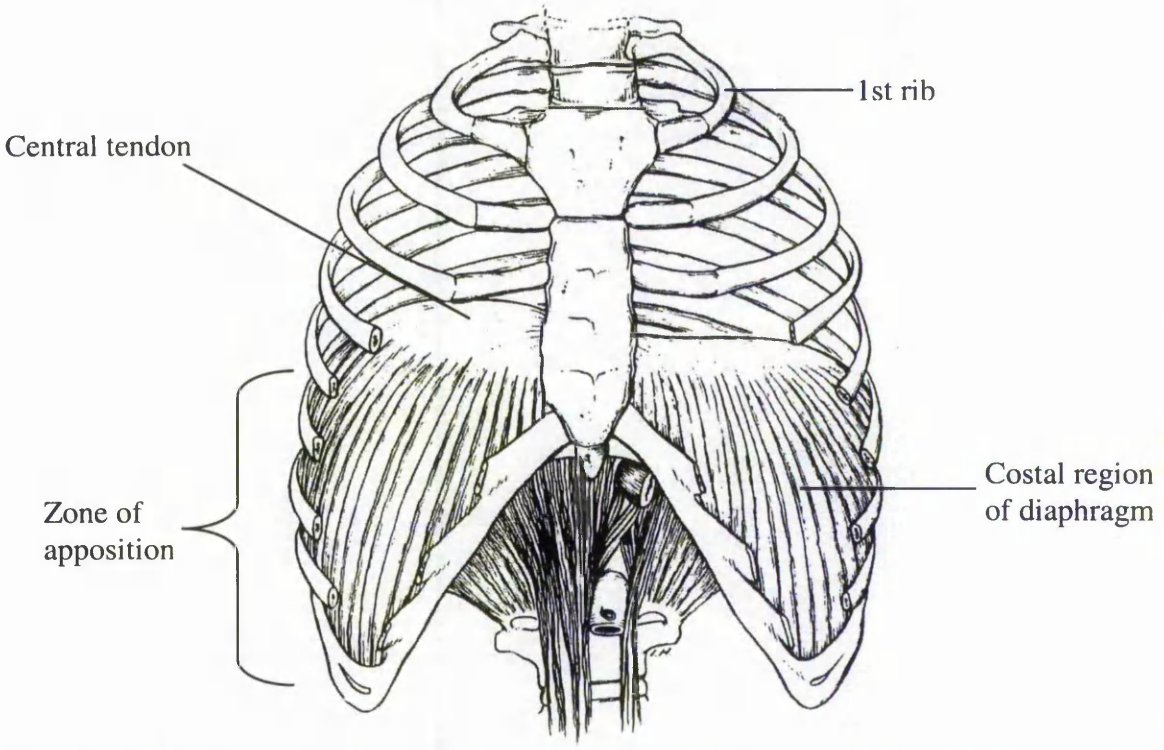

Figure 1.2 The human diaphragm at functional residual capacity. In this position the diaphragm resembles an elliptical cylindroid capped by a dome. (From Rochester et al. 1981).

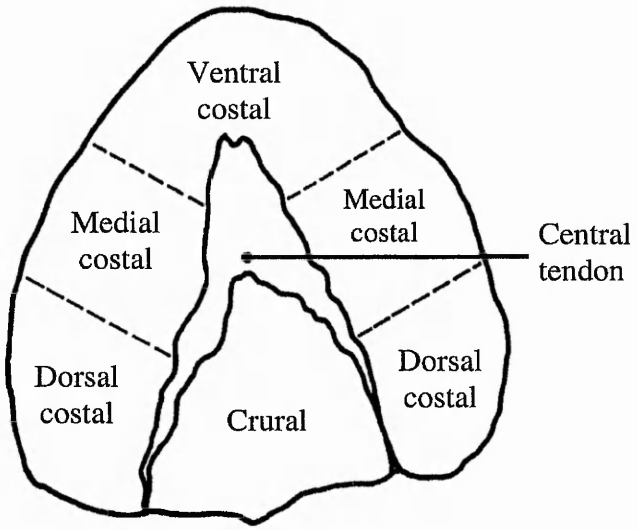

Figure 1.3 Superior view of the rodent diaphragm depicting the crural diaphragm and the ventral, medial, and dorsal regions of the costal diaphragm. (From Poole et al. 1997).

Diaphragm contraction causes a fall in Ppl and thus an increase in lung volume. Simultaneously, Pab increases and the abdominal wall is displaced ventrally. The mechanical action of the diaphragm on the rib cage has 2 components (De Troyer and Estenne 1988). The "appositional" component describes how Pab is conveyed through the zone of apposition when the diaphragm contracts, thus causing the lower rib cage to expand. The "insertional" component describes how the costal diaphragm applies a cranially orientated force onto the lower six ribs to which it inserts, thus causing them to rotate up and outwards. The abdominal viscera enhance this action through providing a solid fulcrum against which the diaphragm pushes in order to elevate the ribs.

1.2.3 INTERCOSTALS

The intercostals occupy the intercostal spaces and are anatomically described as internal and external (the latter being superficial to the former). The internal intercostals are further divided into parasternal and internal interosseous intercostals (De Troyer and Estenne 1988).

The parasternal intercostals (Figure 1.4) are phasically active during resting breathing, acting to elevate the attached ribs, thus they are primary inspiratory muscles (De Troyer and Estenne 1984). The action of the external and internal interosseous intercostals (Figure 1.5) on the ribs depends on: (I) the different moments that the muscle exerts on the attached upper and lower ribs; and (II) the resistance of the upper ribs to caudal motion relative to the resistance of the lower ribs to cranial motion (Wilson et al. 2001). Generally, the first point dictates that the majority of the external and internal interosseous intercostals are inspiratory and expiratory muscles, respectively, although note that only the former are active at rest (De Troyer and Estenne 1988). However, with regards to the second point, such resistances are not uniform throughout the rib cage, thus there exists topographic differences in the respiratory effects of the external and internal intercostals. Indeed, the external intercostals in the ventral half of the lower interspaces actually impart an expiratory effect on the rib cage (Wilson et al. 2001), thus it would be erroneous to describe all external intercostals as inspiratory.

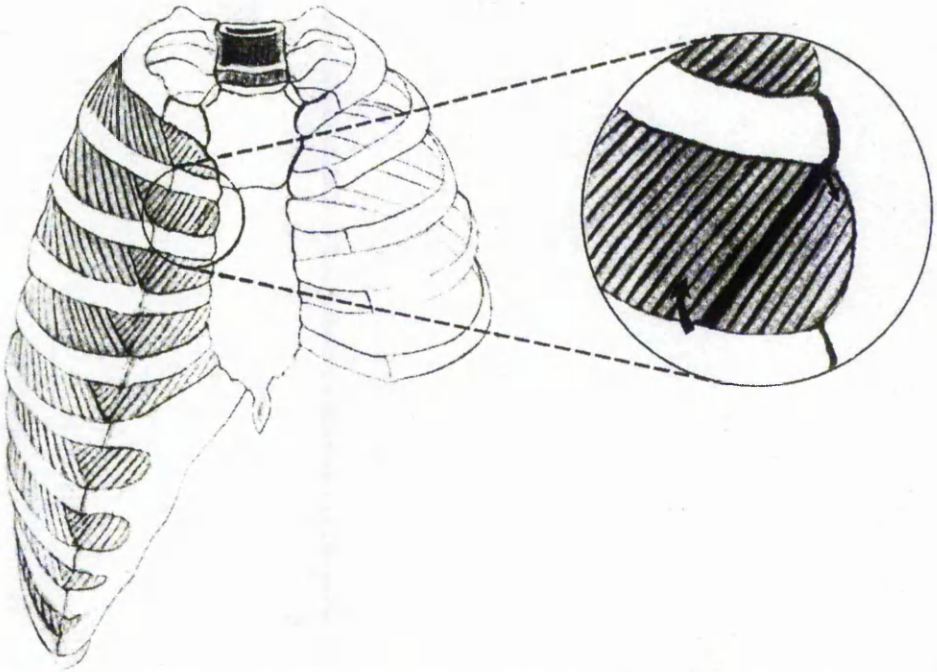

Figure 1.4 Functional anatomy of the parasternal intercostals. (From De Troyer and Estenne 1988).

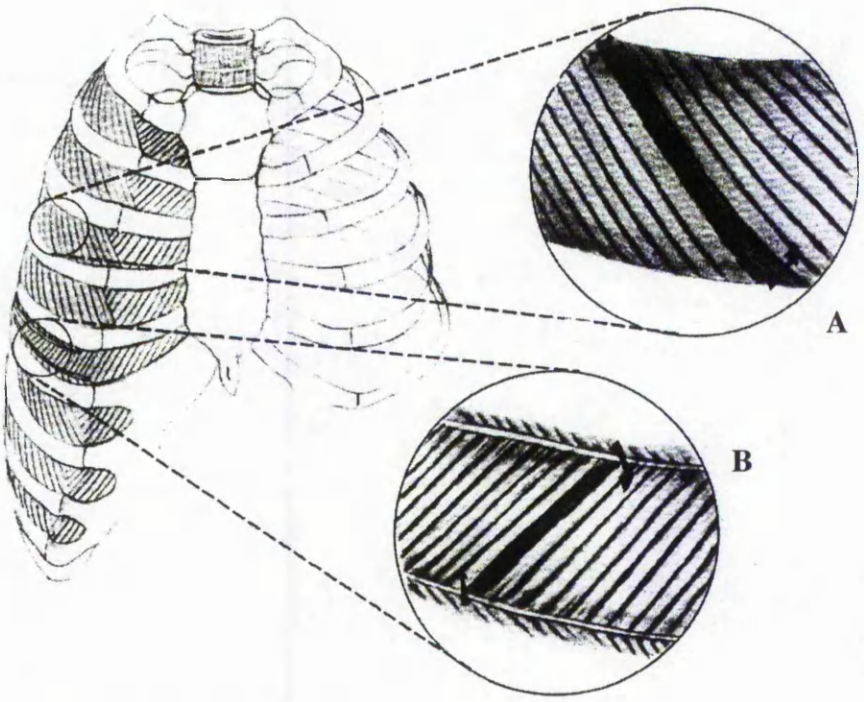

Figure 1.5 Functional anatomy of the external (A) and internal (B) interosseous intercostals. (From De Troyer and Estenne 1988).

1.2.4 SCALENES

The scalenes (Figure 1.6) are often classified as accessory inspiratory muscles (Cotes 1993; West 2000); however, studies utilising electromyographic recordings from needle electrodes have recorded phasic inspiratory activity by the scalenes during resting breathing in humans (De Troyer and Estenne 1984), which makes them primary inspiratory muscles. When active, the scalenes stabilise the upper rib cage, which is displaced inwards if the diaphragm contracts alone (De Troyer and Estenne 1984).

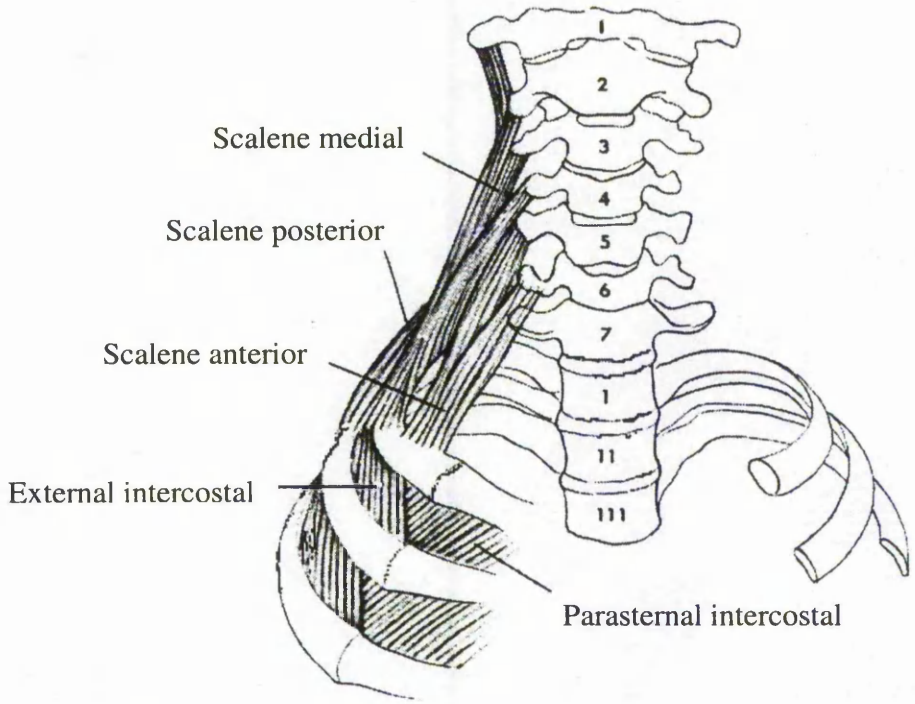

Figure 1.6 Anterior view of the upper rib cage depicting the scalenes. (From De Troyer and Estenne 1988).

1.2.5 Accessory respiratory muscles

When the ventilatory demand is increased, such as in exercise, accessory respiratory muscles are recruited. When V_T exceeds approximately 65% VC the sternomastoids (Figure 1.7), which are perhaps the most important accessory inspiratory muscles in humans, are recruited to assist expansion of the upper rib cage (Raper et al. 1966). The levator costae (Figure 1.8) show phasic inspiratory activity during quiet breathing, although these muscles are not prime movers since activity declines if lung volume is maintained above FRC (Goldman et al. 1985). The triangularis sterni (Figure 1.9) pulls the ribs caudally during expiration (De Troyer and Estenne 1988). The origins and insertions of other muscles suggest they may also have, albeit small, a respiratory function. For example, trapezius, serratus superior, and the pectoral muscles may exert an inspiratory effect on the rib cage, whereas serratus inferior may exert an expiratory effect (Loring and De Troyer 1985).

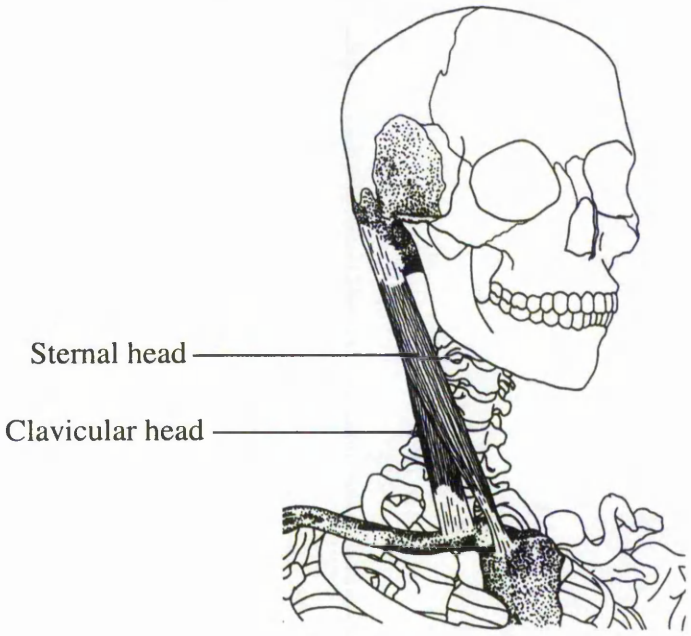

Figure 1.7 Three-quarter anterior view depicting the sternomastoids. (From Stone and Stone 2000).

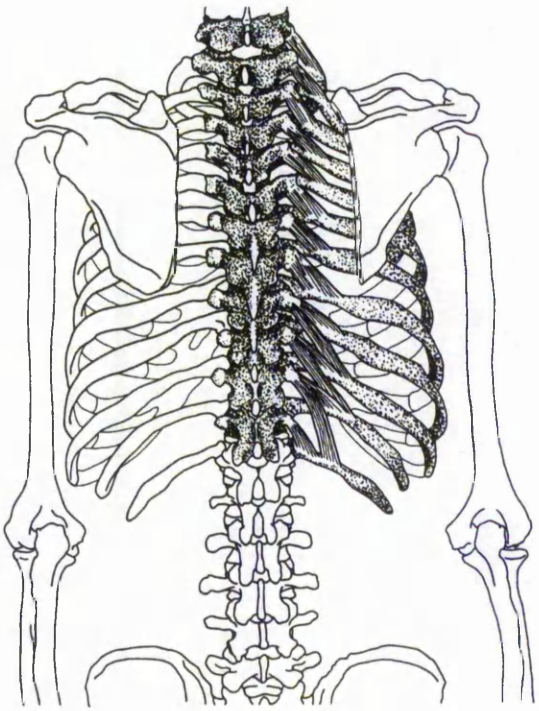

Figure 1.8 Posterior view of the trunk depicting the levator costae. (From Stone and Stone 2000).

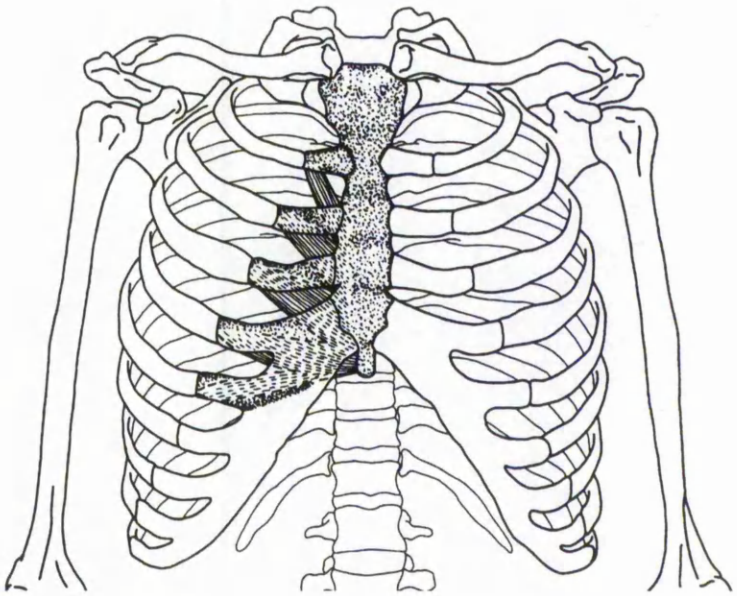

Figure 1.9 Anterior view of the trunk depicting the triangularis sterni. (From Stone and Stone 2000).

1.2.6 ABDOMINAL MUSCLES

Under resting conditions expiration is passive, relying on the release of elastic energy stored in the lung parenchyma, abdominal contents, and chest wall. However, during exercise there is increased recruitment of the abdominal muscles, which represent powerful muscles of expiration. The primary abdominal muscles that contribute to increasing $\dot{V}_E$ are rectus abdominis, external and internal obliques, and transversus abdominis (Figure 1.10) (De Troyer and Estenne 1988). Abdominal muscle contraction increases Pab and forces the abdominal contents and the diaphragm cranially. This displacement augments Ppl and reduces lung volume (De Troyer and Estenne 1988). Because of its circumferential orientation, transversus abdominis constricts the abdominal contents directly when active and is thus mechanically the most effective at increasing Pab and having an influential role on expiration and the operating length of the diaphragm (De Troyer et al. 1990). Abdominal muscle action may also assist inspiratory muscle function in two ways. Firstly, expiratory displacements of the diaphragm and rib cage lengthen the

inspiratory muscles to a more favourable portion of their length-tension curve, thus less activation generates a given V_T. Secondly, forcing the diaphragm cranially into the thoracic cavity can reduce lung volume below the passive end-expiratory volume, thus passive descent of the diaphragmatic dome at end-expiration increases lung volume prior to inspiratory activity (De Troyer and Estenne 1988).

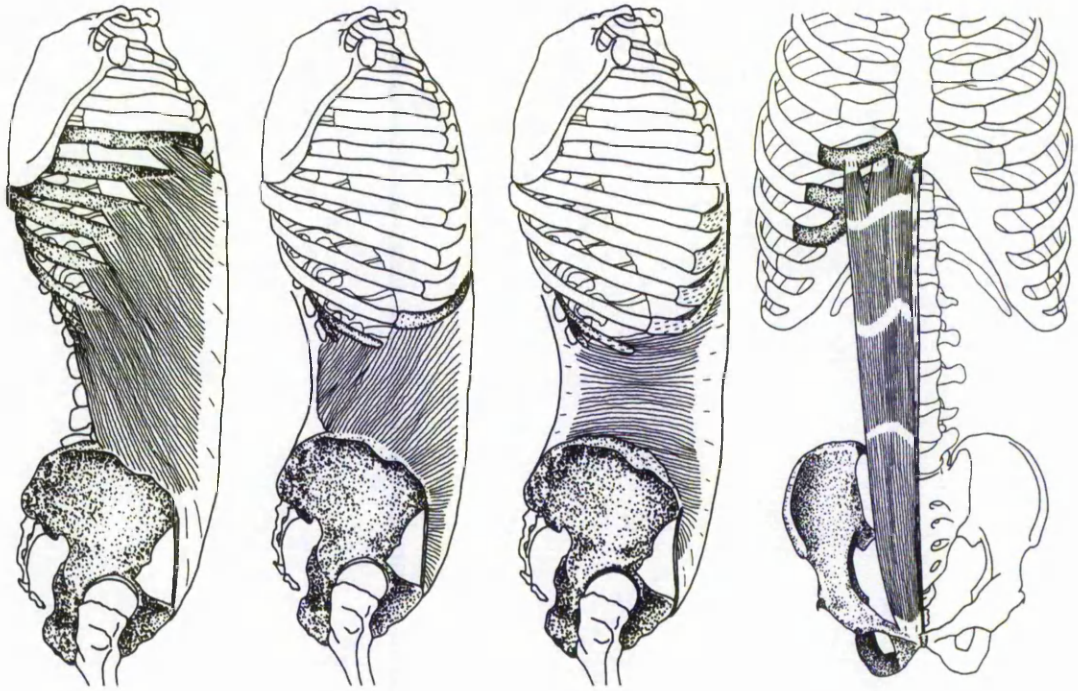

Figure 1.10 Muscles of the anterior abdominal wall. From left to right: external oblique, internal oblique, transversus abdominis, rectus abdominis. (From Stone and Stone 2000).

1.2.7 MORPHOLOGY OF THE RESPIRATORY MUSCLES

Using histochemical staining for myosin ATPase activity, the costal diaphragm of healthy males (post-mortem) aged 17-51 years has been shown to comprise 49% (SEM 3) type I, 28% (SEM 6) type IIA, and 23% (SEM 4) type IIX fibres, which is comparable to that reported for limb skeletal muscle (vastus lateralis) (Mizuno and Secher 1989). The external and internal intercostals comprise 62 (SEM 3) and 64% (SEM 3) type I, 17 (SEM 1) and 35% (SEM 3) type IIA, and 22 (SEM 2) and 1% (SEM 1) type IIX fibres,

respectively (Mizuno and Secher 1989). The sternomastoids and abdominal muscles comprise approximately 35 and 54% type I fibres, respectively, whereas the fibre type distribution of the scalenes is thought to resemble that reported for the external intercostals (Mizuno 1991). The preponderance of oxidative fibres (type I and type IIA) within the respiratory muscles provides many fatigue-resistant motor units to sustain $\dot{V}_E$ during periods of rest and exercise. Type IIX fibres are probably recruited for more forceful contraction such as trunk stabilisation during lifting manoeuvres (McCool et al. 1997), heavy exercise, sneezing and coughing (Edwards and Faulkner 1985). Although the percentage composition of type I fibres is higher in the intercostal muscles compared to the diaphragm, and despite diaphragm muscle fibre type composition being comparable to limb muscle, the diaphragm displays a greater capillary-to-fibre ratio, a smaller fibre area supplied by each capillary (Mizuno and Secher 1989), greater mitochondrial density (Hoppeler et al. 1981), and greater oxidative enzyme activities (Mizuno 1991). Thus as the only "essential" skeletal muscles the biochemical characteristics of the respiratory muscles bear greater resemblance to cardiac than locomotor muscles (Dempsey 1986).

With multiple arterial sources and a vast capillary network, the diaphragm is superbly adapted to accommodate substantial perfusion rates and exchange substances such as gases and energy substrates (Hussain 1996). Indeed, blood flow to the equine diaphragm may surpass 300 $ml \cdot min^{-1} \cdot 100g^{-1}$ during maximal exercise, which exceeds that observed for all other respiratory muscles and the primary locomotor muscles; in fact, only the myocardium receives greater blood flow (Manohar 1990; Manohar 1986). Venous drainage through the inferior phrenic, intercostal, and internal mammary veins also provides excellent removal rates of metabolic waste products (Hussain 1996), which could impair muscle function (Johnson et al. 1996).

1.3 BREATHING MECHANICS DURING EXERCISE

The mechanics of breathing are determined by the interactions of the lung, chest wall, and respiratory muscles. The work of breathing can be divided into 2 categories: (I) elastic work, which involves work done against lung elastic recoil, chest wall recoil, and surface tension; and (II) resistive work, which is required to overcome resistance to air flow in the airways (Sheel et al. 2004). The act of breathing is accomplished by respiratory muscle contraction, which causes changes in intrathoracic pressure, air flow rate and lung volume. During whole-body exercise the increase in $\dot{V}_E$ is achieved by increasing both f_R and V_T. During light and moderate intensity exercise $\dot{V}_E$ is increased primarily through increasing V_T, which encroaches into both inspiratory and expiratory reserve volumes, thus resulting in reduced and increased end-expiratory and end-inspiratory lung volumes, respectively. During more intense exercise V_T plateaus at approximately 60% of VC and further increases in $\dot{V}_E$ are achieved through increasing f_R (Wetter and Dempsey 2000). The advantage of initially increasing V_T is that it limits dead space ventilation. The expansion of V_T during progressive exercise is achieved by recruiting the diaphragm, inspiratory and expiratory rib cage muscles, and the abdominal muscles (Aliverti et al. 1997; Johnson et al. 1993).

From rest to exercise, however, there is a marked change in the pattern of respiratory muscle recruitment. Aliverti et al. (1997) and Kenyon et al. (1997) gauged respiratory muscle recruitment patterns at rest and during progressive cycling exercise at 0, 30, 50, and 70% $\dot{W}$ max by measuring changes in the shape and volume of the three chest wall compartments: (I) the lung-apposed rib cage compartment; (II) the diaphragm-apposed rib cage compartment; and (III) the abdomen. Note that prior to these investigations chest wall kinematics were typically analysed using Konno and Mead's (1967) two-compartment chest wall model composed of the rib cage and abdomen.

Because these compartments move in a somewhat unitary manner during resting breathing, the chest wall can be regarding as having just two ways to move, or two degrees of freedom. However, because the chest wall moves with more than two degrees of freedom during exercise (i.e. there is distortion of the chest wall), the two-compartment model is not appropriate for the study of chest wall kinematics during exercise (Aliverti et al. 1997; Grimby et al. 1976). Therefore, Aliverti et al. (1997) and Kenyon et al. (1997) used the two-compartment rib cage model, which considers that the lung and diaphragm-apposed rib cage compartments are exposed to substantially different pressures on their inner surface during inspiration, and that the diaphragm and inspiratory rib cage muscles act only on the diaphragm-apposed and largely on the lung-apposed parts of the rib cage, respectively. A three-dimensional optical reflectance motion analysis system was used to measure the volumes of the three chest wall compartments. Figure 1.11 shows the placement of the reflective markers on the chest wall and division of the chest wall into the three compartments. The boundary between the lung-apposed and diaphragm-apposed rib cage compartments was taken at the level of the xiphoid, whereas the boundary between the diaphragm-apposed rib cage compartment and the abdomen was along the lower costal margin anteriorly and at the level of the lowest point of the lower costal margin posteriorly. Pressures developed by the inspiratory and expiratory rib cage muscles were estimated as the difference between the dynamic Ppl-lung-apposed rib cage volume loop (Ppl determined from Poes measured using a balloon catheter) and the relaxation pressure-volume curve of the lung-apposed rib cage compartment. Pressure generation by the diaphragm was measured by Pdi (Pab – Ppl, where Pab is inferred from gastric pressure measured using a balloon catheter). Pressure developed by the abdominal muscles was measured as the difference between the dynamic Pab-abdominal volume loop and the relaxation pressure-volume curve of the abdomen.

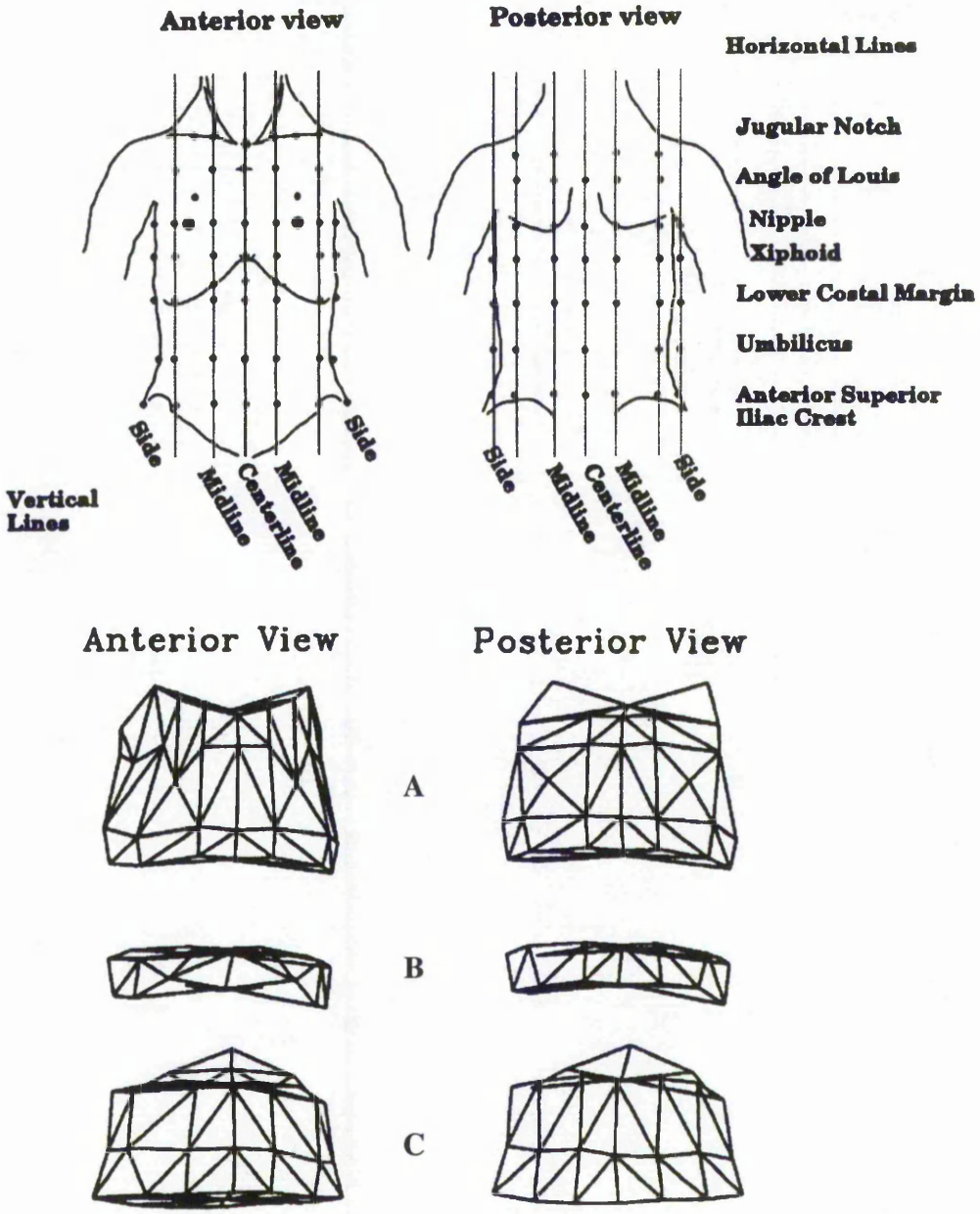

Figure 1.11 Top: placement of reflective markers on chest wall. Bottom: division of chest wall into three volume compartments: lung-apposed rib cage compartment (A), diaphragm-apposed rib cage compartment (B), and abdomen (C). (From Kenyon et al. 1997).

At rest the diaphragm produced the highest peak pressure (approximately 10 cmH_2O), whereas during exercise from 0-70% $\dot{W}$ max the abdominal muscles and inspiratory rib cage muscles produced the greatest peak pressures. At 70% $\dot{W}$ max the abdominal muscles, inspiratory rib cage muscles, diaphragm, and expiratory rib cage muscles produced peak pressures of approximately 39, 30, 19, and 10 cmH_2O,

respectively. From rest to exercise, there is thus a shift in the pattern of respiratory muscle tension development, which thereafter remains constant, but with increasing gain, as exercise intensity, and thus $\dot{V}_E$, increases (Aliverti et al. 1997). Figure 1.12 shows changes in chest wall volume (i.e. the sum of the three chest wall compartments) and changes in the volume of the three chest wall compartments during progressive exercise. As is evident, from 0-70% $\dot{W}$ max the end-expiratory volumes of the lung- and diaphragm-apposed rib cage compartments remained constant, whereas abdominal volume declined progressively. Thus the reduction in end-expiratory chest wall volume, and thus lung volume, was almost exclusively related to a decrease in end-expiratory abdominal volume. Conversely, end-inspiratory abdominal volume remained constant during progressive exercise, whereas the end-inspiratory volumes of the lung- and diaphragm-apposed rib cage compartments increased. Thus the increase in end-inspiratory lung volume was almost entirely due to rib cage expansion. These changes in thoraco-abdominal configuration during exercise are thought to reflect an innate tendency to coordinate the force and timing of both inspiratory and expiratory muscle activity in such a manner as to minimise rib cage distortion and the work of breathing (Aliverti et al. 1997; Kenyon et al. 1997). For example, the capacity of the diaphragm to generate force is critically dependent upon its operating length, which depends on lung volume and chest wall configuration. From TLC to FRC, the diaphragm lengthens and demonstrates an increasing ability to generate pressure, whereas the force-length relation is relatively flat over lengths between FRC and RV (Braun et al. 1982). Moreover, during an isovolume inspiratory manoeuvre, the diaphragm may shorten considerably going from the belly-in to belly-out configuration, thus demonstrating how chest wall configuration influences diaphragm length and subsequently its force generating capacity. Accordingly, decreasing end-expiratory chest wall volume through decreasing end-expiratory abdominal volume lengthens the diaphragm and places it on a more

favourable portion of its force-length curve, whereas a constant end-inspiratory abdominal volume prevents excessive diaphragm shortening during inspiration (Aliverti et al. 1997). Aliverti et al. (1997) highlight, however, that the optimal length of the inspiratory rib cage muscles for force (i.e. pressure) generation is shorter than the FRC length. Thus with increasing rib cage expansion during inspiration, the inspiratory rib cage muscles progressively approach their optimal length, whereas maintenance of the end-expiratory volumes of the lung- and diaphragm-apposed rib cage compartments during expiration prevents excessive lengthening of the inspiratory rib cage muscles prior to the succeeding inspiration.

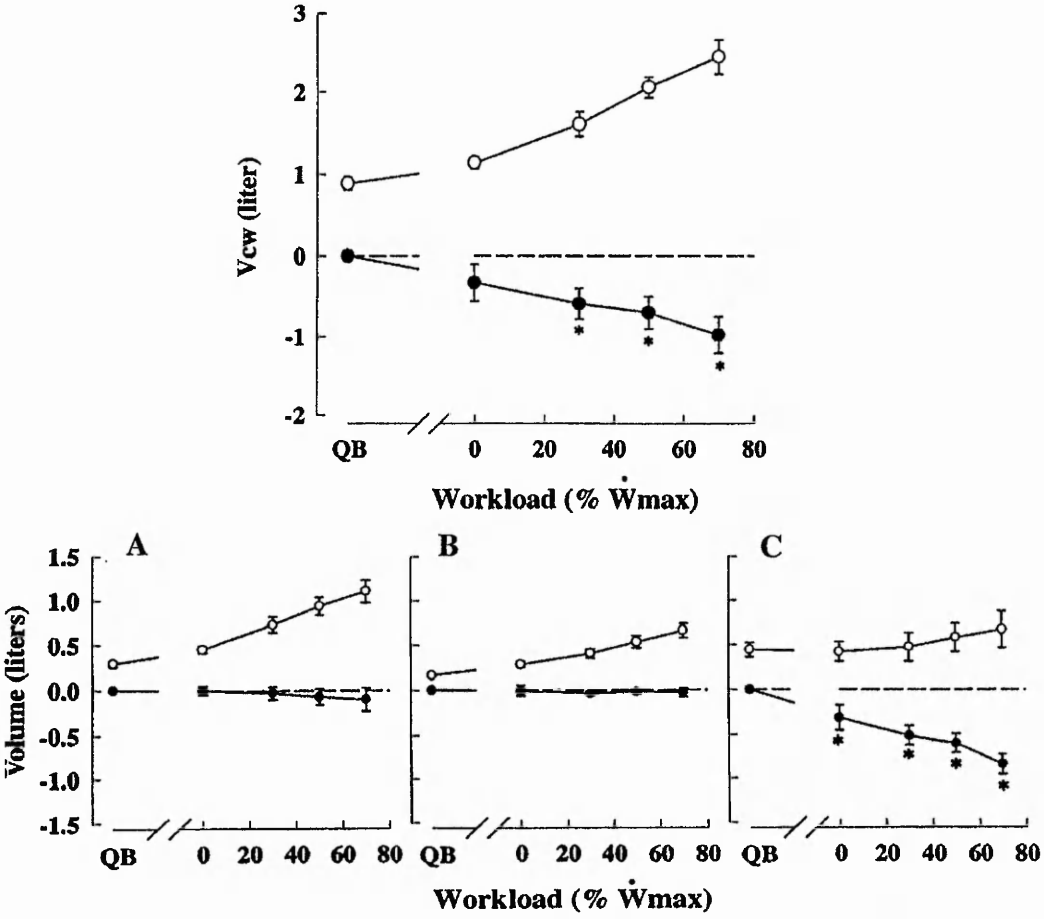

Figure 1.12 Top: Chest wall volume (Vcw) changes during progressive exercise. Difference between end-inspiratory (○) and end-expiratory (●) Vcw is tidal volume. Dashed horizontal line is end-expiratory Vcw during quiet breathing (QB), which was set to zero. Bottom: Changes in lung-apposed rib cage volume (A), diaphragm-apposed rib cage volume (B), and abdominal volume (C) during progressive exercise. All values are mean ± SEM. *Significantly different from end-expiratory volume during quiet breathing ($P < 0.05$). (From Aliverti et al. 1997).

However, note that the mechanical interactions described by Aliverti et al. (1997) and Kenyon et al. (1997) relate to leg cycling, and that they may not be entirely applicable to other modes of exercise, such as running, in which the motor control strategies for regulating $\dot{V}_E$ may differ due to the differing roles that various respiratory muscles may have in postural control, the different gravitational forces that may act on the abdominal wall, and the effects of repetitive torso movement. Indeed, compared to cycling there is greater tonic activation of the abdominal muscles during running (Henke et al. 1988), which probably contributes to postural control. Importantly, this tonic activation may modify abdominal wall compliance and perhaps diaphragmatic function. It is also unclear whether the minimum energy expenditure paradigm of ventilatory control is influenced by the locomotor-respiratory coupling that often accompanies exercise in which the act of locomotion tends to deform the thorax (Bramble and Carrier 1983).

The assertion that the spontaneously regulated ventilatory control system ensures a 'minimum work response' is further supported by studies comparing responses to voluntary and involuntary hyperpnoea. Klas and Dempsey (1989) had 5 subjects voluntarily mimic under resting conditions the flow-volume loop, duty cycle, and end-expiratory lung volume demonstrated during maximal exercise (4 subjects cycling, 1 running). The work of breathing was defined as the integrated area of the Poes-volume loop. [Note that the common definition of work is the product of a force applied through a distance. In a fluid system, however, the force is measured in terms of the pressure applied and displacement in terms of any volume change (Milic-Emili 1991)]. During maximal exercise, subjects met or only slightly exceeded the effort-independent portion of the maximal expiratory flow-volume loop (described below), and the maximal effective expiratory pressures were also either met or only slightly exceeded. However, during the mimic trial Poes throughout most of expiration exceeded that achieved spontaneously

during exercise by 5-15 cmH_2O. Therefore, despite the presence of expiratory flow limitation (described below), subjects were producing ineffective, wasteful expiratory pressures, subsequently resulting in a 15-40% increase in the work of breathing per breath compared to that observed in exercise. These data are consistent with the idea that in the spontaneous ventilatory control system expiration is reflexively terminated, perhaps due to the influence of airway mechanoreceptors, when maximal effective expiratory pressures are reached or approached, thus avoiding dynamic compression of airways (Pellegrino et al. 1993). However, when the cortex exerts voluntary control over hyperpnoea, such regulation is overridden and the work of breathing is increased. Klas and Dempsey (1989) speculate that the inefficiency of voluntary hyperpnoea may be explained, in part, by insufficient "learning" of the respiratory muscle effort required for a given ventilatory load. This theory is consistent with observations on healthy subjects who demonstrated gradual increases in MIP and inspiratory muscle endurance during threshold loading when tests were repeated on successive days (Clanton et al. 1987; Eastwood et al. 1998). Such improvements suggest a learning effect whereby coordination of the respiratory muscles is improved to achieve a more efficient breathing pattern.

In another study, Coast et al. (1993) compared the work of breathing (established from integrated Poes-volume loops) and respiratory muscle $\dot{V}O_2$ under 3 conditions: (I) voluntary isocapnic hyperpnoea at rest with $\dot{V}_E$ elevated to 20, 40, 70, and 100 $l \cdot min^{-1}$; (II) leg cycling exercise at intensities sufficient to increase $\dot{V}_E$ close to that observed in condition I; and (III) voluntary isocapnic hyperpnoea at rest with $\dot{V}_E$, V_T, f_R, and duty cycle matched to that demonstrated during exercise. It was found that when the breathing pattern during voluntary hyperpnoea was matched with the breathing pattern associated with exercise, the work of breathing and respiratory muscle $\dot{V}O_2$ was not different between the two conditions. Conversely, when subjects adopted a self-selected breathing

pattern during voluntary hyperpnoea, the work of breathing and respiratory muscle $\dot{V}O_2$ was greater than in exercise, with this difference becoming greater with increases in $\dot{V}_E$. More specifically, at a $\dot{V}_E$ of 60 l·min^{-1} the difference in the work of breathing between exercise and voluntary hyperpnoea was negligible. Conversely, if $\dot{V}_E$ were increased to 120 l·min^{-1} the work of breathing and respiratory muscle $\dot{V}O_2$ would be predicted to be greater during voluntary hyperpnoea compared to exercise by approximately 29% and 70 ml·min^{-1}, respectively. Unlike Klas and Dempsey's (1989) observations these results were not explained by excessive expiratory pressures since Poes-volume loops were not greatly different between the two conditions. Instead, the increased work of breathing and respiratory muscle $\dot{V}O_2$ was attributed to a more tachypnoeic breathing pattern during voluntary hyperpnoea (V_T being around 1.8 and 1.3 l during exercise and voluntary hyperpnoea, respectively). A greater f_R for a given $\dot{V}_E$ implies that greater inspiratory and expiratory airflow rates, and thus by virtue greater velocities of respiratory muscle shortening, are being achieved. Because the energy requirements of muscle depend upon the tension generated, the energy of activation, and the velocity of muscle shortening, an increase in the velocity of respiratory muscle shortening is likely to increase the metabolic cost of respiratory muscle action (Collett et al. 1985; McCool et al. 1986). Further evidence that increasing inspiratory flow rate augments the work of breathing is provided by studies demonstrating decreased inspiratory muscle endurance against a constant inspiratory resistive or pressure-threshold load when inspiratory flow is increased (Clanton 1995). Why the subjects in Coast et al.'s (1993) study did not generate excessive expiratory pressures during voluntary hyperpnoea, whereas those in Klas and Dempsey's (1989) study did, is probably explained by the different levels of $\dot{V}_E$: Coast et al. (1993) used levels of $\dot{V}_E$ that could be maintained during submaximal steady state exercise (≤100

$l \cdot min^{-1}$), whereas Klas and Dempsey (1989) used maximal exercise $\dot{V}_E$ (range 106 – 179 $l \cdot min^{-1}$). In the former scenario in which submaximal exercise $\dot{V}_E$ is mimicked, the tidal flow-volume loop is likely to remain within the confines of the maximal flow-volume loop, thus if expiratory pressures were increased under these conditions, flow rate and volume would also increase and the target $\dot{V}_E$ would no longer be mimicked. It is thus reasonable to presume that had Coast et al. (1993) measured the work of breathing at higher levels of $\dot{V}_E$ where the tidal flow-volume loop intersected the maximal flow-volume loop, then the work of breathing would have been greater, regardless of whether breathing pattern was controlled.

As an increase in f_R becomes the dominant strategy for further increasing $\dot{V}_E$ during heavy exercise, inspiratory and expiratory flow rates are increased, and inspiratory and expiratory durations decline. The highest flow rates that can be generated thus predominantly determine the highest achievable $\dot{V}_E$. However, greater flow rates result in increased turbulent airflow, which increases flow-resistive work (Wetter and Dempsey 2000). Trained endurance athletes achieve a greater $\dot{V}_E$ during heavy endurance exercise compared to sedentary or less well-trained individuals (Babcock et al. 1996). These greater ventilatory demands are not, however, matched by a training-induced increase in the maximal flow-volume loop (Johnson et al. 1992), which represents the relationship between maximal possible flow rate and lung volume across the range of volume from TLC to RV. In sedentary and moderately trained individuals tidal flow-volume loops during even maximal exercise usually remain within the confines of the maximal flow-volume loop (Babcock et al. 1996; Olafsson and Hyatt 1969). However, in endurance trained male athletes capable of generating a $\dot{V}_E$ during maximal exercise that may be twofold greater than that of sedentary subjects, there is often a degree of overlap between

expiratory tidal and maximal flow-volume loops (Johnson et al. 1992; McClaran et al. 1999) (Figure 1.13). This phenomenon is termed expiratory flow limitation, and is thought to represent dynamic narrowing or closure of airways during forceful expiratory efforts sufficient to cause an increase in intra-thoracic pressure above atmospheric. Consequently, transmural pressure across the airway increases and narrows the airway (West 2000). The maximal flow-volume loop during expiration thus defines the boundary to maximal flow rate at any lung volume. In other words, expiratory flow limitation indicates that the maximum effective expiratory pressure (i.e. that beyond which further pressure development does not increase expiratory flow) over a given lung volume has been attained, and that further expiratory effort would be 'wasteful'. Maximal expiratory flow decreases with lung volume because the difference between alveolar and intrapleural pressure decreases and the airways become narrower (West 2000).

In male endurance athletes expiratory flow limitation is usually observed only in heavy exercise (Figure 1.13). For example, Johnson et al. (1992) failed to detect expiratory flow limitation in male endurance athletes [$\dot{V}O_2$peak = 73 (SEM 1) $ml \cdot kg^{-1} \cdot min^{-1}$] running at intensities up to 61% $\dot{V}O_2$peak; however, at 83% $\dot{V}O_2$peak with $\dot{V}_E$ increased to 117 (SEM 7) $l \cdot min^{-1}$, there was a 24% (SEM 7) overlap between expiratory tidal and maximal flow-volume loops as lung volume approached end-expiration. During maximal exercise with $\dot{V}_E$ increased to 169 (SEM 5) $l \cdot min^{-1}$, 61% (SEM 9) of the expiratory tidal flow rate was flow limited, and in some cases there was a 75% overlap between expiratory tidal and maximal flow-volume loops.

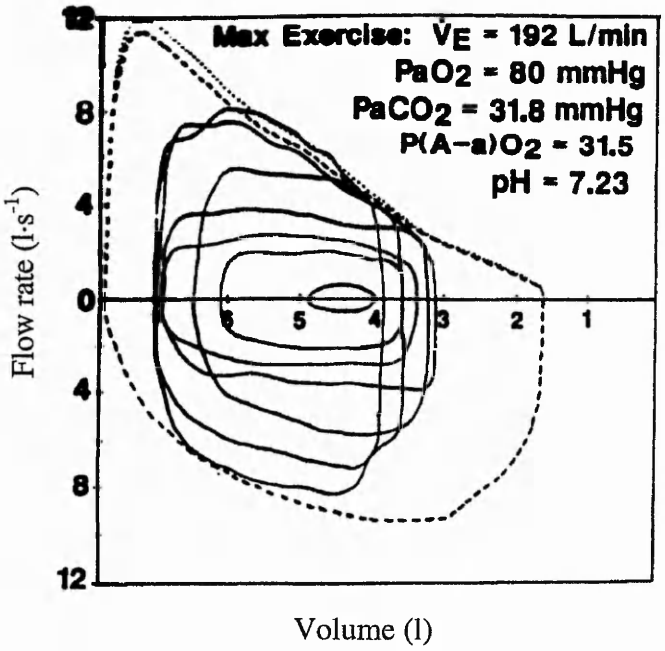

Figure 1.13 Tidal flow-volume loop for an individual subject ($\dot{V}O_2$ peak = 80 ml·kg^{-1}·min^{-1}) during moderate-through-maximal running exercise. Tidal loops are shown relative to the maximal flow-volume loop (dashed line). P(A-a)O_2, alveolar-to-arterial oxygen difference. (From Johnson et al. 1992).

The mechanical constraints presented by the airways are thought to limit exercise hyperpnoea, as evidenced by an unchanged $\dot{V}_E$ during maximal exercise when the chemoreceptor drive to breathe was increased through either dead space loading, hypercapnia (F_ICO_2 = 0.04-0.06), or hypoxia (F_IO_2 = 0.16) (Johnson et al. 1992; McClaran et al. 1999). Heliox (HeO_2) also provides a useful tool to examine the issue of whether expiratory flow limitation limits exercise hyperpnoea. Heliox is a low-density gas, and therefore reduces airway flow resistance and increases the maximal available flow-volume loop, thereby attenuating flow limitation during expiration. During maximal exercise in the presence of expiratory flow limitation [43% (SD 8) overlap between expiratory tidal and maximal flow-volume loops], competitive male cyclists demonstrated a $\dot{V}_E$ of approximately 170 l·min^{-1}, but were incapable of further increases in $\dot{V}_E$ when dead space loading (1 litre) was superimposed on exercise. However, when exercise was performed using a heliox inspirate $\dot{V}_E$ increased during dead space loading from approximately 185

to 210 $l \cdot min^{-1}$ (McClaran et al. 1999). These data provide evidence of expiratory flow limitation effectively limiting the ability of trained endurance athletes to increase $\dot{V}_E$ during heavy exercise. Interestingly, because of their smaller lung volumes, intrathoracic airway diameter, and lower maximal expiratory flow rates, female athletes are, compared to height-matched males, at increased risk for expiratory flow limitation (McClaran et al. 1998). If sufficient to constrain $\dot{V}_E$, this may contribute to excessive widening of the $P(A\text{-}a)O_2$ and partly contribute to the high prevalence, compared to men, of exercise-induced arterial hypoxaemia in female athletes (Harms et al. 1998a). Note, however, that mechanical constraints on hyperpnoea are never sufficient to cause retention of carbon dioxide in healthy subjects (men and women) (Johnson et al. 1992; McClaran et al. 1998).

As previously mentioned, it is thought that the onset of, or even impending, expiratory flow limitation, probably triggers a reflex termination of expiration, thus preventing the maximum effective expiratory pressure being exceeded (Pellegrino et al. 1993). As a consequence, end-expiratory lung volume progressively increases back towards the resting value. This response is paralleled by a gradual increase in end-inspiratory lung volume, which at maximal workloads may reach 85-100% TLC (Johnson et al. 1992; McClaran et al. 1999; Mota et al. 1999). Through moving V_T to a higher lung volume (i.e. hyperinflation) and thus away from the flow-limiting pressures attained at lower lung volumes, subjects are able to further increase expiratory flow rate and thus $\dot{V}_E$. However, hyperinflation increases the elastic load on the inspiratory muscles and places the diaphragm on a less advantageous portion of its length-tension curve. It also places the less efficient inspiratory rib cage muscles, which are increasingly recruited at high lung volumes, at a mechanical disadvantage (Brancatisano et al. 1993). Subsequently, because the maximum force that the inspiratory muscles can develop declines with increasing lung volume, for a given load a greater fraction of MIP would have to be developed by the

inspiratory muscles, thus increasing their metabolic demands (Collett and Engel 1986). Indeed, Aaron et al. (1992) noted the highest oxygen cost per litre of exercise $\dot{V}_E$ to occur in subjects with significant expiratory flow limitation. Forcing the inspiratory muscles to contract at a greater fraction of MIP would thus make them more susceptible to fatigue (Roussos et al. 1979; Tzelepis et al. 1988). Roussos et al. (1979) demonstrated the inspiratory mouth pressure as a fraction of MIP that could be maintained indefinitely to decline from approximately 60% MIP at FRC to approximately 30% MIP at FRC plus one-half inspiratory capacity. Note also that the capacity for inspiratory pressure generation is further attenuated during heavy exercise because of increased inspiratory flow rates (Johnson et al. 1992). Thus collectively, during moderate-through-maximal exercise the inspiratory muscles are 'functionally weakened': peak tidal inspiratory Ppl progressively reaches a greater fraction of the maximal dynamic capacity (Figure 1.14), and during maximal exercise the pressure generated by the inspiratory muscles may represent 80-100% of their capacity (Johnson et al. 1992; McClaran et al. 1999).

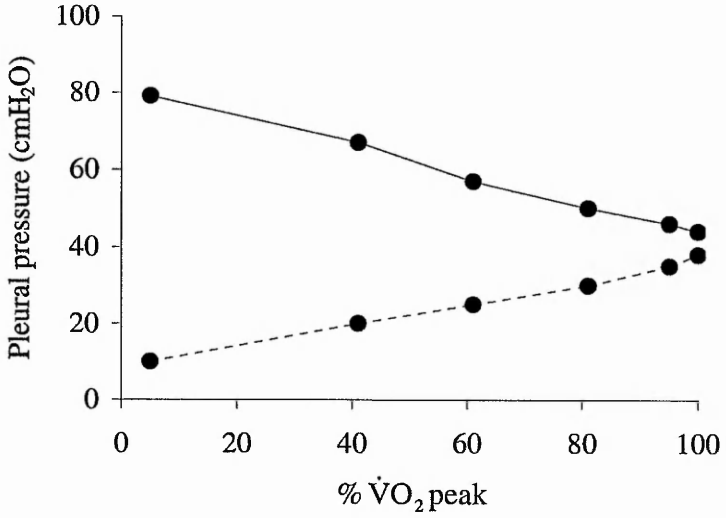

Figure 1.14 Peak inspiratory pleural pressure during tidal breathing (dotted line) and capacity of inspiratory muscles for pressure generation (solid line) with progressive exercise. Values are means from 8 endurance runners. (From Johnson et al. 1992).

1.4 CONSEQUENCES OF THE WORK OF BREATHING DURING EXERCISE

During short-term, heavy/maximal endurance exercise $\dot{V}_E$ commonly exceeds 200 $l \cdot min^{-1}$ in endurance athletes (Secher 1993). During more prolonged (30-60 min), submaximal exercise, including that which extends for more than 8 h (e.g. an ultra-marathon or iron-man event), $\dot{V}_E$ may be much lower (approximately 50-100 $l \cdot min^{-1}$), but the respiratory muscles are required to maintain this ventilatory output for a much longer duration. Under both conditions, the work of breathing may have significant physiological consequences (Ker and Schultz 1996; Perret et al. 2000), which may subsequently impact on exercise performance. The following subsections address the consequences of exercise hyperpnoea, and the implications for whole-body endurance exercise performance.

1.4.1 EXERCISE-INDUCED RESPIRATORY MUSCLE FATIGUE: CHANGES IN STRENGTH

In trained endurance athletes achieving a $\dot{V}_E$ of 200-250 $l \cdot min^{-1}$ (Secher 1993), the respiratory muscles are required to produce a power output of around 50 W (Milic-Emili 1991), which is substantial considering their small size. Furthermore, as highlighted above, the cumulative work performed by the respiratory muscles during prolonged submaximal exercise can be substantial despite a relatively low $\dot{V}_E$. A recognised consequence of such great breathing efforts is exercise-induced respiratory muscle fatigue, defined as "a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest" (National Heart, Lung, and Blood Institute 1990).

Due to the location of the respiratory muscles, objective measures of respiratory muscle fatigue are rarely adopted because they involve invasive techniques that give rise to technical and/or ethical constraints. Therefore, non-invasive volitional measures of respiratory muscle function, such as MIP/MEP, pre- and post-exercise are more commonly

employed. Using MIP as an index of global inspiratory muscle strength Loke et al. (1982) reported a decline in MIP from 166 (SD 11) to 138 (SD 8) cmH_2O (-16%) in endurance runners following a marathon (42.2 km, mean exercise time = 3 h 24 min). Similarly, competitive female rowers experienced an 11.2% (SD 2.6) decrement in MIP from a baseline value of 104 (SD 8) cmH_2O following a 6 min all-out rowing exercise (Volianitis et al. 2001). From baseline MIP's of 104.9 (SEM 8.3) and 99.8 (SEM 7.9) cmH_2O, trained cyclists demonstrated 18 (SEM 2) and 14% (SEM 2) reductions following 20- and 40-km time-trials, respectively, and values remained below baseline during 30 min recovery (Romer et al. 2002a). Collectively, these studies demonstrate the susceptibility of even highly trained endurance athletes to global exercise-induced inspiratory muscle fatigue. The severity of respiratory muscle fatigue is also influence by exercise modality. A study performed on triathletes showed greater reductions in MIP and inspiratory muscle endurance capacity following cycling compared to running, despite both activities being performed at the same metabolic intensity (Boussana et al. 2001). Hill et al. (1991) suggest that the crouched position of cycling increases abdominal impedance and thus diaphragmatic work, which may contribute to greater diaphragmatic fatigue. Perret et al. (1999) reported no change in MIP following exhaustive cycling at 85% $\dot{V}O_2$peak in 12 healthy subjects. Note, however, that baseline MIP was approximately 170 cmH_2O (around 173% of the predicted value; Wilson et al. 1984), and that an inverse relationship exists between baseline MIP and the extent to which MIP declines following exercise (McConnell et al. 1997). Strong inspiratory muscles thus offer some protection against global inspiratory muscle fatigue, perhaps by reducing the force contribution from each active inspiratory myofibre or by recruiting fewer of them, which might also cause a delay in the recruitment of inefficient type IIX fibres (Tanaka and Swensen 1998).

The force generating capacity of the expiratory muscles, as quantified from MEP, is not usually reduced following heavy endurance exercise (Coast et al. 1999; Hill et al. 1991; Perret et al. 1999), although Loke et al. (1982) noted an approximate 28% reduction in MEP following a marathon. That the same exercise causes decrements in MIP but not MEP (Boussana et al. 2001; Coast et al. 1999; Hill et al. 1991) suggests the inspiratory muscles are more susceptible to fatigue during exercise than expiratory muscles. However, because inspiratory muscle endurance capacity exceeds expiratory muscle endurance capacity (Clanton 1995), distinct levels of activation probably explain the different levels of fatigue. Specifically, as previously discussed (section 1.3), the onset of expiratory flow limitation during heavy exercise results in hyperinflation and an increase in inspiratory muscle work. Conversely, expiratory muscle activity is reflexively constrained by expiratory flow limitation, as evidenced by a Ppl throughout expiration that rarely exceeds the maximal effective Ppl (Johnson et al. 1992; Pellegrino et al. 1993).

Although exercise-induced decrements in MIP/MEP are often taken as evidence of respiratory muscle fatigue, other, potentially confounding factors should also be considered when employing such measures. For example, volitional measurements ultimately depend on subject motivation and aptitude, thus an exercise-induced reduction in MIP/MEP might simply reflect a lack of motivation or coordination rather than respiratory muscle fatigue per se. Due to an increase in thoracic blood volume, RV may increase by up to 0.5 litres following maximal exercise and require more than 30 min to return to baseline (Cordain et al. 1994). This would shorten the inspiratory muscles and reduce their force generating capacity. Volitional mouth pressure tests are also unable to discriminate between the weakness/fatigue of the different respiratory muscles. Maximal volitional pressures may also provide a poor indicator of long-lasting low-frequency fatigue since the high neuronal firing frequency associated with such manoeuvres means that high-frequency fatigue is

primarily assessed (Supinski et al. 2002). Finally, volitional respiratory muscle performance measures cannot discriminate between central or peripheral components of fatigue. Given these considerations, it would be erroneous to diagnose the presence/absence of respiratory muscle fatigue using only MIP/MEP measurements.

1.4.2 EXERCISE-INDUCED RESPIRATORY MUSCLE FATIGUE: CHANGES IN ENDURANCE

Strength is functionally and physiologically distinguishable from endurance. This distinction is emphasised when concurrent strength and endurance tests are used to assess respiratory muscle fatigue. Perret et al. (1999) observed no change in MIP following exhaustive cycling exercise at 85% $\dot{V}O_2$peak in 12 healthy subjects, but a 40% decline in inspiratory muscle endurance during a constant-load (80% MIP) inspiratory resistive breathing test. Ker and Schultz (1996) measured MIP and inspiratory muscle endurance before and 3 days after an ultra-marathon (87 km, mean time = 8 h 29 min). To assess inspiratory muscle endurance subjects sustained 75% MIP at RV for 5 s, followed by 5 s rest. The task was continued until subjects could not sustain or reach the target pressure. Although MIP had fully recovered 3 d after the ultra-marathon, inspiratory muscle endurance time was still reduced by 26.5% [62.3 (SD 42) vs. 16.5 (SD 20) s]. Such a prolonged impairment in muscle function may reflect damaged muscle fibres (Jones 1996). A more durable reduction in inspiratory muscle endurance compared to inspiratory muscle strength probably relates to these performance measures assessing, primarily, low- and high-frequency fatigue, respectively (Supinski et al. 2002). Whereas low-frequency fatigue may take hours or even days to recover, the loss of force in high-frequency fatigue is usually restored within minutes (Jones 1996).

Interestingly, Perret et al. (2000) reported similar reductions in inspiratory muscle endurance performance following exhaustive cycling exercise at 65, 75, 85, or 95%

$\dot{V}O_2$peak. In contrast, a positive correlation exists between the severity of exercise-induced diaphragm fatigue and exercise intensity (Johnson et al. 1993). These discrepancies are probably due to the different respiratory muscle performance tests utilised. Whereas Johnson et al. (1993) used phrenic nerve stimulation techniques to objectively assess diaphragm fatigue, Perret et al. (2000) used an inspiratory resistive breathing task, which involves the preferential recruitment of inspiratory rib cage muscles (McCool et al. 1992).

The effects of endurance exercise on expiratory muscle endurance remain relatively unexplored and our understanding is limited to the findings of Fuller et al. (1996), who evaluated expiratory muscle endurance in trained cyclists before and after heavy cycling exercise at 75% maximal exercise $\dot{V}_E$ (average duration = 17 min). Expiratory muscle endurance was assessed using repeated MEP efforts at end-expiratory lung volume. Each effort was sustained for 6 s and separated by a 10 s rest interval. Duty cycle was thus set at 0.38 to allow subjects to take 2-4 breaths during each 10 s recovery. Expiratory muscle fatigue was quantified by the decline in the area under the pressure-time curve. Following exercise, both the pressure-time area and peak expiratory pressure declined at faster rates and to lower levels compared to pre-exercise values. These changes also paralleled reductions in rectus adbominis and external oblique electromyographic activities, thus suggesting a central component of fatigue. However, Fuller et al. (1996) suggest that the extent of abdominal muscle activation during exercise is unlikely to result in a magnitude of fatigue sufficient to cause impairment of expiratory airflow, pulmonary ventilation, or endurance exercise performance. However, recent evidence suggests that expiratory muscle fatigue may have implications for cardiac output distribution during exercise (see section 1.4.7), thus we should not discount its importance in contributing to exercise tolerance.

1.4.3 EXERCISE-INDUCED DIAPHRAGM FATIGUE

The technique of bilateral phrenic nerve stimulation in conjunction with Pdi measurement provides a non-subjective, non-motivation-dependent test to assess diaphragm function, thereby overcoming the limitations associated with volitional respiratory muscle function tests. Note that hereafter low-frequency fatigue describes a reduction in force generation in response to low-frequency stimuli (1-20 Hz), whereas high-frequency fatigue describes a reduction in force generation in response to high-frequency stimuli (50-100 Hz) (Supinski et al. 2002). Using phrenic nerve stimulation techniques, Johnson et al. (1993) provided the first objective evidence of low-frequency diaphragm fatigue subsequent to whole-body endurance exercise in healthy humans of varied endurance training status ($\dot{V}O_2$peak = 40-80 ml·kg^{-1}·min^{-1}). Prior to exercise at 85 and 95% $\dot{V}O_2$peak, Pdi at FRC averaged 27.5 and 24.9 cmH_2O using a supramaximal twitch, 55 and 46 cmH_2O with 10 Hz stimulation, and 90 and 78 cmH_2O with 20 Hz stimulation, respectively. Following exercise at 95% $\dot{V}O_2$peak, reductions in twitch Pdi values averaged between 9 (SEM 2) and 33% (SEM 8) progressing from RV to TLC. Diaphragm fatigue was thus more discernable at shorter muscle lengths with the greatest reduction in Pdi occurring at approximately 80% TLC. There were also 21 (SEM 3) and 13% (SEM 2) reductions in Pdi values obtained at FRC with 10 and 20 Hz stimulation, respectively. Post-exercise recovery of twitch Pdi required 70 (SEM 4) min. That diaphragm fatigue was more pronounced at higher lung volumes (i.e. shorter diaphragm length) is probably explained by how diaphragm muscle fibres were recruited during exercise. More specifically, the force output and velocity of shortening of the diaphragm during exercise was greatest at approximately 80% TLC. Therefore, at this diaphragm muscle fibre length (i.e. lung volume) faster, more fatigable, muscles fibres were probably recruited, thus contributing to a greater fall in twitch Pdi at this lung volume (Johnson et al.

1993). Diaphragm fatigue was less consistent following exercise at 85% $\dot{V}O_2$peak, such that a 15% decrement was observed only in twitch Pdi at FRC. Johnson et al. (1993) subsequently concluded that diaphragm fatigue occurs in healthy humans when exercise intensity exceeds 85% $\dot{V}O_2$peak and is sustained to the limit of volitional tolerance. In the same year, Mador et al. (1993) also demonstrated exercise-induced diaphragm fatigue in sedentary subjects [$\dot{V}O_2$peak = 2.67 (SD 0.50) $l \cdot min^{-1}$] following exhaustive cycling exercise at 80% $\dot{W}$ max. Specifically, twitch Pdi at FRC declined by approximately 17% [28.9 (SD 3.7) vs. 23.9 (SD 5.1) cmH_2O] 10 min following exercise and values required 60 min to return to baseline.

Babcock et al. (1998) extended these observations by demonstrating high-frequency diaphragm fatigue in healthy humans following exhaustive exercise at 95% $\dot{V}O_2$peak. Because humans cannot tolerate tetanic stimulation, Babcock et al. (1998) assessed high-frequency diaphragm fatigue using a paired stimulation technique with varied intervals between stimuli. An assumption of this method is that the Pdi response to the first stimulation is the same as a twitch Pdi response, whereas the response to the second stimulation reflects the response of the muscle to numerous stimuli at that frequency. High-frequency fatigue is thus reflected by a reduction in the response to the second stimulation. Subjects received at FRC two supramaximal stimuli of the phrenic nerves at a constant current and the time interval between stimuli was varied from 10 ms (100 Hz) to 100 ms (10 Hz). Following exercise high-frequency diaphragm fatigue was confirmed by reductions in Pdi values at 50, 70, and 100 Hz. However, Pdi values at these stimulation frequencies were resolved following 30 min of recovery, although Pdi values at 10 and 20 Hz (reflective of low-frequency diaphragm fatigue) remained below baseline up to 60 min post-exercise. These observations are consistent with the knowledge that high-frequency fatigue recovers more quickly than low-frequency fatigue (Jones 1996).

The identification of high- and low-frequency diaphragm fatigue implies two different types of excitation-contraction coupling failure (Jones 1996). Force loss in high-frequency fatigue is probably related to disruption of action potential propagation along the muscle membrane or transmission along the T-tubule. This may result from increased extracellular potassium concentration, which causes depolarisation of the plasma membrane and subsequently a slow inactivation of voltage-gated sodium channels, which further reduces the excitability of the muscle (Sejersted and Sjøgaard 2000). Blocking of action potentials by potassium may also be exacerbated within the lumen of the transverse-tubule because of its small volume (Jones 1996), and because it possesses fewer sodium-potassium ATPase molecules (i.e. the sodium-potassium pump) compared to the surface membrane (Åstrand et al. 2003). However, changes in extracellular potassium concentration are readily reversible and perhaps explain why high-frequency fatigue is rapidly reversed. Force loss in low-frequency fatigue is perhaps due to a reduction in calcium release from the sarcoplasmic reticulum and/or damaged muscle fibres caused by excessive stretch (Jones 1996). Because the relationship between force and intracellular calcium concentration is sigmoidal, a reduction in calcium release from the sarcoplasmic reticulum would only account for reductions in force at low frequencies of stimulation (Jones 1996).

It is possible that diaphragm fatigue occurs relatively early in heavy endurance exercise. Johnson et al. (1993) obtained an index of respiratory muscle force production during heavy (85% $\dot{V}O_2$ peak) endurance exercise by calculating the time integral of the Ppl (reflective of total respiratory muscle recruitment and inferred from Poes) and Pdi (reflective of diaphragm recruitment) waveforms multiplied by f_R. The change in time integral of Pdi and Poes thus provides an indication of diaphragmatic versus total respiratory muscle recruitment. Using these techniques, Johnson et al. (1993) demonstrated

dissociation between diaphragmatic and total respiratory muscle pressure output during heavy exercise; following an initial, sudden increase in pressure output by the diaphragm and the total respiratory muscle system, there was a plateau in diaphragmatic pressure output relative to total respiratory muscle pressure output, as reflected by a falling Pdi/Poes ratio (Figure 1.15). Thus despite a rising $\dot{V}_E$ and increasing negativity of Poes the relative contribution of the diaphragm to total inspiratory effort declines as heavy exercise progresses. This suggests that the hyperpnoea of heavy endurance exercise involves the preferential recruitment of accessory inspiratory muscles, thus effectively "sparing" the diaphragm. That diaphragm fatigue accounts for the decay in Pdi/Poes is supported by the observation that pressure output is maintained when proportional assist ventilation is used to unload the inspiratory muscles (Babcock et al. 2002). Moreover, in sedentary subjects a declining diaphragm pressure output during the last 3 min of heavy exercise is only observed in those that subsequently demonstrate post-exercise reductions in twitch Pdi (Mador et al. 1993).

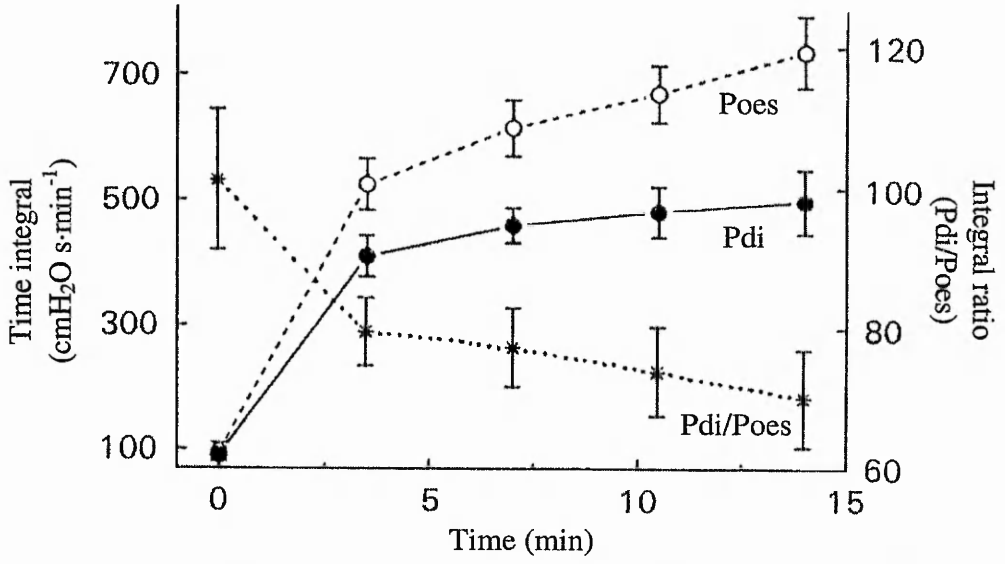

Figure 1.15 Time integrals for oesophageal pressure (Poes) and transdiaphragmatic pressure (Pdi) and the Pdi/Poes ratio during exhaustive cycling exercise at 95% $\dot{V}O_2$ peak. Values are means ± SEM. (From Johnson et al. 1993).

Intriguingly, the fatiguing mechanical work performed by the diaphragm during heavy exercise is not usually fatiguing when mimicked voluntarily under resting conditions. Babcock et al. (1995) observed a 27.2% (SEM 6.3) reduction in twitch Pdi following exercise at 85-90% $\dot{V}O_2$peak, but no reduction when subjects voluntarily mimicked at rest the $\dot{V}_E$, V_T, f_R, time integral of Pdi, and duty cycle achieved during the final stages of the endurance exercise test. Thus factors attributable to whole-body exercise must account for a significant portion of the respiratory muscle fatigue observed under these conditions. Competition for the available cardiac output between respiratory and locomotor muscles, and greater concentrations of circulating, potentially fatiguing, metabolic by-products secondary to locomotor muscle activity, have both been implicated (Johnson et al. 1996). Note, however, that these influences may not be mutually exclusive since competition for blood flow between locomotor and respiratory muscles could influence metabolite production and/or uptake in both muscle groups.

Although the work of breathing during heavy exercise is insufficient to cause diaphragm fatigue when mimicked at rest, it is influential when accompanied by whole-body exercise. Although Babcock et al. (1995) observed diaphragm fatigue following heavy endurance exercise, force output was maintained in a stimulated hand muscle exposed to the same exercise milieu as the diaphragm. Moreover, the 20-30% decline in twitch Pdi following heavy (80-85% $\dot{V}O_2$peak) exercise is prevented when proportional assist ventilation is used to unload the inspiratory muscles during exercise (Babcock et al. 2002). Therefore, the aetiology of exercise-induced diaphragm fatigue seems dependent upon an interaction between the pressure generating power output of the diaphragm and a competition for blood flow between respiratory and active locomotor muscles.

1.4.4 CENTRAL/SUPRASPINAL COMPONENTS OF RESPIRATORY MUSCLE FATIGUE

A decline in the force generating capacity of a muscle under load can result from a disruption in any one of the steps comprising the "chain of command" for voluntary force production. Disruption in a step prior to the neuromuscular junction is otherwise referred to as central fatigue, which is defined as "a progressive reduction in voluntary activation of muscle during exercise" (Gandevia 2001). Supraspinal fatigue is a subset of central fatigue and is defined as a "failure to generate output from the motor cortex" (Gandevia 2001).

Although bilateral phrenic nerve stimulation provides an objective measure of peripheral diaphragm fatigue, it does not address central components of fatigue. Indeed, recent evidence suggests that central inhibition may have a mechanistic role in shaping diaphragm force output and fatigue during exercise. Most notably, the diaphragm appears more susceptible to central fatigue than limb locomotor muscles. For example, subsequent to maximal exercise (Babcock et al. 2002), isocapnic MVV, or maximal inspiratory resistive or pressure threshold loading (Guleria et al. 2002; Luo et al. 2001), the decline in twitch Pdi rarely exceeds 20-30%. Conversely, maximal knee-extensor exercise can readily cause a decline in twitch amplitude of the quadriceps that exceeds 40% (Guleria et al. 2002). Thus when faced with an increased load, there appears to be less central drive to the diaphragm compared to limb muscle. Guleria et al. (2002) suggest that this response protects the diaphragm from severe contractile failure. This notion has received empirical support from studies measuring the diaphragm motor-evoked potential during transcranial magnetic stimulation following heavy endurance exercise. Verin et al. (2004) performed phrenic and femoral nerve stimulation and transcranial magnetic stimulation before and after incremental treadmill exercise performed to volitional tolerance to establish the extent of peripheral and supraspinal fatigue, respectively, in diaphragm and quadriceps muscles. Following exercise, twitch responses to phrenic and femoral nerve stimulation were not

different to resting values, thus there was no evidence of peripheral fatigue. However, during transcranial magnetic stimulation, motor-evoked potentials for the diaphragm and quadriceps were significantly reduced 5 min post-exercise to about 60% of baseline values, thus indicating supraspinal fatigue. The motor-evoked potentials for the quadriceps had partially recovered after 20 min recovery, and following 60 min values had returned to resting levels. Conversely, a further reduction to 45% of baseline values was recorded in the diaphragm motor-evoked potential following 20 min of recovery. Therefore, the reduction in diaphragm motor-evoked potential amplitude following heavy endurance exercise was more pronounced than that observed for the quadriceps following 20 min of recovery. Following 60 min of recovery, the diaphragm motor-evoked potential had partially recovered but still only represented about 80% of the resting value, thus supraspinal fatigue was also more durable for the diaphragm compared to the quadriceps. In another study, Jonville et al. (2005) performed transcranial and cervical magnetic stimulation on healthy volunteers before and after two separate 16 min cycling exercise bouts at 55% $\dot{V}O_2$ peak. During one of the exercise bouts subjects breathed through an inspiratory threshold valve set at 10% MIP. The breathing pattern during loaded exercise was not different to that observed during normal exercise. There was also no change in twitch inspiratory mouth pressure during cervical magnetic stimulation following either exercise bout, thus excluding peripheral diaphragm fatigue. Similarly, the motor-evoked potential amplitude of the rectus femoris was also unaffected by both exercise bouts, thus peripheral quadriceps fatigue was not present. However, following normal exercise the motor-evoked potential amplitude of the diaphragm was significantly reduced approximately 45% from resting values. Perhaps more intriguing, however, is that this reduction was not apparent following loaded exercise, even though loading exacerbated dyspnoea. Thus superimposing a modest inspiratory load during moderate exercise

appeared to protect the diaphragm from supraspinal fatigue. Jonville et al. (2005) propose that the addition of an inspiratory load during exercise modifies respiratory muscle recruitment, such that extradiaphragmatic muscles are preferentially recruited, which subsequently preserves diaphragm excitability.

Central fatigue probably serves to protect against contraction-induced muscle damage or homeostatic disturbances that might result from muscular exercise (Gandevia 2001). However, because marked supraspinal diaphragm fatigue may occur in the absence of peripheral diaphragm fatigue, this mechanism appears somewhat excessive for this muscle. Guleria et al. (2002) propose that the diaphragm actually has a 'normal' central control system, but evolutionary pressures (i.e. hunting/fleeing predators) have overridden this influence in locomotor muscles. Such a profound central inhibition may, however, be detrimental to endurance exercise performance. For example, the decline in diaphragmatic pressure output relative to total respiratory muscle pressure output during heavy exercise (Johnson et al. 1993) could, in part, reflect central inhibition. Subsequently, less efficient accessory rib cage muscles would be recruited to maintain adequate $\dot{V}_A$, which would presumably increase the metabolic requirements of the respiratory pump (Johnson et al. 1996). Additionally, the increased recruitment of extradiaphragmatic muscles is also associated with an exacerbation of dyspnoea (Jonville et al. 2005; McConnell and Romer 2004b), which may further curtail endurance exercise performance.

1.4.5 Effects of endurance training status on exercise-induced respiratory muscle fatigue

Regular whole-body endurance training promotes significant increases in oxidative and antioxidant enzyme activities in respiratory muscles (Powers et al. 1997). Although these cellular adaptations have been primarily observed in animal models, similar changes

are likely to explain why whole-body endurance training in humans promotes significant improvements in respiratory muscle endurance (Robinson and Kjeldgaard 1982; Clanton et al. 1987). By virtue of these training-induced adaptations, it is attractive to speculate that the respiratory muscles of endurance athletes would be more resistant to fatigue during exercise, and indeed, several reports support this assertion. Coast et al. (1990) recorded a 10-17% drop in MIP in sedentary subjects [$\dot{V}O_2$peak = 42.0 (SEM 3.0) $ml \cdot kg^{-1} \cdot min^{-1}$] following maximal, incremental cycling exercise, but no reduction in a group of cross-country skiers [$\dot{V}O_2$peak = 71.8 (SEM 3.8) $ml \cdot kg^{-1} \cdot min^{-1}$]. Bender and Martin (1985) noted a greater decrement in MVV_{60} in recreationally active subjects compared to endurance trained runners following a high-intensity 60 min running exercise, even though similar levels of $\dot{V}_E$ were achieved during exercise. Although these findings suggest that endurance athletes are less susceptible than non-athletes to exercise-induced respiratory muscle fatigue, note that decrements in respiratory muscle function have been consistently observed in highly trained triathletes (Boussana et al. 2001), marathon runners (Loke et al. 1982), competitive rowers (Volianitis et al. 2001), and cyclists (Romer et al. 2002a) following participation in specific events. Thus rather than endurance training status, exercise-induced reductions in MIP are perhaps more dependent upon other factors, such as baseline inspiratory muscle strength (McConnell et al. 1997). It is worth noting that although baseline MIP in Coast et al.'s (1990) study was not significantly different between athletes and non-athletes, there was a trend for MIP to be 14.5% greater in the athletes [158 (SEM 7) vs. 138 (SEM 15) cmH_2O]. Volitional respiratory muscle performance tests are also highly dependent upon subject motivation, which might be particularly relevant when testing sedentary subjects. Furthermore, different studies have assessed respiratory muscle function following exercise of different modality, duration and intensity, which are all likely to influence the severity of respiratory muscle fatigue (Hill et

al. 1991; Johnson et al. 1993; Romer et al. 2002a). Therefore, these studies do not fully resolve the issue of whether endurance training status influences the extent of respiratory muscle fatigue during exercise.

However, Babcock et al. (1996) used phrenic nerve stimulation techniques to objectively assess the role of endurance training status on exercise-induced diaphragm fatigue. During 60 min of recovery from exercise at 95% $\dot{V}O_2$peak, comparable reductions in twitch Pdi and Pdi during repeat stimulations at 10 and 20 Hz were recorded in highly trained and moderately trained subjects ($\dot{V}O_2$peak = 61-79 and 40-59 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). However, despite attaining similar levels of diaphragm fatigue, $\dot{V}_E$ was 145.5 (SEM 6.7) and 121.7 (SEM 7.9) $l \cdot min^{-1}$ in highly trained and moderately trained subjects, respectively, and the highly trained subjects produced a diaphragmatic force output during the initial 60% of exercise that was 28% greater than that observed in moderately trained subjects. Furthermore, during the final 3 min of exercise the highly trained and moderately trained subjects reached 90 and 40%, respectively, of their dynamic capacity to generate inspiratory pressure. Thus for the same degree of diaphragm fatigue, highly trained endurance athletes perform significantly greater amounts of diaphragmatic work compared to their less-trained contemporaries (Babcock et al. 1996). Thus at matched levels of respiratory muscle power outputs, trained endurance athletes would probably incur less diaphragm fatigue than less-trained individuals.

1.4.6 CARDIO-PULMONARY INTERACTIONS DURING EXERCISE

It is estimated that during maximal exercise the respiratory muscles of endurance athletes demand around 15% of both the $\dot{V}O_2$peak (Aaron et al. 1992) and cardiac output (Harms et al. 1998b), the latter being comparable to that measured directly in the

maximally exercising pony (Manohar 1990, 1986). Under these conditions the respiratory muscles are thus competing with other metabolically active tissues (i.e. locomotor muscles) for the available cardiac output. Through employing mechanical loading and unloading of the inspiratory muscles during high-intensity endurance exercise, studies have been able to investigate the interaction between respiratory muscle work and limb blood flow, as determined using the thermodilution technique. Harms et al. (1997) increased, via mesh screens placed in the inspiratory line, or decreased, via proportional assist ventilation, the work of breathing to 128.2% (SEM 25.2) and 36.7% (SEM 26.6) of control, respectively, in trained cyclists performing 2.5 min of maximal exercise, and compared physiological responses to control measures. During inspiratory loading leg vascular resistance increased from 13.6 (SEM 0.4) to 14.6 (SEM 0.3) $mmHg \cdot l^{-1} \cdot min^{-1}$, and this was significantly related to noradrenaline spillover across the limb, which increased 78% (SEM 5) above control. Subsequently, due to a local vasoconstriction, leg blood flow declined from 18.4 (SEM 0.3) to 17.1 (SEM 0.2) $l \cdot min^{-1}$, and because oxygen extraction across the limb did not change, leg $\dot{V}O_2$ also declined from 3.41 (SEM 0.10) to 3.13 (SEM 0.06) $l \cdot min^{-1}$ (Figure 1.16). Conversely, with inspiratory unloading leg vascular resistance declined to 13.1 (SEM 0.3) $mmHg \cdot l^{-1} \cdot min^{-1}$ (note that control values are given above) following an 11% (SEM 3) reduction in noradrenaline spillover across the limb musculature. Leg blood flow increased to 19.2 (SEM 0.3) $l \cdot min^{-1}$ due to a local vasodilation, and leg $\dot{V}O_2$ increased to 3.51 (SEM 0.07) $l \cdot min^{-1}$ (Figure 1.16), which was due entirely to an increase in muscle perfusion as oxygen extraction was unchanged.

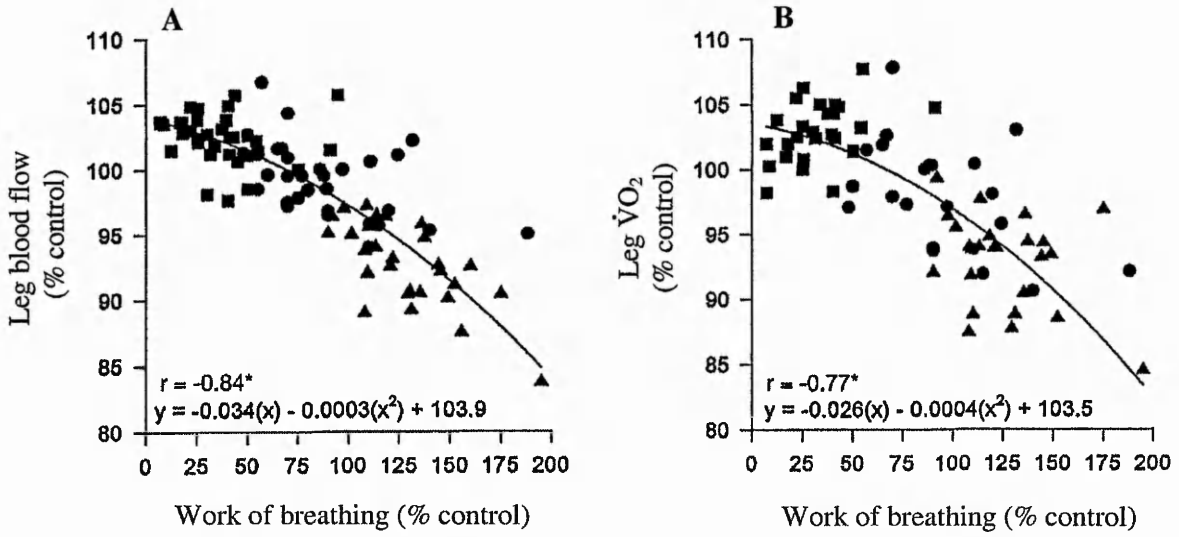

Figure 1.16 Relative effects of changing the work of breathing on leg blood flow (A) and leg oxygen uptake ($\dot{V}O_2$) (B) during maximal cycling exercise in 7 trained cyclists. (From Harms et al. 1997).

The findings of Harms et al. (1997) thus suggest that the metabolic requirements of the respiratory muscles during maximal exercise are partly met at the expense of locomotor muscle perfusion. Such changes are likely to explain why mechanical loading and unloading of the inspiratory muscles during high-intensity cycling exercise increases and decreases, respectively, the severity of quadriceps muscle fatigue (Romer et al. 2004) and exercise tolerance (Harms et al. 2000).

In contrast, leg vascular resistance, noradrenaline spillover, leg blood flow, and leg $\dot{V}O_2$ were unchanged from control when inspiratory muscle loading/unloading was imposed on submaximal (50 and 75% $\dot{V}O_2$peak) exercise workloads (Wetter et al. 1999). Indeed, some suggest that changing respiratory muscle work may only influence cardiac output distribution during maximal exercise. Using transcutaneous near-infrared spectroscopy across the vastus lateralis, Kowalchuk et al. (2002) measured tissue oxyhaemoglobin and reduced haemoglobin concentrations during high-intensity exercise [end-exercise $\dot{V}O_2$ = 94% (SD 7) $\dot{V}O_2$peak; average exercise duration = 11.9 (SD 1.4) min], in which subjects breathed through an inspiratory resistive load (perplex block with

internal diameter of 10 mm presenting a resistance of 6.5 $cmH_2O \cdot l^{-1} \cdot s^{-1}$) for 3-4 min. During submaximal exercise when there is reserve for cardiac output and muscle oxygen extraction to increase, a decline in leg blood flow should result in an increased oxygen extraction across the muscle, and subsequently an increase in the near-infrared muscle deoxygenation signal (i.e. increased concentrations of reduced haemoglobin). However, Kowalchuk et al. (2002) failed to observe such an increase during loaded breathing, thus leg blood flow did not appear to be compromised secondary to a respiratory 'steal' phenomenon. There were also no changes in heart rate, $\dot{V}O_2$, or $\dot{V}_E$ during loaded breathing. Kowalchuk et al. (2002) proposed that blood flow redistribution from non-working tissues supplied the newly recruited respiratory muscle mass with adequate perfusion, and that limitations on the available cardiac output only exist at maximal workloads. Note, however, that near-infrared spectroscopy does not always provide an accurate measurement of oxygen saturation (McCully and Hamaoka 2000). Furthermore, this technique only measures oxygen saturation over a very small area and at a tissue depth that approximates just 1.5 cm (McCully and Hamaoka 2000). The findings of Kowalchuk et al. (2002) also differ from those derived during submaximal (50% $\dot{V}O_2$peak) and high-intensity (90% $\dot{V}O_2$peak) exercise in which inspiratory loading consistently resulted in an increase in pulmonary $\dot{V}O_2$, presumably to meet the increased metabolic demands of the respiratory muscles (Wetter et al. 1999; Harms et al. 2000). It is unclear why Kowalchuk et al. (2002) failed to observe an increase in pulmonary $\dot{V}O_2$ during loaded breathing, since an increase in respiratory muscle $\dot{V}O_2$ would be expected. Importantly, however, reductions in leg blood flow due to fatiguing respiratory muscle work appear to result from a respiratory muscle metaboreflex (see below) (Harms et al. 1997), which may be activated, even during submaximal exercise, by metabolite accumulation within the

interstitium of the respiratory muscles (Rodman et al. 2003). It is thus possible that a respiratory muscle metaboreflex was not activated in the study of Kowalchuk et al. (2002).

The possibility that leg blood flow only increases when inspiratory muscle unloading is superimposed on high-intensity/maximal exercise may explain why submaximal (70-80% $\dot{V}O_2$max) exercise tolerance in moderately trained subjects remained unchanged with mechanical unloading of the respiratory system (Gallagher and Younes 1989; Krishnan et al. 1996; Marciniuk et al. 1994). Moreover, as noted by Harms et al. (2000), the work of breathing in moderately trained subjects would be less than that observed in highly trained athletes (in whom high-intensity exercise tolerance increases with inspiratory unloading – Harms et al. 2000), thus the effect of alleviating inspiratory muscle work would probably be less discernable. The importance of exercise intensity in determining the efficacy of respiratory muscle unloading on exercise endurance was demonstrated by Aaron et al. (1985), who assessed cycling endurance times at 80-85 and 90-95% $\dot{V}O_2$max in highly trained athletes breathing either air or normoxic helium. The latter reduces ventilatory work (due to the reduced gas density) and expands the maximal flow-volume loop, which enables a higher $\dot{V}_E$ to be achieved by those susceptible to expiratory flow limitation (see section 1.3). With helium breathing, cycling endurance improved (+40%) only at intensities >90% $\dot{V}O_2$max, and compared to air breathing, was accompanied by an increased $\dot{V}_E$ (171 vs. 149 l·min^{-1}), and reduced $\dot{V}O_2$ (4.48 vs. 4.90 l·min) and dyspnoeic ratings (6.9 vs. 8.0).

1.4.7 The respiratory muscle metaboreflex

During dynamic steady-state exercise, skeletal muscle blood flow increases in direct proportion to local metabolic demands. Additionally, due to vasoconstriction blood

flow is re-distributed from inactive tissues to vasodilated working muscle, with mean arterial pressure being maintained within 10-15 mmHg of resting levels (Dempsey et al. 2002). For limb skeletal muscle, this precise cardiovascular regulation is dually mediated by feed-forward (i.e. central command) and feedback (mechano- and metaboreflexes) mechanisms (Thomas and Segal 2004). Powerful metabolic vasodilatory substances such as lactate, H^+, potassium, adenosine, and nitric oxide also accumulate in skeletal muscle during exercise and contribute to local vasodilation (Clifford and Hellsten 2004). Evidence for central command exerting an effect on vasoconstrictor outflow in humans is provided by studies that have dissociated motor command from muscle force production. Victor et al. (1995) studied muscle sympathetic nerve activity (MSNA), as recorded from the peroneal nerve, during near-maximal isometric handgrip exercise with and without partial neuromuscular blockade using curare. After curare administration, near maximal voluntary efforts were still associated with high levels of MSNA, despite muscle force production being attenuated to <25% of the initial maximum voluntary contraction. Mechanoreflexes from contracting skeletal muscle mediate an increase in sympathetic outflow following mechanical deformation of the musculotendinous unit or through venous distension, whereas metaboreflexes are activated by metabolite accumulation within working muscle, which signals a mismatch between muscle perfusion and metabolic demand (Dempsey et al. 2002). Metabolite accumulation in skeletal muscle thus plays a dual role in causing local vasodilation and metaboreflex activation. Both mechano- and metaboreflexes are thought to originate in thinly myelinated group III and unmyelinated group IV nerve endings (Dempsey et al. 2002).

Although the aforementioned studies were primarily performed on limb skeletal muscle, evidence suggests that feedback from the lung and respiratory muscles also influences the autonomic control of blood flow in the systemic circulation during exercise.

For example, discharge of group IV phrenic afferents increases during electrically stimulated contractions of the rat diaphragm sufficient to cause fatigue (Hill 2000). Furthermore, as discussed previously, reductions in leg vascular conductance with inspiratory muscle loading during maximal exercise correlate inversely with changes in noradrenaline spillover across the limb, which implies a sympathetically mediated response (Harms et al. 1997). Several studies have also examined the effects of isolated respiratory muscle work on MSNA in the resting limb, as measured with intraneural electrodes in the peroneal nerve. St Croix et al. (2000) and Sheel et al. (2001) recorded MSNA and resting leg blood flow, respectively, whilst subjects inspired against a resistive load to a target mouth pressure of 60% MIP, with f_R and duty cycle held at 15 breaths·min^{-1} and 0.70, respectively. Subjects were instructed to isolate the diaphragm during inspiratory efforts and the protocol was continued until the target mouth pressure could not be maintained. This protocol caused diaphragm fatigue, as quantified by a reduction in mouth twitch pressure to 56.5% (SD 24.8) of control during phrenic nerve stimulation. During inspiratory loading, a time-dependent increase and decrease in MSNA and leg blood flow, respectively, was observed, and at the point of task failure MSNA had increased 77% (SD 51) above control, leg blood flow had declined from 0.441 (SD 0.166) to 0.337 (SD 0.111) l·min^{-1} (-23%), and leg vascular resistance had increased from 0.217 (SD 0.070) to 0.314 (SD 0.095) mmHg·ml^{-1}·min^{-1} (+45%). These changes were also apparent despite the inhibitory influence of a 12 (SD 7) mmHg increase in arterial blood pressure. These changes thus demonstrate how increases in sympathetic outflow to the resting limb may arise with fatiguing diaphragmatic work. However, when this protocol was mimicked without added inspiratory resistance, MSNA and leg blood flow were not affected. Indeed, so long as diaphragm fatigue was avoided, large voluntary increases in inspiratory motor output (generation of large inspiratory pressures or flow rates) had no

effect on MSNA, leg vascular resistance, or leg blood flow. Moreover, note that MSNA was actually attenuated during inspiration, i.e. when inspiratory motor output was highest. A potential reason for this is that the strong inhibitory influence of an increased systemic blood pressure that accompanies inspiration is perhaps more important in modifying respiratory modulation of MSNA than central respiratory motor output (Sheel et al. 2001; St Croix et al. 2000). These findings suggest, unlike that observed for limb skeletal muscle, a minimal role for central command in shaping sympathetic vasoconstrictor activity during fatiguing inspiratory muscle work. Furthermore, Sheel et al. (2002) have identified, similar to that observed for limb skeletal muscle, a threshold for activation of MSNA during fatiguing inspiratory muscle work. Specifically, sympathetic outflow to the resting limb only occurs with the onset of diaphragm fatigue, which presumably coincides with local metabolite accumulation. Similar responses are also observed when fatiguing expiratory muscle work is performed under resting conditions (Derchak et al. 2002). Accordingly, these observations suggest that the vasoconstrictor response to fatiguing respiratory muscle work in resting humans is primarily mediated by a respiratory muscle metaboreflex.

It is thus clear that in the intact human, volitional fatigue of the respiratory muscles under resting conditions exerts a significant influence on sympathetic vasomotor outflow to the resting limb. Recent studies performed on exercising dogs also suggest that the respiratory muscle metaboreflex is capable of overcoming the powerful vasodilatory substances that accumulate in locomotor muscles during whole-body exercise. Rodman et al. (2003) injected a bolus of lactic acid into the phrenic or deep circumflex iliac arteries (to activate diaphragm and abdominal expiratory muscle metaboreflexes, respectively) of dogs performing steady-state exercise sufficient to increase cardiac output, hindlimb blood flow, and vascular conductance 159 (SD 106), 276 (SD 309), and 299% (SD 90), respectively, above resting values. Note that similar responses were evoked from

diaphragm and abdominal muscle metaboreflexes, thus the results were combined for statistical analysis. The response to lactic acid injection was almost immediate as an increase in arterial pressure and decline in hindlimb blood flow was observed 5 (SD 7) s after the start of injection, and peak responses were observed 9 (SD 3) s after the changes began. During lactic acid injection, mean arterial pressure increased 17% (SD 10), which coincided with a 9% (SD 2) decline in cardiac output. Hindlimb blood flow declined 13% (SD 9), and total systemic and hindlimb conductance were also reduced 18 (SD 4) and 23% (SD 10), respectively. Rodman et al. (2003) also confirmed the respiratory muscle metaboreflex to be sympathetically mediated by demonstrating, at rest, a complete elimination of such cardiovascular adjustments following adrenergic receptor blockade via phentolamine and propranolol administration. Although these data strongly suggest a prominent role for respiratory muscle metaboreflexes during moderate exercise, Rodman et al. (2003) speculate that the respiratory muscle metaboreflex would be less effective during high-intensity exercise in which substantial local vasodilator influences would accumulate in working locomotor muscle. Moreover, it is unclear whether respiratory and locomotor muscles differ in their sensitivities to vasoconstrictor outflow during exercise, although preliminary work has shown that arterioles isolated from rodent diaphragm constrict less when exposed to noradrenaline compared to a portion of the gastrocnemius that is similar in fibre type and oxidative capacity (Aaker and Laughlin 2002). This latter observation suggests that blood flow to the diaphragm, and perhaps other respiratory muscles, may be prioritised during exercise-induced increases in MSNA. Indeed, Seals (2001) suggests that as the "vital organ" responsible for supporting pulmonary ventilation, perfusion of the respiratory muscles during exercise assumes priority over locomotor muscles. Such precedence would allow pulmonary ventilation to continue unabated, thus ensuring proper regulation of arterial blood gases, pH, and subsequently cellular homeostasis.

1.4.8 IMPLICATIONS FOR ENDURANCE EXERCISE PERFORMANCE

Theoretically, respiratory muscle fatigue could predispose alveolar hypoventilation, thus posing a threat to systemic oxygen transport and carbon dioxide elimination. However, despite the diaphragm making a lesser contribution to total respiratory muscle pressure output as high-intensity exercise progresses, the recruitment of accessory respiratory muscles defends $\dot{V}_A$ (Johnson et al. 1993). However, changes in respiratory muscle recruitment pattern could promote chest wall distortion and a mechanical inefficiency of breathing that increases the metabolic and blood flow demands of the respiratory muscles, possibly at the expense of locomotor muscle perfusion (Harms et al. 1997; Harms et al. 2000). Moreover, respiratory muscle fatigue and/or the recruitment of extradiaphragmatic muscles could exacerbate dyspnoea (Jonville et al. 2005; McConnell and Romer 2004b), which would further contribute to the overall sensory perception of exercise exertion. However, independent of respiratory muscle recruitment pattern, a greater central motor command would be required to generate a given mechanical output from fatigued respiratory muscles, thus further increasing the sensation of respiratory effort (McConnell and Romer 2004b). Given that the diaphragm (Johnson et al. 1993) and occasionally the expiratory muscles (Fuller et al. 1996) fatigue during heavy endurance exercise, it is attractive to speculate that a respiratory muscle metaboreflex is instrumental in determining the distribution of systemic blood flow during exercise. A decline in leg blood flow secondary to a local vasoconstriction would reduce substrate delivery and metabolic by-product removal. Working locomotor muscles may thus be required to function increasingly under anaerobic conditions, thus increasing the production of metabolic by-products and augmenting the breakdown of muscle glycogen. Such circumstances would intensify limb discomfort, augment muscle fatigue, and probably contribute to exercise intolerance (Harms et al. 2000).

1.5 SPECIFIC RESPIRATORY MUSCLE TRAINING

As highlighted in the preceding section, the work of breathing during heavy endurance exercise may result in respiratory muscle fatigue, an exacerbation of dyspnoea, and a potential 'steal' of blood flow from the active locomotor muscles. Furthermore, whereas the voluntary induction of respiratory muscle fatigue impairs subsequent high-intensity endurance exercise tolerance (Mador and Acevedo 1991; Martin et al. 1982), the use of proportional assist ventilation to reduce inspiratory muscle work, and thus the severity of inspiratory muscle fatigue and dyspnoea, prolongs cycling endurance at near maximal intensities (Harms et al. 2000). Respiratory muscle work thus appears to have significant physiological consequences that impact on endurance exercise performance. Therefore, in an attempt to overcome, or at least alleviate, some of these deleterious influences, a growing number of studies have focused their attention on the ergogenic effects of specific respiratory muscle training (RMT). The following subsections address RMT techniques, and the effects on respiratory muscle function and endurance exercise tolerance.

1.5.1 RESPIRATORY MUSCLE TRAINING TECHNIQUES

A commonly employed RMT technique is voluntary isocapnic hyperpnoea, which represents a form of respiratory muscle endurance training. Subjects usually sustain a $\dot{V}_E$ of around 50-80% MVV (with fixed V_T and f_R) for a typical duration of 30 min, and sessions are usually performed 3-5 $d \cdot wk^{-1}$ for 3-5 weeks (McMahon et al. 2002; Morgan et al. 1987; Sonetti et al. 2001; Spengler et al. 1999). Voluntary isocapnic hyperpnoea training probably provides the greatest specificity in terms of replicating exercise hyperpnoea. However, to maintain isocapnia during volitional hyperpnoea, monitoring of pulmonary gas exchange is required, which makes the technique difficult to implement

outside the laboratory. Furthermore, McConnell and Romer (2004a) caution that high flow rates during volitional hyperpnoea could promote drying of airways, thus promoting bronchoconstriction in susceptible individuals.

In order to improve inspiratory muscle strength, other studies have utilised inspiratory flow resistive loading (Gething et al. 2004a; Gething et al. 2004b; Hanel and Secher 1991), which requires subjects to inspire, usually from RV to TLC (or as close to there as possible), through an orifice of variable diameter, thus for a given flow rate a smaller orifice provides a greater resistive load. However, because inspiratory pressure (analogous to inspiratory muscle force generation) is inversely related to inspiratory flow (analogous to muscle shortening velocity), the training load with flow resistive loading is also dependent upon the inspiratory flow rates achieved. Therefore, breathing pattern or inspiratory mouth pressure must be monitored to ensure that subjects adhere to the prescribed training intensity.

A RMT technique that is growing in popularity is inspiratory pressure-threshold loading, which requires the continuous application of inspiratory pressure above a preset threshold so that inspiration can proceed. The advantages of this technique are that several devices are commercially available, training intensity is easily quantified, inspiratory efforts are virtually flow independent, and training sessions do not require additional equipment. The training load can be adjusted to make training sessions short (e.g. 5 min), thus focusing on strength development, or prolonged (e.g. 30 min), thus focusing on endurance development. Inspiratory manoeuvres are performed from RV to TLC (or as close to there as possible), and sessions are usually performed 5-7 $d \cdot wk^{-1}$ for a period of 4-6 weeks (McConnell and Sharpe 2005; Romer et al. 2002a; Williams et al. 2002), although some studies have extended training over 11 weeks (Volianitis et al. 2001).

1.5.2 Effects on respiratory muscle function

Respiratory muscle strength usually remains unchanged following volitional hyperpnoea training (Leith and Bradley 1976; Tzelepis et al. 1999), probably because hyperpnoea is primarily focused on the flow (velocity) axis of the pressure-flow relationship (Romer and McConnell 2003), and several physiological adaptations associated with endurance training (Holloszy and Coyle 1984) are opposite to those that occur following resistive-type training (Tesch 1988). Expectedly, however, volitional hyperpnoea training does improve ventilatory endurance. Leith and Bradley (1976) reported a 19% increase in maximal sustainable ventilatory capacity following a 5 week volitional hyperpnoea training regimen in 4 sedentary subjects. A comparable improvement (approximately 11%) was also reported in 5 trained cyclists following a 4 week volitional hyperpnoea training regimen (no change observed in the control group) (Fairbarn et al. 1991). These latter findings suggest that respiratory muscle endurance can be improved beyond that derived from whole-body endurance training alone. Other studies have also consistently observed improvements in ventilatory endurance following volitional hyperpnoea training. For example, following 40 × 30 min training sessions performed at 60% MVV over 15 weeks, ventilatory endurance at 70% MVV increased in 13 sedentary subjects from a median (group mean not reported) of 4.6 (range 2.0-10.2) to 40.0 (range 15.5-40.0) min (note that tests were terminated by the investigators at 40 min), with no change being observed in the control group [from 5.2 (range 2.0-10.4) to 3.9 (1.5-19.6) min] (Markov et al. 2001). Similarly, ventilatory endurance at 74% MVV improved in 10 trained cyclists by approximately 250% [8.3 (SD 2.0) vs. 29.8 (SD 13.6) min] following 20 × 30 min volitional hyperpnoea training sessions performed at 60% MVV; again no change was observed in the control group (McMahon et al. 2002).

Without exception, strength-based RMT studies consistently observe improvements in respiratory muscle strength. Leith and Bradley (1976) had 4 untrained subjects complete a 5 week RMT regimen comprising MIP and MEP manoeuvres at 20% intervals over the VC range for 30 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$. Following training both MIP and MEP measured at FRC increased by approximately 55% (baseline values were 110 and 132 cmH_2O, respectively). Gething et al. (2004a) employed inspiratory flow resistive loading [10 inspiratory manoeuvres at 80% MIP (recorded by computer and displayed in real-time), 3 $d \cdot wk^{-1}$ for 6 weeks] in 21 healthy subjects, and observed a 37 (SD 25) cmH_2O (+35%) increase in MIP from a baseline of approximately 110 cmH_2O. Similar improvements (34-45%) in MIP have been documented in highly trained endurance athletes following pressure threshold inspiratory muscle training comprising 30 inspiratory manoeuvres performed twice daily at 50% MIP for 6-11 weeks (Romer and McConnell 2003; Romer et al. 2002a; Romer et al. 2002c; Volianitis et al. 2001). There does however appear to be a plateau in strength and power development in response to pressure threshold inspiratory muscle training at 6 weeks (Romer and McConnell 2003; Volianitis et al. 2001). This observation is consistent with the assertion that all physiological systems have an upper limit, or genetic ceiling, for change (Kraemer 2000). Intriguingly, Suzuki et al. (1993) observed similar improvements in MIP and volitional Pdi (34.9 and 31%, respectively) following pressure-threshold inspiratory muscle training, thus increased diaphragmatic strength may explain most of the improvement in MIP. However, negative abdominal pressures (Clanton et al. 1990) and submaximal diaphragm activation (Hershenson et al. 1988) were observed in humans performing Mueller efforts, thus suggesting preferential activity of the accessory rib cage muscles during inspiratory efforts against large resistances. Hershenson et al. (1988) speculate that deactivation of the diaphragm during forceful inspiratory efforts may serve to better match the relative strengths of the

diaphragm and rib cage muscles. Because diaphragm strength exceeds rib cage muscle strength, the former may be deactivated during Mueller efforts to prevent collapse of the rib cage. It thus remains a possibility that in some individuals the rib cage musculature, rather than the diaphragm, receives the majority of the training stimulus with inspiratory muscle strength training. This notion is, in part, consistent with the findings of Hart et al. (2001) who failed to demonstrate an increase in twitch Pdi during phrenic nerve stimulation following a pressure threshold inspiratory muscle training regimen (30 inspiratory manoeuvres performed twice daily at 50% MIP for 6 weeks), although MIP increased significantly by 12.2%. Irrespective of the improvement in MIP, on the basis that diaphragm contractility was unchanged Hart et al. (2001) concluded inspiratory muscle training to be ineffective. However, others highlight that an increase in twitch Pdi would require hypertrophy of diaphragm muscle fibres, which would not be expected during the initial 6-8 weeks of strength training (Caine and Sharpe 2002). Moreover, by their own admission, Hart et al. (2001) state that, because of the within-subject variability associated with the twitch Pdi technique, noteworthy increases in Pdi may have gone undetected due to the small sample size (n = 6 in both RMT and control groups). The efficacy of inspiratory muscle training to improve diaphragm strength thus remains unclear.

In contrast to the impressive (+50%) improvements in MIP frequently documented following inspiratory muscle strength training, Sonetti et al. (2001) reported a somewhat smaller MIP improvement (approximately 8%) in trained cyclists following a 5 week training regimen incorporating concurrent inspiratory muscle strength (pressure threshold loading) and endurance (voluntary isocapnic hyperpnoea) training. Note, however, that concurrent strength and endurance training can be antagonistic, with inhibition focused on strength development (Leveritt et al. 1999). This inhibition is perhaps explained by the inability of muscle to effectively adapt to the inconsistent adaptations associated with

strength and endurance training (i.e. the chronic hypothesis). Alternatively, fatigue from endurance training bouts may compromise effective tension development during subsequent strength training bouts (i.e. the acute hypothesis) (Leveritt et al. 1999). Alternatively, it is worth noting that the magnitude of adaptation within a physiological system subsequent to training is highly dependent upon the baseline status of the system, or natural endowment (Kraemer 2000). Therefore, since it is well-established that baseline MIP is not related to endurance training status, height, nor body mass (Coast et al. 1990; Eastwood et al. 2001; McConnell et al. 1997; McCool et al. 1997; Robinson and Kjeldgaard 1982), it is possible that the high baseline MIP reported by Sonetti et al. (2001) [about 144% of predicted (Wilson et al. 1984)] reflected a genetic predisposition and that this presented a smaller window for physiological adaptation with inspiratory muscle training.

An important consideration when assessing changes in MIP due to inspiratory muscle training is that related to the considerable learning effect that sometimes accompanies MIP manoeuvres. For example, 16 female swimmers underwent a 2 week learning period (3 sessions·wk^{-1}) that was designed to accustom subjects to the MIP manoeuvre prior to an inspiratory muscle training regimen. During this learning period MIP increased from 108 (SD 20) to 142 (SD 28) cmH_2O (+31%) (Clanton et al. 1987). Although neural adaptation might account for some of this improvement, a little caution appears warranted when using MIP measurements to assess inspiratory muscle strength.

An additional benefit of inspiratory muscle strength training is that inspiratory muscle endurance may also improve. Williams et al. (2002) utilised a 4 week pressure threshold inspiratory muscle training regimen in 7 endurance-trained runners. Training sessions were performed 4-5 d·wk^{-1}, lasted approximately 25 min and comprised 5-7 sets of 4-5 min loaded breathing (50% MIP) with 1-2 min rest between each set. Inspiratory

muscle endurance was assessed at 60% MIP with duty cycle and f_R set at 0.5 and 22 breaths·min^{-1}, respectively. A measure of MIP was made every minute and breathing endurance time was recorded as the time to which two consecutive MIP recordings were ≤80% of baseline MIP. Following training MIP and inspiratory muscle endurance increased by 31 and 128%, respectively. These findings concur with other studies in which leg strength training was shown to improve cycling and running endurance (Tanaka and Swensen 1998). It is likely that the maximal sustainable force, or critical power, is relative to maximum force, thus an increase in MIP should result in an increase in maximum sustainable MIP, and subsequently inspiratory muscle endurance to a given submaximal load (Clanton 1995). Improved muscular endurance following strength training may reflect fewer myofibres being recruited for a given load, and/or in a reduction in the force contribution from each active myofibre (Tanaka and Swensen 1998). The consequence of this is that there would be less metabolic disturbance experienced by the muscle as a whole.

1.5.3 Specificity of respiratory muscle training

Training specificity, which refers to the distinct adaptations that occur within a physiological system in response to the training stimuli, is an established training principle for peripheral skeletal muscle (Morrissey et al. 1995). The force-velocity relationship for concentric muscle action describes how force production is inversely related (hyperbolically) to muscle shortening velocity, probably because of a progressively smaller number of cross-bridge contacts on actin filaments (Hunter 2000). Training-induced changes in this relationship are dependent upon the type of muscle contraction performed. For example, isometric resistance training improves maximal muscle force but has little effect on maximal muscle shortening velocity. However, as a result of the increase in

maximal muscle force, there is also an increase in the velocity of muscle shortening against, primarily, high resistances, with smaller improvements being observed towards maximal muscle shortening velocity (Duchateau and Hainaut 1984). Conversely, dynamic training with low resistances and high velocities of muscle shortening increases maximal muscle shortening velocity, but causes only minor improvements in maximal muscle force. However, in contrast to that observed for isometric training, muscle shortening velocity increases, primarily, against light resistances, with improvements becoming less discernable towards maximal muscle force (Duchateau and Hainaut 1984).

The force-velocity relationship described for limb skeletal muscle is proportional to the pressure-flow relationship for the inspiratory muscles contracting in synergy. Moreover, analogous to that described for limb skeletal muscle, adaptations to inspiratory muscle training are dependent upon the pressure-flow (i.e. force-velocity) characteristics of the training stimulus. For example, daily training comprising 10 sets of three MIP manoeuvres at RV over 9 weeks improved MIP by 41%, but had no effect on PIF. Conversely, daily training comprising maximal, dynamic inspiratory efforts from RV to TLC with no added external resistance (i.e. low-pressure, high-flow manoeuvres) improved PIF by 18%, but had no effect on MIP (Romer and McConnell 2003).

Inspiratory muscle strength training is also lung volume specific, with greater improvements in MIP occurring at the lung volume at which training takes place (Tzelepis et al. 1994). This finding is analogous to the length specificity of training described for limb skeletal muscle, for which strength gains resulting from isometric resistance training are greatest at the angle at which training takes place, with smaller strength gains being achieved about this angle (Thépaut-Mathieu et al. 1988). Jones and Round (1990) propose that for a compound muscle group (i.e. comprised of several muscles), each muscle demonstrates an individual force-length relationship, which combine to produce the force-

length relationship for the entire group. Training at specific muscle lengths may thus result in preferential adaptation within one of the muscles, subsequently causing an alteration in the force-length relationship of the whole muscle group (Figure 1.17).

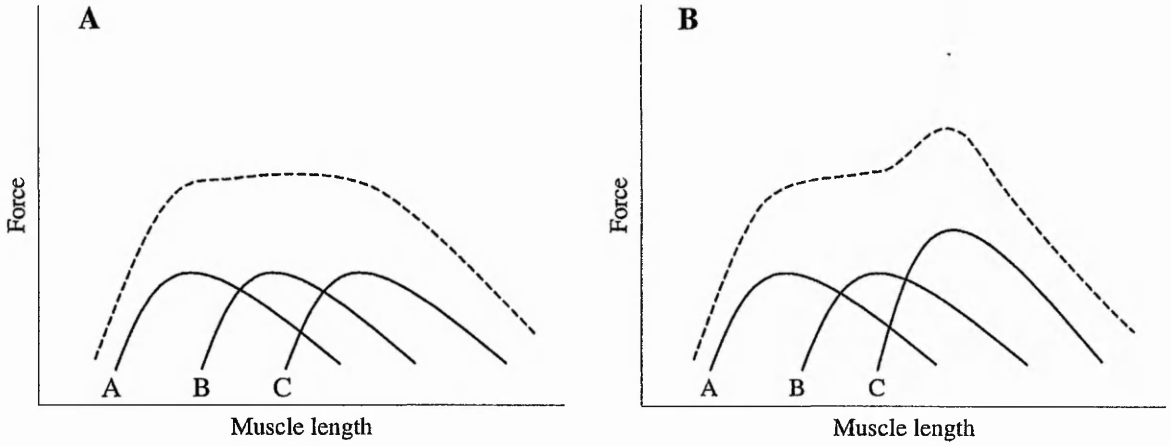

Figure 1.17 The potential effect of selective muscle adaptation on the force-length relationship of a compound muscle group. The muscle is composed of 3 muscles, A, B, and C, each possessing individual force-length properties, which combine to form the force-length relationship for the muscle group (dashed line). A, pre-training; B, post-training. (From Jones and Round 1990).

1.5.4 RESPIRATORY MUSCLE STRENGTH TRAINING: NEURAL VS. HYPERTROPHIC ADAPTATIONS

Significant improvements in MIP are often observed within just 1-4 weeks of inspiratory muscle strength training (Huang et al. 2003; Kellerman et al. 2000; Suzuki et al. 1993; Volianitis et al. 2001). Such an abrupt increase in strength during the early stages of strength training is usually ascribed to neural adaptation, with muscle hypertrophy progressively predominating after 4-6 weeks of training (Sale 1988). The view that neural adaptation accounts for most of the increase in strength during the early stages of training is supported by several observations. For example, increases in strength usually precede muscle hypertrophy during the first few weeks of training (Enoka 1997). Additionally, studies have recorded, via surface electrodes placed over the active muscle, an increase in

electromyographic activity (i.e. more motor units recruited and/or active motor units firing at higher rates) following strength training (Sale 1988). Further support for neural adaptation comes from the observation that unilateral strength training increases contralateral strength by about 8% (Munn et al. 2004). This phenomenon, known as "cross education", occurs without any change in the characteristics of the contralateral muscle fibres. Perhaps the most intriguing finding that substantiates neural adaptation is that observed in subjects performing "imagined" training. Yue and Cole (1992) had subjects train a left hand muscle (abductor digiti minimi) 5 $d \cdot wk^{-1}$ for 4 weeks. One training group performed 15 maximal isometric contractions with 20 s rest between efforts, whereas another training group performed 15 imagined maximal contractions with 20 s rest between each imagined effort. Electromyographic recordings were made to ensure that imagined contractions did not result in muscular contraction of the test muscle. Following training maximum isometric force of the trained muscle increased 30 and 22% in the contraction and imagining group, respectively.

Although neural adaptation is an acknowledged consequence of strength training regimens, the mechanisms responsible are less well understood. Moreover, empirical studies have focused exclusively on limb skeletal muscle, thus we can only speculate that similar mechanisms explain early improvements in inspiratory muscle strength during inspiratory muscle training. One possible mechanism of neural adaptation could reside in an attenuation of reciprocal inhibition, which describes the co-contraction of antagonists during contraction of agonists. This adaptation may indeed contribute to early increases in MIP since for limb skeletal muscle, a reduction in reciprocal inhibition has been noted following just 1 week of strength training (Carolan and Cafarelli 1992). Alternatively, strength training may attenuate the influence of autogenic inhibition, which describes the inhibitory mechanisms in the neuromuscular system that prevent excessive, potentially

damaging forces being applied by muscles to bones and connective tissues (Wilmore and Costill 2004). For example, the influence of Golgi tendon organs might partly explain why many untrained individuals are incapable of activating all motor units (particularly high threshold units) in prime movers during maximum voluntary contractions (Enoka 1997). A reduction in the activity of Golgi tendon organs could thus account, in part, for the attenuation of autogenic inhibition (quantified by an increase in electromyographic activity) following strength training (Aagaard et al. 2000), which subsequently allows for greater activation of motor units. Another potential mechanism of neural adaptation could reside in the principle of training specificity. For instance, improvements in strength are often greatest when training and strength evaluation exercises are similar (Morrissey et al. 1995), which suggests more optimal patterns of muscle recruitment are achieved for tasks that resemble the training task.

In contrast to the rapid manifestation of neural adaptation with strength training, adaptive changes within muscle (i.e. hypertrophy) occur more slowly and, for limb skeletal muscle, are not usually detected until 4-6 weeks of training (Sale 1988). This might explain why MIP does not improve further when pressure threshold inspiratory muscle training is performed beyond 6 weeks (Romer and McConnell 2003; Volianitis et al. 2001). Although speculative, these latter observations could further suggest that most of the increase in MIP is related to neural mechanisms. However, note that the initial hypertrophic response of a muscle fibre may involve an increase in size at the expense of the extracellular space (Åstrand et al. 2003). Therefore, despite no change in muscle cross-sectional area, muscle hypertrophy may actually occur prior to 4-6 weeks of training. Indeed, preliminary evidence suggests that inspiratory muscle hypertrophy may result from inspiratory muscle training. Rollier et al. (1998) examined the effects of an 8 week inspiratory flow resistive training regimen (30 $min \cdot d^{-1}$, 3 $d \cdot wk^{-1}$, at a resistance approximating –2.5 cmH_2O) on

diaphragmatic fibre dimensions in the rat. Compared to controls, trained animals demonstrated a 44, 34, and 32% greater cross sectional area of type I, IIA, and IIX fibres, respectively. However, species differences make it difficult to extrapolate these findings to humans.

The only published study to investigate the effects of inspiratory muscle strength training on human inspiratory muscle morphology is that of Ramírez-Sarmiento et al. (2002). These authors had chronic obstructive pulmonary disease patients perform intermittent inspiratory flow resistive loading (3 min training periods with 2 min rest intervals) for 30 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$ for 5 weeks, at an intensity of 40-50% MIP. Open biopsies taken from the external intercostals before and after training revealed significant increases in the proportion of type I fibres (approximately 38%) and the size of type II fibres (approximately 21%). These adaptations were likely to have contributed to the approximate 29 and 100% increases in, respectively, MIP and breathing endurance time against a constant inspiratory load of 80% MIP. Given that muscle hypertrophy is thought to require at least 4-6 weeks of strength training, it is somewhat surprising that Ramírez-Sarmiento et al. (2002) observed an increase in type II fibre size following just 5 weeks of RMT. Furthermore, it is worthy of note that hypertrophy of inspiratory muscle fibres had probably occurred prior to the fifth training week. However, the notion that muscle hypertrophy requires 4-6 weeks of resistance training is based primarily on studies performed on limb skeletal muscle in which 2-3 training sessions were performed per muscle/muscle group, per week. Muscle fibre hypertrophy is not only dependent upon exercise intensity, but also the number of workouts performed, with hypertrophy of limb skeletal muscle typically requiring more than 16 training sessions (Staron et al. 1994). Thus because inspiratory muscle training studies typically comprise 5-7, and sometimes 14, training sessions per week, muscle hypertrophy might occur much earlier than

previously anticipated. Alternatively, given their status as essential skeletal muscles, the respiratory muscles may be more plastic and capable of a quicker hypertrophic response than other skeletal muscles. These possibilities warrant further study.

1.5.5 Effects on endurance exercise performance

Since RMT studies have utilised different RMT regimens, different tests of exercise performance, and subjects of varied endurance training status, it is unsurprising that the effects of RMT on exercise endurance are somewhat controversial (McConnell and Romer 2004a). Preliminary RMT studies utilising 3-4 weeks of voluntary hyperpnoea training reported no significant change in high-intensity (90% $\dot{W}$max or 95% $\dot{V}O_2$peak) cycling endurance (Fairbarn et al. 1991; Morgan et al. 1987). These studies were, however, limited by small sample sizes (4-5 RMT subjects and 5 control subjects), which would have reduced the statistical power and increased the probability of making a type II error. Indeed, although reported as non-significant, cycling endurance time in Fairbarn et al.'s (1991) study improved from 342.2 to 427.8 s (+25%). In contrast to these initial investigations, subsequent studies performed by Boutellier and co-workers have demonstrated consistent improvements in cycling endurance following voluntary hyperpnoea training. For example, Markov et al. (2001) noted an increase in cycling endurance at 70% $\dot{W}$max from 35.6 to 44.2 min (+24%) following 40 sessions of voluntary hyperpnoea training performed over 15 weeks in 15 sedentary subjects. These improvements exceeded changes in a non-training control group. Boutellier and Piwko (1992) demonstrated an increase in cycling endurance at 80% physical working capacity 170 (i.e. the exercise intensity corresponding to a heart rate of 170 beats·min^{-1} during cycling) from 26.8 to 40.2 min (+50%) in 4 sedentary subjects. However, the small sample size and lack of a control group somewhat undermines the significance of these results. In

an uncontrolled study, Boutellier et al. (1992) had 8 trained cyclists/triathletes perform 4 weeks of voluntary hyperpnoea training consisting of breathing for 30 min at the highest sustainable $\dot{V}_E$ (range 85-160 $l \cdot min^{-1}$), 5 $d \cdot wk^{-1}$. Cycling endurance at approximately 77% $\dot{V}O_2$ peak increased following training from 22.8 to 31.5 min (+38%). Spengler et al. (1999) reported a comparable improvement [20.9 (SD 5.5) vs. 26.6 (SD 11.8) min, +27%) in cycling endurance at 85% $\dot{W}$ max in trained endurance athletes following an identical training regimen. Intriguingly, these improvements exceed the 15% (SD 4) increase in high-intensity (90% $\dot{V}O_2$ peak) cycling endurance observed when inspiratory muscle work was reduced by about 50% using proportional assist ventilation (Harms et al. 2000). Some suggest that it seems inconceivable that the effects of RMT would exceed those observed with mechanical unloading (Sonetti et al. 2001). However, the effects of mechanical unloading were examined at near-maximal exercise intensities, whereas the aforementioned RMT studies employed exercise tests of more moderate intensity. Furthermore, the mechanisms by which these distinct interventions contribute to improving exercise tolerance may differ. These considerations therefore preclude direct comparison between these studies.

It is crucial to note that in contrast to Fairbarn et al. (1991) and Morgan et al. (1987), who evaluated exercise endurance at near-maximal intensities, the aforementioned RMT studies evaluated exercise endurance at more moderate work-rates. It is thus possible that the intensity of the exercise performance evaluation test partly mediates the efficacy of RMT. Indeed, McConnell and Romer (2004a) propose that improvements in high-intensity endurance exercise tolerance following RMT are, if at all present, likely to be small. This is because high-intensity tests have end-points that are primarily determined by intolerable sensations associated with limitations to both oxygen transport and the mechanisms responsible for maintaining a tolerable, cellular environment that does not limit muscle

contraction. Accordingly, the efficacy of RMT might be expected to be more apparent when the performance evaluation test is submaximal and prolonged.

Thus far, the efficacy of RMT has been primarily assessed using leg cycling ergometry exercise. Williams et al. (2002), on the other hand, examined the effects of pressure threshold inspiratory muscle training (see section 1.5.2 for details of training protocol) on running endurance at 85% $\dot{V}O_2$peak in 7 endurance trained athletes [$\dot{V}O_2$peak = 59.9 (SD 11.7) $ml \cdot kg^{-1} \cdot min^{-1}$]. Although MIP and inspiratory muscle endurance increased by 31 and 128%, respectively, running performance remained unchanged at about 20 min. Subsequently, Williams et al. (2002) speculated that their highly trained athletes were, by virtue of their impressive baseline MIP (140% of the predicted value), perhaps already resistant to exercise-induced inspiratory muscle fatigue, and therefore derived minimal benefit from inspiratory muscle training. However, this contention remains highly speculative since Williams et al. (2002) did not assess exercise-induced inspiratory muscle fatigue pre- and post-RMT. Nevertheless, the possibility exists that subjects with high baseline MIP experience smaller training effects with inspiratory muscle strength training, both in terms of MIP (Sonetti et al. 2001) and whole-body endurance exercise performance. That small RMT-induced improvements in inspiratory muscle strength preclude substantial improvements in exercise endurance might not be surprising given that the relative reduction in specific markers of exercise intolerance, such as $[lac^-]_B$, and perceived limb and respiratory effort, are inversely related to the relative improvement in MIP (Romer et al. 2002c; Williams et al. 2002). However, it is also possible that the choice of performance evaluation test (constant-load test vs. time-trial) influences the outcome of RMT studies (McConnell and Romer 2004a), thus Williams et al. (2002) may have observed an improvement in exercise performance had a time-trial performance measure been utilised (this possibility is discussed in chapter 4). Another

possible explanation for the results of Williams et al. (2002) is that the efficacy of RMT may depend, in part, on the exercise modality of the performance evaluation test. Specifically, since greater inspiratory muscle fatigue is observed following heavy cycling compared to running, even when both activities are performed at the same relative intensity (Boussana et al. 2001), RMT may benefit cycling performance more than running performance. However, this assertion is grounded on assumption that reduced exercise-induced respiratory muscle fatigue is the primary mechanism by which RMT improves exercise endurance. This, however, remains unconfirmed.

A criticism relevant to all RMT studies cited above is that they evaluated exercise endurance using fixed work-rate exercise tests. Although such tests are useful when examining physiological changes following an intervention, they may be criticised for their poor reproducibility and lack of external validity. Jeukendrup et al. (1996) reported a coefficient of variation of 26.6% for 6 cycling tests performed to volitional tolerance at a fixed work-rate of 75% $\dot{V}O_2$peak, whereas the coefficient of variation for 6 tests requiring the completion of a preset amount of work (approximately 1 h duration) was only 3.35%. A low coefficient of variation (1.0-1.1%) was also reported in trained cyclists performing three 20 and 40 km time-trials (Palmer et al. 1996), whereas Hill and Smith (1999) reported a 27% increase in cycling endurance in the second of two trials at critical power (fixed work-rate test). This latter improvement, ascribed to a practice effect, is comparable to that observed in most voluntary hyperpnoea RMT studies. In addition, since constant-load tests also bear little resemblance to competitive performance, the external validity of the findings is somewhat limited. A further limitation pertinent to the above RMT studies is that they utilised either no control group, or a control group that did not perform an intervention, which makes it difficult to demarcate improvements in performance due to RMT, and improvements in performance due to placebo and/or practice effects.

In light of these experimental deficiencies, several recent studies have thus employed true placebo groups and time-trial based tests to evaluate performance. Following an 11 week pressure threshold inspiratory muscle training regimen, Volianitis et al. (2001) observed a 3.5% improvement in the distance covered during a 6 min all-out rowing test in trained female rowers, which exceeded the 1.6% improvement observed in the placebo group. Romer et al. (2002b) examined the effects of 6 weeks pressure threshold inspiratory muscle strength training or placebo on 20 and 40 km cycling time-trial performances in trained cyclists. Following RMT, 20 and 40 km time-trial performances improved by 3.5 and 3.4%, respectively, whereas no changes were observed in the placebo group. Changes in performance were also different between groups. It has been suggested that for an elite athlete, a performance enhancement is worthwhile if it is at least one half of the typical variation between individual performances (Hopkins et al. 1999). For time-trials lasting approximately 30-60 min competitive male cyclists have a typical variation in performance of approximately 1-3.4% (Jeukendrup et al. 1996; Palmer et al. 1996; Paton and Hopkins 2001), thus the improvement in performance reported by Romer et al. (2002b) would likely constitute a worthwhile enhancement.

Although the above RMT studies were rigorously designed and well-controlled, a potential limitation may reside in their placebo design, which involved training with negligible inspiratory resistance. This "minimal exercise" training regimen has been criticised by some investigators (Sheel 2002; Sonetti et al. 2001) on the basis that it may not activate important placebo factors, such as expectation, although no studies have attempted to measure this directly. However, in this context it is worth noting that significant placebo effects have been previously associated with the administration of anabolic steroids (Ariel and Saville 1972) and sports drinks (Clark et al. 2000). In the latter study trained cyclists demonstrated a 1.5% improvement in 40 km cycling time-trial

performance when a sham sports drink was ingested during exercise. Therefore, to resolve this ambiguity, Sonetti et al. (2001) examined whether placebo effects partly account for improvements in exercise endurance following RMT. Trained cyclists breathed through an inspiratory pressure threshold device (resistance removed) that contained aquarium gravel, which was purported to reduce the oxygen content of inspired air, thus mimicking intermittent hypoxic (i.e. altitude) exposure. Training was performed for 30 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$ for 5 weeks. The RMT group performed concurrent respiratory muscle strength and endurance training. Exercise performance, evaluated using an 8 km cycling time-trial, improved significantly after RMT by 1.8% (8 of 9 subjects improving performance), whereas no significant improvement was observed in the placebo group. However, 5 of 8 placebo subjects did improve performance and overall changes in performance were not different between RMT and placebo groups. Sonetti et al. (2001) thus concluded a placebo effect to be a primary mechanism by which RMT improves endurance performance. It is intriguing that the performance enhancement observed by Sonetti et al. (2001) is comparable to that observed following the ingestion of a sham sports drink (Clark et al. 2000). However, it is also interesting that breathing endurance at 90% MVV increased non-significantly by 74 [pre vs. post:- 0.66 (SD 0.33) vs. 1.15 (SD 0.94) min] and 78% [2.00 (SD 2.93) vs. 3.55 (SD 4.39) min] following RMT and placebo regimens, respectively. McConnell and Romer (2004a) speculate that the improvement in ventilatory endurance observed in the placebo group could be representative of a training effect (due to the small resistance inherent to the placebo training device), which subsequently contributed to improving exercise endurance in some subjects. However, by their own admission Sonetti et al. (2001) state that breathing endurance tests performed prior to the intervention were highly variable between trials conducted on different days (coefficient of variation ± 163%). This may explain the relatively large, but non-significant, improvement

in breathing endurance. It is possible that such variability was related to the lack of control of breathing pattern during the breathing endurance tests (only $\dot{V}_E$ was controlled). Clanton (1995) suggests that breathing endurance at high $\dot{V}_E$ is partly dependent upon breathing strategy (respiratory muscle recruitment patterns, adopted V_T and f_R, and end-expiratory lung volume), which may change with repeat testing and contribute to improved endurance. The changes in breathing endurance reported by Sonetti et al. (2001) may thus be somewhat misleading. Nevertheless, the issue of whether placebo effects explain the efficacy of RMT could perhaps be resolved using well-controlled animal studies, in which placebo effects are removed.

A recently completed study (McConnell and Sharpe 2005) examined the effects of pressure threshold inspiratory muscle training on the maximal lactate steady state cycling power. It is attractive to speculate that RMT increases maximum lactate steady state, since cycling time-trial performances of approximately 30-60 min duration are intuitively performed close to this intensity (Harnish et al. 2001), and Romer et al. (2002b) showed significant improvements in 20 and 40 km cycling time-trials following pressure threshold inspiratory muscle training. Moreover, due to the hyperbolic relationship between work-rate and time to exercise intolerance (Hill 1993), only small increases in maximum lactate steady state would be necessary for marked improvements in submaximal exercise tolerance to be observed, with performance gains becoming less discernable with progressive increments in work-rate. An increase in maximum lactate steady state work-rate could thus explain the greater ergogenicity of RMT when performance evaluation tests are prolonged and submaximal (McConnell and Romer 2004a). However, despite this possibility McConnell and Sharpe (2005) observed no change in the maximum lactate steady state cycling power following inspiratory muscle training. However, the authors speculate that only subtle changes in maximum lactate steady state (i.e. <2.5%) may occur

with inspiratory muscle training, and that such minor alterations may go undetected. It is unclear, however, whether such a minor increase would constitute a worthwhile improvement, since there is limited knowledge of the interrelationship between minor increases in maximal lactate steady state and performance enhancement.

In summary, although the reserve to improve respiratory muscle function through RMT is substantial, the effects of such training on endurance exercise performance remain somewhat debated. These discrepancies are not, however, surprising considering the diverse methodologies employed in different studies. The ergogenicity of RMT appears more discernable when the performance evaluation test is of moderate intensity, with performance gains becoming less discernable at higher work-rates. Thus there appears to be certain conditions under which RMT will enhance performance. Significant improvements in rowing and cycling time-trials have been noted following pressure threshold inspiratory muscle training, although in some studies similar improvements have been observed in placebo-trained participants. However, the general consensus is that under certain conditions RMT may provide a beneficial adjunct to regular whole-body training regimens. Further study should examine whether time-trial based performance is improved following voluntary hyperpnoea training.

1.5.6 Mechanisms of action

What mechanisms explain the ergogenic effects of RMT? The preceding section highlighted the potential significance of placebo effects, although more research is warranted to further investigate this phenomenon. It is generally agreed that RMT does not improve $\dot{V}O_2$peak, maximal exercise $\dot{V}_E$, cardiovascular function (stroke volume, heart rate, and cardiac output), blood-gas concentrations, or SaO_2 during exercise (McConnell and Romer 2004a). The following subsections thus address several other possibilities.

1.5.6.1 DECREASED BLOOD LACTATE CONCENTRATION

Several studies have reported a 1-2.5 $mmol \cdot l^{-1}$ reduction in the exercise $[lac^-]_B$ following both respiratory muscle endurance training (voluntary hyperpnoea) and inspiratory muscle strength training (pressure threshold loading) (McConnell and Sharpe 2005; Romer et al. 2002c; Spengler et al. 1999; Volianitis et al. 2001). Spengler et al. (1999) propose that following voluntary hyperpnoea training the respiratory muscles possess greater capacity to consume and metabolise lactate. However, a training-induced increase in intramuscular lactate clearance primarily results from increased lactate oxidation (Bergman et al. 1999), thus if voluntary hyperpnoea training resulted in greater lactate clearance by respiratory muscles, this would probably occur through increasing mitochondrial density and associated constituents, such as mitochondrial lactate dehydrogenase and monocarboxylate transporter proteins (MCT's) (Bergman et al. 1999; Dubouchaud et al. 2000). At a given level of ventilatory work, these mechanisms would also contribute to decreased lactate production and efflux from respiratory muscles. Whether RMT increases mitochondrial density within respiratory muscles is unknown. However, since regular whole-body endurance training augments the oxidative capacity of the respiratory muscles (Powers et al. 1997), it seems inconceivable that isolated respiratory muscle endurance training would not promote similar, if not greater, adaptation.

That $[lac^-]_B$ declines following pressure threshold inspiratory muscle strength training is intriguing and difficult to explain. A reduced $[lac^-]_B$ during leg cycling exercise has also been observed following leg strength training (Marcinik et al. 1991). Although enzymatic adaptations within muscle are an unlikely consequence of strength training regimens (Tesch 1988), recent studies have shown an increased expression of MCT1 and MCT4 proteins in human skeletal muscle following high-intensity exercise training. Juel et al. (2004) had subjects perform 8 weeks of one-legged knee extensor exercise training 3-5

times·wk^{-1} (the untrained leg serving as control). Each training session comprised 15 × 1 min exercise bouts at 150% of thigh $\dot{V}O_2$peak, separated by 3 min of rest. Following training, membrane contents of MCT1 and MCT4 were 115 and 111%, respectively, of the vastus lateralis of the trained compared to the control leg. During a subsequent incremental, single leg exercise test, the trained leg demonstrated greater rates of lactate release, despite the lactate gradient from muscle to blood being lower in the trained compared to the untrained leg. Moreover, during a submaximal (30 W) single leg exercise test there was greater lactate clearance by the trained compared to the untrained leg. These findings suggest that lactate transport capacity can be augmented following strength-based training regimens. However, as already discussed increased lactate clearance following training primarily results from increased intramuscular lactate oxidation, but there is little evidence of increased mitochondrial density in limb skeletal muscle following strength training (Tesch 1988). It thus remains unclear whether inspiratory muscle strength training increases mitochondrial density and mitochondrial MCT1 contents in inspiratory muscles.

An alternative/additional mechanism by which RMT reduces $[lac^-]_B$ may reside in a change in locomotor muscle blood flow. If RMT improved ventilatory efficiency, then the reduction in metabolic demand could free blood flow to active locomotor muscles (Sheel 2002). Additionally, if respiratory muscle fatigue causes, via a metaboreflex, an attenuation of leg blood flow (Harms et al. 1997), then attenuating such fatigue could serve to maintain limb perfusion. These changes may increase the extra-to-intramuscular lactate gradient and thus augment lactate uptake by working limb muscles. However, whether respiratory muscle recruitment patterns or cardio-pulmonary interactions are influenced by RMT remains to be addressed.

Although reduced $[lac^-]_B$ is frequently observed following RMT, it is unclear whether this makes a major contribution to enhancing exercise endurance. For example,

Kohl et al. (1997) observed a lower $[lac^-]_B$ during exercise following RMT, but no change in cycling endurance. Conversely, McMahon et al. (2002) and Sonetti el. (2001) observed significant post-RMT improvements in endurance exercise performance in the absence of any change in $[lac^-]_B$.

1.5.6.2 Attenuated respiratory muscle fatigue

Given the potential physiological implications of exercise-induced respiratory muscle fatigue (see section 1.4), the avoidance or delay of such fatigue could represent a key underlying mechanism by which RMT improves exercise endurance. Unfortunately, no objective measures of respiratory muscle function, such as Pdi during phrenic nerve stimulation, have been performed pre- and post-RMT. Instead, studies have relied on volitional MIP measurements to assess the effects of RMT on inspiratory muscle fatigue. Volianitis et al. (2001) recorded, at baseline, an 11.2 and 11.1% reduction in MIP following a 6 min all-out rowing exercise in pressure threshold inspiratory muscle training and placebo groups, respectively. Subsequent to the fourth training week, these values were 3.1 and 10.7%, respectively, thus suggesting a reduction of inspiratory muscle fatigue in the RMT group. Romer et al. (2002a) observed a 17 and 18% reduction in MIP following a 20 km cycling time-trial in pressure threshold inspiratory muscle training and placebo groups, respectively. Following the intervention these values were 10 and 17%, respectively, thus again suggesting an attenuation of inspiratory muscle fatigue post-RMT. A reduction in inspiratory muscle fatigue following pressure threshold inspiratory muscle strength training might be expected since for a given ventilatory load the inspiratory muscles would be working at a lower fraction of maximum force. Although the effects of voluntary hyperpnoea training on exercise-induced respiratory muscle fatigue have not been addressed, improvements in volitional measures of ventilatory endurance (see section

1.5.2) suggest that the respiratory muscles are indeed more resilient to fatigue following training. Moreover, tachypnoea is often observed during exercise that is preceded by voluntary fatigue of the respiratory muscles (Mador and Acevedo 1991), thus less tachypnoea during exercise following RMT (Boutellier 1998; Volianitis et al. 2001) could also be taken as evidence of a reduction in respiratory muscle fatigue.

Inspiratory muscle endurance largely depends on both the pressure generated, as a fraction of the maximum, and the duty cycle. The product of these ratios is commonly defined as the tension-time index (Clanton 1995). For a given inspiratory load the tension-time index would be lower if, following RMT, maximum inspiratory muscle force production was increased and/or duty cycle was decreased. The rate of inspiratory muscle pressure development and PIF has been shown to increase following pressure threshold inspiratory muscle training (Romer and McConnell 2003). During exercise, this may cause a reduction in duty cycle and allow expiration to be prolonged. This could serve to maintain end-expiratory lung volume, which increases during heavy endurance exercise due to expiratory flow limitation (Johnson et al. 1992) and subsequently makes the inspiratory muscles more susceptible to fatigue (Roussos et al. 1979; Tzelepis et al. 1988).

1.5.6.3 Attenuated perceptual response to exercise

A common finding is that both the perception of limb and respiratory effort during exercise are reduced following RMT. When averaged across all workloads, Romer et al. (2002b) observed a 16 and 18% reduction in dyspnoea and perceived limb discomfort, respectively, during incremental cycling exercise following pressure threshold inspiratory muscle training. Similarly, reductions of 7.9 and 7.2% in dyspnoea and limb discomfort, respectively, were observed throughout 20 min of shuttle running following pressure threshold inspiratory muscle training (Romer et al. 2002c). The extent to which exercise-

induced dyspnoea declines following pressure threshold training depends, in part, on the relative improvement in MIP (Romer et al. 2002c; Williams et al. 2002). Surprisingly, few studies have examined the effects of voluntary hyperpnoea training on the perceptual response to exercise; however, in one study subjects reported that they were experiencing less breathlessness during their habitual training (Boutellier et al. 1992).

The mechanisms by which RMT reduces the perceptual response to exercise are not readily forthcoming. It is suggested that acidosis activates group III and IV intramuscular nerve afferents, which contributes to the sensation of muscular effort during exercise (Westerblad et al. 2002). Studies showing an attenuated perceptual response to exercise following RMT often note a concurrent decline in $[lac^-]_B$, which implicates a concomitant reduction in $[H^+]$ (Robergs et al. 2004). Accordingly, a reduced accumulation of metabolic end-products in respiratory and locomotor muscles might provide less sensory input into the central nervous system, and therefore result in a reduction in the overall sensation of effort. However, the conscious perception of muscular effort is likely to involve not only feedback (i.e. sensory) mechanisms related to muscle contraction, but also feedforward mechanisms related to central motor command (American Thoracic Society 1999). Indeed, dyspnoea is perhaps mediated by a conscious awareness of the outgoing motor command via corollary discharge from respiratory neurones to the sensory cortex (El-Manshawi et al. 1986). It is perhaps not surprising, therefore, that dyspnoea is exacerbated during exercise that is either preceded by voluntary fatigue of the respiratory muscles (Mador and Acevedo 1991), or which is performed with inspiratory muscle loading (Harms et al. 2000), since a greater motor drive to the inspiratory muscles would be required to achieve the same mechanical output. These observations probably explain why strengthening of the inspiratory muscles attenuates the perception of respiratory effort, since the proportion of the maximum force utilised for a given inspiratory load, and subsequently the outgoing

motor command, would be lowered (Kellerman et al. 2000). Consistent with this notion, Huang et al. (2003) noted a 22% reduction in inspiratory motor drive [measured as the pressure generated after the first 0.1 s of inspiration when the airway is briefly occluded (i.e. $P_{0.1}$)] following a 4-week pressure threshold inspiratory muscle training regimen in healthy humans. Furthermore, the decline in $P_{0.1}$ was significantly correlated with changes in MIP ($r = -0.45$). Thus at least under resting conditions, the motor drive to the inspiratory muscles is lower following strengthening of the inspiratory muscles. It thus seems reasonable to speculate that a reduction in inspiratory motor drive might explain, in part, reduced dyspnoea during exercise even when $\dot{V}_E$ is unchanged (Romer et al. 2002b).

Dyspnoea may also arise through altered respiratory muscle recruitment, such as increased recruitment of extradiaphragmatic muscles (Johnson et al. 1996). If the plateau in diaphragm pressure output relative to total respiratory muscle pressure output during heavy exercise (Johnson et al. 1993) were a consequence of diaphragm fatigue, then reducing such fatigue might delay/reduce extradiaphragmatic muscle recruitment. However, Wetter and Dempsey (2000) propose that an RMT-mediated decline in dyspnoea would only benefit performance if dyspnoea contributed to exercise limitation prior to RMT. Note, however, that although most healthy subjects report leg discomfort as the primary cause of exercise cessation, dyspnoea may curtail performance even though it is not always consciously perceived as being a limiting factor.

1.5.6.4 IMPROVED BREATHING EFFICIENCY

Several studies have reported a lower $\dot{V}_E$ during submaximal endurance exercise following RMT (McConnell and Sharpe 2005; Boutellier 1998). Potentially, reducing $\dot{V}_E$ would reduce the metabolic requirements of the respiratory muscles, thus 'freeing' blood flow to the locomotor muscles (Sheel 2002). However, it also seems likely that during

submaximal exercise in which there is spare capacity to increase cardiac output, the locomotor muscles are already receiving adequate perfusion and that increased available blood flow would present minimal benefit in terms of increasing oxygen delivery to locomotor muscles (Wetter et al. 1999). Indeed, although the effects of RMT on cardio-pulmonary interactions during exercise are unknown, a reduced $\dot{V}_E$ does not always accompany an improvement in exercise endurance (McMahon et al. 2002; Spengler et al. 1999), thus the significance of this adaptation is probably minor.

1.5.7 Summary

Though a body of literature supports the ergogenicity of RMT, the mechanisms explaining improvements in exercise performance remain obscure, although they are likely to be multifaceted and involve a complex interplay between respiratory muscles, active locomotor muscles, and the brain (Figure 1.18). Underlying mechanisms may also differ depending on the modality of RMT, the endurance training status of the subject, the baseline physiological status of the respiratory muscles (including genetic predisposition), and whether the subject is healthy or of clinical status.

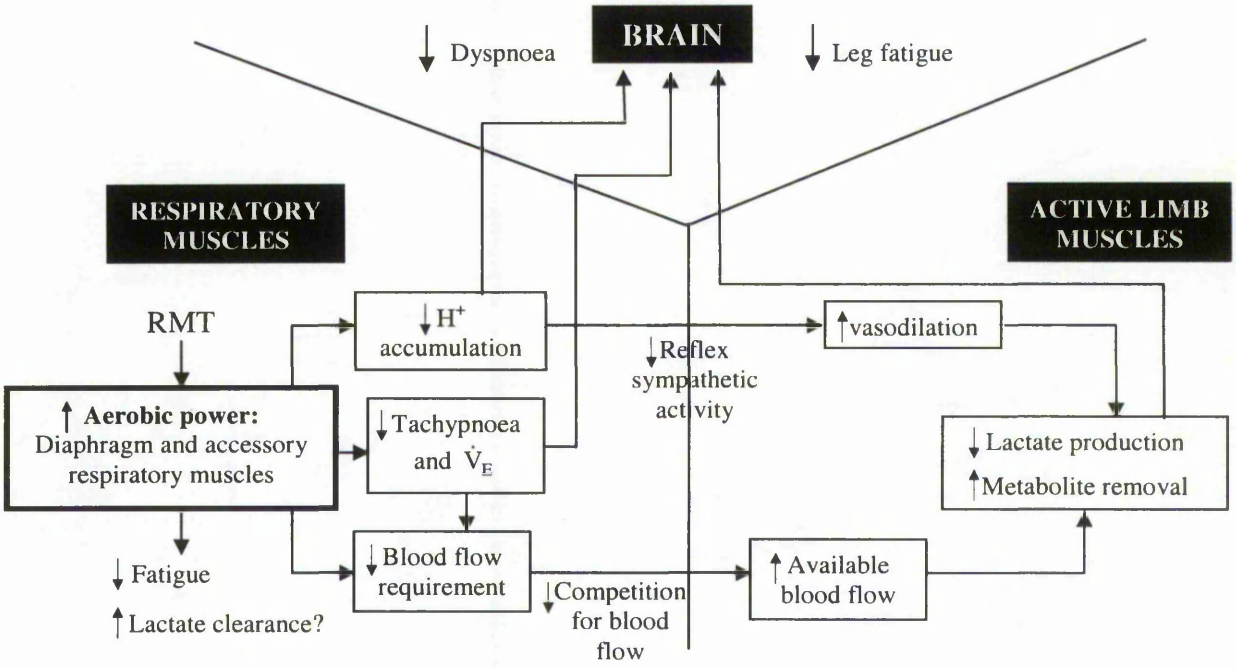

Figure 1.18 Possible effects of RMT on parameters that may affect endurance exercise tolerance. (From Wetter and Dempsey 2000).

1.6 Contribution of the respiratory muscles to lactate turnover during exercise

Landmark studies performed by the pioneer physiologists Archibald Vivian (A.V.) Hill and Otto Meyerhof in the 1920's laid the foundations upon which our knowledge of lactate has evolved (Bassett 2002), although modern perspectives differ considerably from those adopted almost a century ago. Up to the early 1970's, lactate (or lactic acid as it was more commonly described) was considered a dead-end metabolite of anaerobic glycolysis and the primary cause of muscle fatigue and the O_2 debt (Gladden 2004). However, a "lactate revolution" has occurred since the 1970's (Gladden 2004), and traditional beliefs have been challenged, and quite often disproved, subsequently leading to a shift in paradigm.

During dynamic whole-body exercise, active locomotor muscles represent the dominant influence on whole-body lactate kinetics (Bergman et al. 1999). However,

breathing also represents a form of muscular exercise, and the enormity of ventilatory work during maximal exercise is epitomised by the substantial metabolic demands of the respiratory muscles (Aaron et al. 1992; Harms et al. 1998b). Whether under these conditions the respiratory muscles become net lactate producers is highly debated. The following subsections address current concepts in lactate metabolism and kinetics during exercise, and subsequently, based on the results of animal and human studies, whether the respiratory muscles contribute to lactate turnover during exercise.

1.6.1 CURRENT CONCEPTS IN LACTATE METABOLISM AND KINETICS DURING EXERCISE

Conventional belief is that lactic acid, not lactate, is initially produced and that because of its low dissociation constant (pKa 3.9), this immediately dissociates at physiological pH to lactate and H^+ (Gladden 2004), the latter being primarily responsible for the metabolic acidosis observed in exercise (this disturbance in acid-base balance has been termed "lactic acidosis"). Lactate production is thus often linked with intracellular acidosis and muscle fatigue (Fitts 1994). However, a close examination of chemical structures shows that lactate, not lactic acid, is produced during glycolysis (Robergs et al. 2004). Moreover, recent advances in the biochemistry of muscle metabolism suggest that lactate production lowers, rather than increases, $[H^+]$. Specifically, in the lactate dehydrogenase reaction two electrons and a proton are removed from NADH, and an additional proton is utilised from solution to support the two electron and two proton reduction of pyruvate to lactate (Robergs et al. 2004). Thus the lactate dehydrogenase reaction effectively functions as a buffer against metabolic acidosis, which is thought to result primarily from ATP hydrolysis together with the hexokinase, phosphofructokinase, and glyceraldehyde 3-phsophate dehydrogenase reactions of glycolysis (Robergs et al. 2004). Lactate formation may further retard intracellular acidosis through its transportation

out of the cell, which involves the co-transportation of a proton (H^+) in a 1:1 ratio (Juel 2001). Thus lactate production and release plays an integral role in muscle pH regulation during exercise. However, whether lactate therefore adopts a protective role against muscle fatigue is unclear, since at physiological temperatures acidosis does not impair muscle function (Westerblad et al. 2002).

Traditionally, lactate production was attributed to muscle hypoxia and therefore a lack of oxygen for the energy requirements of the contracting muscles (Gladden 2004). Such thinking provided the basis of the "anaerobic threshold" concept, which theoretically describes the point during incremental exercise when metabolic acidosis and accompanying changes in pulmonary gas exchange and $\dot{V}_E$ occur because of an abrupt reliance on anaerobic metabolism (Wasserman et al. 1973). However, although glycolysis, and thus lactate production, accelerates when oxygen availability is insufficient to promote pyruvate and fat metabolism in the mitochondria (Gladden 2004), lactate production also occurs under fully aerobic conditions in which oxygen availability to muscle is not limiting (Brooks 1986). Several authors therefore refute the anaerobic threshold concept on the basis that muscle tissue is not oxygen-limited during submaximal exercise (Brooks 1985; Connett et al. 1986). For example, using proton magnetic resonance spectroscopy to determine myoglobin saturation (and thus an estimate of intracellular PO_2), Richardson et al. (1998) observed a constant intracellular PO_2 of rectus femoris muscle during single leg knee-extensor exercise (50-100% muscle $\dot{V}O_2$peak), but a linear relationship between net lactate efflux and $\dot{V}O_2$. Moreover, intracellular PO_2 remained above the critical mitochondrial O_2 tension (0.1-0.5 Torr), i.e. the PO_2 below which the maximal mitochondrial respiratory rate cannot be supported (Brooks 1985). These findings suggest that lactate production is simply a consequence of glycolytic flux, with a quantity of pyruvate always being converted to lactate according to the law of mass action. With

increments in work-rate glycolytic flux accelerates to match the demand for ATP, thus progressively more pyruvate is converted to lactate. The acceleration of glycolysis under fully aerobic conditions has subsequently been termed "aerobic glycolysis" (Conley et al. 1998). Brooks (1985) suggests that the conversion of pyruvate to lactate in fully oxygenated muscle represents an inescapable process due to lactate dehydrogenase, which has a greater catalytic activity than other glycolytic enzymes providing alternative pathways for pyruvate metabolism. Furthermore, because the K_M for pyruvate conversion to lactate is about 0.08 mmol, the maximal catalytic activity of lactate dehydrogenase can be supported by the concentrations of intramuscular pyruvate observed during even submaximal exercise. Brooks (1985) therefore describes lactate production to be "as much a part of carbohydrate metabolism as is the production of carbon dioxide from respiration", although lactate is an intermediate product rather than a waste product.

Our understanding of lactate transport has also advanced in recent years. Transmembrane lactate transport was once thought to occur by simple diffusion; however, today's consensus is that lactate transport is bi-directional (depending on transmembrane lactate and H^+ concentration gradients) and carrier-mediated by sarcolemmal monocarboxylate transporter proteins (MCT's) (Juel 2001). Moreover, because lactate transport also involves the co-transportation of a proton in a 1:1 ratio, MCT's play an important role in muscle pH regulation (Juel 1998). There are two isoforms of MCT present in human skeletal muscle, MCT1 and MCT4 (Juel 2001), which dominate in oxidative and glycolytic fibres, respectively (Pilegaard et al. 1999). Although this might suggest these transporters are specialised for lactate uptake and lactate efflux, respectively, it is now thought that both MCT1 and MCT4 are important for cell-to-cell lactate shuttling, and that lactate clearance in vivo is most likely controlled by mitochondrial uptake and oxidation (Bergman et al. 1999; Dubouchaud et al. 2000).

The facilitated exchange of lactate between lactate producing and lactate consuming cells is implicit in the cell-to-cell lactate shuttle paradigm (Figure 1.19). Originally introduced by Brooks (1986) as simply the "lactate shuttle", this hypothesis posits that lactate production during rest and exercise represents an integral mechanism by which different tissues share carbohydrate for oxidation and other processes such as gluconeogenesis. More specifically, lactate released into the interstitium (from primarily fast-twitch muscle fibres or transiently from red fibres) can be consumed and oxidised by adjacent lactate consuming muscle fibres (i.e. oxidative fibres), or alternatively, lactate released into the circulation may reperfuse active muscle beds where it is consumed and oxidised by highly oxidative fibres. Some lactate released into the circulation may also be taken up by the heart and oxidised, or the liver and kidneys where it serves as a gluconeogenic precursor.

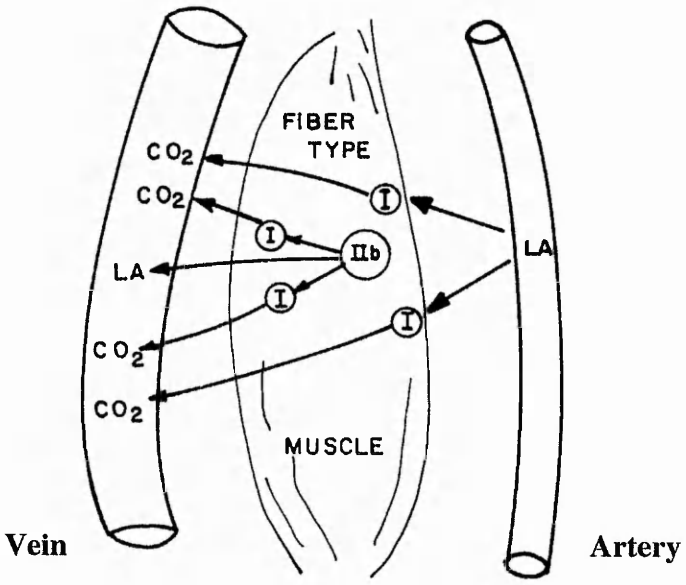

Figure 1.19 The cell-to-cell lactate shuttle. (From Brooks 1986).

Skeletal muscle represents the primary regulatory component of the lactate shuttle (Brooks 2000). Lactate tracer studies performed on humans have shown that during

moderate steady-state leg cycling exercise around 50% of lactate disappearance is explained by active-limb lactate uptake (Bergman et al. 1999). Moreover, because skeletal muscle contains a mixture of oxidative and glycolytic muscle fibres, active locomotor muscles readily engage in simultaneous lactate extraction and release during exercise (Bergman et al. 1999).

Oxidation represents the main fate of lactate during exercise and recovery. Lactate tracer studies performed on humans and rats have shown that during, and following, steady-state exercise around 75-80% of lactate removed from the circulation is oxidised (Bergman et al. 1999; Brooks and Gaesser 1980), with most of the remainder converted to glucose in the liver (Bergman et al. 2000). The notion that an increased metabolic rate increases net lactate utilisation is supported by studies showing increased muscle lactate metabolism and an accelerated decline in $[lac^-]_B$ during active, compared to passive, recovery from heavy exercise (Bangsbo et al. 1994; Dodd et al. 1984). The importance of lactate as a respiratory fuel is further exemplified by the observation that it competes successfully with glucose as a carbohydrate fuel source during moderate-intensity exercise, with lactate flux often exceeding glucose flux (Brooks 2000). Lactate thus represents a useful metabolic intermediate that can be exchanged between tissue compartments, thus providing substrate for oxidation and gluconeogenesis.

Brooks (1998) subsequently extended the cell-to-cell lactate shuttle paradigm and proposed an intracellular shuttle also, which involves cystolic lactate being taken up and oxidised within the mitochondria of the same cell. Support for the intracellular lactate shuttle hypothesis comes from studies that have identified, using a combination of Western blotting and electron microscopy techniques, MCT1 and lactate dehydrogenase in interfibrillar mitochondria of human and rat skeletal muscle (Brooks et al. 1999; Butz et al. 2004; Dubouchaud et al. 2000). Therefore, in addition to its role in cell-to-cell lactate

shuttling, MCT1, being also located in the mitochondrial membrane, also facilitates lactate uptake and oxidation in cells demonstrating high mitochondrial densities. The existence of an intracellular lactate shuttle thus suggests a link between glycolytic and oxidative pathways during exercise, such that the product of the former is the substrate for the latter.

1.6.2 LACTATE KINETICS OF THE RESPIRATORY MUSCLES: ANIMAL STUDIES

For most exercise physiologists, human respiratory muscles are relatively inaccessible during exercise in vivo, thus it is difficult to quantify intramuscular lactate kinetics. Accordingly, more information has been derived from animal experiments. Perhaps the earliest of these experiments was performed by Hollanders (1968), who using a perfused, excised rat diaphragm preparation subjected to a period of tetanus (3000 stimulations·min^{-1}), observed an increased lactate output from approximately 45 to 130 μg·min^{-1} per g wet tissue. It should be noted that the high level of lactate release observed at rest in this study was unlikely to reflect true resting conditions. Rather, because the diaphragm was unperfused (i.e. ischaemic) for approximately 4 min during the preparation period, glycolytic flux and lactate production would have increased subsequent to the reduced oxygen supply. In addition, handling of the rats prior to killing may have induced a stress response, including increased sympathetic stimulation of the adrenal medulla and a subsequent increase in adrenaline secretion. The increased adrenaline may have further stimulated glycolysis and lactate production (Hamann et al. 2001). However, although these influences were not likely to explain the marked lactate release observed during electrical stimulation, the treatment (3000 stimulations·min^{-1}) was somewhat extreme, thus the external validity of the findings is rather limited.

More recent studies have focused on diaphragmatic lactate kinetics during loaded breathing. In unanaesthetised decerebrate rats the application of a large inspiratory resistive

load (32,000 $cmH_2O \cdot l^{-1} \cdot s^{-1}$) up to the point of respiratory arrest (defined as the cessation of inspiratory efforts for 10 s) depleted diaphragm glycogen concentration by approximately 47% and increased diaphragm lactate concentration from 3.1 (SEM 0.3) (control animals) to 6.4 (SEM 0.8) $mmol \cdot g^{-1}$ (Ciufo et al. 2001). Since the $[lac^-]_B$ and lactate concentration within the soleus were not significantly altered by loaded breathing, these increases could not be attributed to, respectively, either an increased lactate extraction from the arterial blood or a non-specific effect associated with loading (e.g. hypoxaemia or increased catecholamine levels). The increased diaphragm lactate concentration was thus attributed to local lactate production. Moreover, that $[lac^-]_B$ did not change despite an increase in diaphragm lactate production suggests that, in accord with the lactate shuttle hypothesis (Brooks 2000), lactate was simultaneously oxidised within the diaphragm. In contrast to these findings, Rochester and Briscoe (1979) exposed anaesthetised dogs to carbon dioxide-induced hyperventilation and inspiratory airflow resistances and concluded, on the basis of arterial and phrenic venous measurements, that the diaphragm engaged in net uptake and utilisation of lactate. Note, however, that hypercapnia may attenuate lactate production and/or release from active tissues (Graham and Wilson 1983), thus these findings remain equivocal. Manohar et al. (1988, 1990, 1991) observed no difference in lactate concentration between arterial and phrenic venous blood in ponies exercising for 4 min at a moderate, heavy, or maximal intensity, or at the maximum workload that permitted 30 min of exercise. These observations suggest that during even maximal exercise the diaphragm refrains from lactate production and release. However, we cannot discount the possibility that lactate was simultaneously formed and oxidised within the active muscle bed (Ciufo et al. 2001). Moreover, because arteriovenous difference measurements across a muscle disregard simultaneous lactate uptake and release (Bergman et al. 1999), the findings may have been somewhat misinterpreted.

The notion that the diaphragm is a major consumer of lactate is supported by studies performed on exercising rats. Fregosi and Dempsey (1986) observed lactate accumulation in rat diaphragm following prolonged, heavy exercise [38 (SEM 3) min at 84% (SEM 2) $\dot{V}O_2$peak], although because diaphragm glycogen concentration was maintained, the elevated lactate was ascribed to uptake and subsequent utilisation from the arterial blood. However, in another study an identical exercise protocol was shown to cause marked glycogen depletion (-43%) within rodent diaphragm (Ianuzzo et al. 1987). Though the reasons for these discrepancies are not obvious, it is worth noting that Fregosi and Dempsey (1986) did observe a 25-75% fall in diaphragmatic glycogen concentration in several animals, thus there appeared to be considerable interanimal variation, which might have been partly related to differences in the endurance training status or fibre type composition of the diaphragm.

Although animal studies provide a more detailed description of respiratory muscle metabolism during exercise, caution is warranted when attempting to extrapolate these observations to humans. Firstly, reports indicate that the horse diaphragm contains only type I and IIA fibres (Cobb et al. 1994), whereas the costal and crural regions of the rodent diaphragm contain, on average, just 3.0 and 7.3% type IIX fibres, respectively (Sugiura et al. 1992). Conversely, type IIX fibres, which demonstrate higher glycolytic rates and are likely to represent the main site of lactate production (Brooks 1986), comprise approximately 23% of the total fibre pool in human costal diaphragm (Mizuno and Secher 1989). Given the paucity of type IIX fibres in rodent and equine diaphragm it is perhaps unsurprising that this muscle produces very little lactate during exercise. However, given the abundance of oxidative fibres in the pony diaphragm, it is also surprising that lactate is not extracted from the arterial blood and utilised as a respiratory fuel.

Another important consideration is that quadruped gait differs considerably from bipedal human gait. Quadruped locomotion imposes certain mechanical constraints that require a degree of locomotor-respiratory coupling; typically, quadrupeds perform one breath cycle per galloping stride, whereas humans are not restricted to a particular locomotor-respiratory coupling ratio (Bramble and Carrier 1983). As a consequence of the propulsive activity characteristic of galloping, the visceral mass of a horse is subjected to horizontal acceleration and deceleration forces (resembling a "visceral piston"), which are applied in phase and synergistically with the action of the respiratory muscles. It is thus suggested that the equine diaphragm adopts a more passive role during exercise with breathing being driven by the movements of locomotion (Bramble and Carrier 1983). However, using electromyogram analysis, studies have shown diaphragm contraction to occur in phase with Poes changes and inspiratory airflow production during exercise in horses, thus suggesting a principle role of the diaphragm in the generation of exercise hyperpnoea (Ainsworth et al. 1997). Furthermore, that the equine diaphragm makes a major contribution to pulmonary ventilation is further supported by its impressive thickness, oxidative capacity, and blood flow capacity (Cobb et al. 1994; Manohar et al. 1990; Poole et al. 2002).

Finally, it is important to highlight that most animal studies have focused their attention on diaphragmatic lactate kinetics; however, many other muscles of the trunk contribute to the exercise hyperpnoea of heavy endurance exercise, and these may make a significant, hitherto unacknowledged, contribution to lactate turnover.

1.6.3 LACTATE KINETICS OF THE RESPIRATORY MUSCLES: HUMAN STUDIES

Given the difficulties associated with measuring human respiratory muscle metabolism during exercise, studies have been resigned to investigating the effects of

changing respiratory muscle work on $[lac^-]_B$. However, note that precise lactate production rates can not be quantified from $[lac^-]_B$ measurements. For example, following endurance training whole-body or muscle lactate production during exercise at the same relative intensity does not change, but $[lac^-]_B$ is lower due to greater lactate clearance rates (Bergman et al. 1999). Furthermore, lactate can be either transported into the mitochondria of the same cell or diffuse towards and be transported into adjacent muscle fibres (Brooks 2000), thus never entering the systemic circulation. Accordingly, changes in $[lac^-]_B$ that result from either changes in respiratory muscle work, or physiological adaptation within the respiratory muscles (e.g. following RMT), warrant cautious interpretation.

Respiratory influences on $[lac^-]_B$ have been primarily examined in resting subjects performing elevated levels of ventilatory work. Eldridge (1966) had subjects exercise their respiratory muscles for 10 min through either: (I) hypoxic breathing (F_IO_2 = 0.15), (II) increased $\dot{V}_E$ (from approximately 7 to 22-25 l·min^{-1}) via 1290 ml added dead space, (III) increased $\dot{V}_E$ via 1290 ml dead space plus an inspiratory load of 21 cmH_2O, or (IV) through treatments I and III combined. There was no change in $PaCO_2$ during any intervention, and despite a decline in PaO_2 being observed during hypoxic breathing trials, values were maintained ≥70 mmHg, thus any affect on SaO_2 was probably small. A significant 0.4 (SD 0.2) mmol·l^{-1} increase in $[lac^-]_B$ was observed only for treatment IV. However, because such loaded breathing protocols fail to mimic the mechanical aspects of breathing during whole-body exercise, the external validity of these findings is limited.

A more detailed description of the effects of respiratory muscle work during exercise on $[lac^-]_B$ can be inferred from studies employing isocapnic voluntary hyperpnoea. Freedman et al. (1983) examined changes in $[lac^-]_B$ in subjects performing 10 min of maximal voluntary, isocapnic hyperpnoea with f_R set at 60 breaths·min^{-1}. $\dot{V}_E$ during volitional hyperpnoea was 108.6 (SEM 4.9) l·min^{-1}, which represented 69% (SEM 4) of the

predicted MVV. Although the former value seems somewhat low for a maximal sustainable $\dot{V}_E$, note that whilst performing these experiments subjects were semi-recumbent, which may have attenuated their ability to perform a MVV manoeuvre (Vilke et al. 2000). Nevertheless, $[lac^-]_B$ increased from 0.84 (SEM 0.09) to 1.91 (SEM 0.33) $mmol \cdot l^{-1}$ during volitional hyperpnoea, although the inter-subject variation was large, with increases ranging from 0-2.7 $mmol \cdot l^{-1}$. This variation was observed despite similar levels of $\dot{V}_E$, thus increases in $[lac^-]_B$ during volitional hyperpnoea may not be related to the magnitude of respiratory muscle work performed. Thus other factors such as differences in respiratory muscle fibre types or recruitment patterns, or endurance training status of the respiratory muscles, might explain these findings. However, a major limitation to this study is that breathing pattern during volitional hyperpnoea was not controlled (i.e. matched to the spontaneous exercise hyperpnoea). It is often assumed that the work of breathing during hyperventilation and whole-body exercise is identical at any given $\dot{V}_E$. As addressed in section 1.3, this may only be the case when breathing pattern (f_R, V_T, duty cycle) is controlled and when $\dot{V}_E$ is sufficiently low so that the tidal flow-volume loop remains within the confines of the maximal flow-volume loop, subsequently avoiding the generation of excessive, wasteful, expiratory pressures and an excessive increase in end-expiratory lung volume (Coast et al. 1993; Klas and Dempsey 1989).

It is thus clear that to accurately evaluate respiratory muscle energetics during exercise, breathing pattern during volitional hyperpnoea must be tightly controlled. This requirement was partially fulfilled by Martin et al. (1984), who had healthy subjects perform volitional hyperpnoea at 100 and 115% maximal exercise $\dot{V}_E$, with f_R matched to the exercise f_R. Maximal exercise $\dot{V}_E$ was recorded at 138 (SEM 7) $l \cdot min^{-1}$ over 1 min during a maximal 2 min running exercise. An increased $[lac^-]_B$ from 0.88 (SEM 0.08) to

1.36 (SEM 0.19) and 1.81 (SEM 0.35) mmol·l^{-1} was observed during volitional hyperpnoea at 100 and 115% maximal exercise $\dot{V}_E$, respectively, thus suggesting that the respiratory muscles may have a small role in lactate turnover during maximal exercise. Again, note the large inter-subject variation: increases in $[lac^-]_B$ during the 100% maximal exercise $\dot{V}_E$ mimic trial ranged from 0-1.7 mmol·l^{-1}, with the greatest increases being observed in subjects utilising the greatest fraction of MVV_{12} ($r = 0.76$). Although Martin et al. (1984) do not discuss this latter relationship, it is possible that the subjects who utilised the greatest fraction of MVV_{12} also utilised the greatest fraction of their maximal respiratory muscle power output, thus resulting in greater metabolic perturbations (i.e. lactate production). However, although Martin et al. (1984) matched $\dot{V}_E$, V_T, and f_R during volitional hyperpnoea to that demonstrated during spontaneous exercise, this does not guarantee the reproduction of respiratory muscle recruitment patterns. These findings therefore remain somewhat inconclusive.

Babcock et al. (1995) performed a slightly more meticulous study by having subjects mimic at rest for approximately 13 min the V_T, f_R, tidal integral of Pdi, and duty cycle achieved over the final third of maximal exercise [maximal exercise $\dot{V}_E$ = 122 (SEM 8.6) l·min^{-1}]. Following the voluntary mimic trial $[lac^-]_B$ was not different to resting values. This study thus suggests that when the breathing pattern and respiratory muscle recruitment patterns are tightly controlled, maximal exercise $\dot{V}_E$ has minimal effect on $[lac^-]_B$. However, studies that utilise volitional hyperpnoea or loaded breathing protocols at rest disregard the influence that whole-body exercise imparts on processes of lactate production, distribution, and disappearance. In particular, under resting conditions there is considerable capacity to match further lactate appearance with a proportional rate of lactate clearance. Therefore, in Babcock et al.'s (1995) study the respiratory muscles may have

produced and released lactate, but concurrent clearance by, primarily, the liver and inactive skeletal muscle, perhaps precluded an increased $[lac^-]_B$. Furthermore, respiratory and locomotor muscles are thought to compete for the available cardiac output during heavy endurance exercise (Harms et al. 1997), which could influence lactate kinetics in both muscle groups. That the respiratory muscles impart no influence on systemic lactate kinetics is also inconsistent with several studies showing an attenuated $[lac^-]_B$ during endurance exercise following specific respiratory muscle training (see section 1.5.6.1). However, even when the work of breathing is increased (via an inspiratory resistance) or decreased (via proportional assist ventilation) by 40-60% during maximal exercise $[lac^-]_B$ is not different to that observed under control conditions (Harms et al. 1998b). Note, however, that respiratory muscle recruitment patterns are likely to differ between exercise hyperpnoea and respiratory muscle loading and unloading (McCool et al. 1992). It is also possible that during maximal exercise, any additional influence that the respiratory muscles have on $[lac^-]_B$ may be masked by the contribution made by the much larger locomotor muscle mass.

Only one published study has examined changes in $[lac^-]_B$ with volitional hyperpnoea superimposed on exercise. Engelen et al. (1995) had 5 healthy, sedentary [$\dot{V}O_2$peak = 2.32 (SD 0.56) l·min^{-1}; maximal exercise $\dot{V}_E$ = 89.4 (SD 16.7) l·min^{-1}] subjects perform maximal isocapnic volitional hyperpnoea for 5 min during exercise at the "lactic acidosis" threshold [i.e. the point during exercise at which $\dot{V}CO_2$ increased out of proportion to $\dot{V}O_2$ (V-slope method): established at 94 (SD 22) W]. Although $\dot{V}_E$ was increased from approximately 38 to 70-80 l·min^{-1} during volitional hyperpnoea, $[lac^-]_B$ remained unchanged at approximately 2.0 mmol·l^{-1}. However, increases in $[lac^-]_B$ during volitional hyperpnoea at rest are often greatest in subjects that utilise the greatest fraction of MVV_{12} (Martin et al. 1984). Although Engelen et al. (1995) do not report MVV data,

the age and height of the subjects would predict MVV to reside within approximately 140-160 $l \cdot min^{-1}$ (Cotes 1993). Thus at most, subjects would have only utilised around 50% MVV during volitional hyperpnoea, which might explain the unchanged $[lac^-]_B$. In contrast to these findings, unpublished observations made by Hartley et al. (reviewed in Åstrand et al. 2003, pg 194) suggest the respiratory muscles may contribute to systemic lactate kinetics during heavy exercise. These authors had five endurance athletes ($\dot{V}O_2$peak = 4.46 $l \cdot min^{-1}$) perform 10 min of cycling exercise close to $\dot{V}O_2$peak. Baseline MVV_{30} was 211 $l \cdot min^{-1}$, whereas maximal exercise $\dot{V}_E$ averaged 159 $l \cdot min^{-1}$, or 76% MVV_{30}. On a separate occasion, during an identical exercise test, subjects voluntarily increased $\dot{V}_E$ during the fifth minute of exercise to approximately 179 $l \cdot min^{-1}$, or 85% MVV_{30}. Subsequently, $[lac^-]_B$ increased significantly from 11.1 to 12.7 $mmol \cdot l^{-1}$. However, note that respiratory alkalosis results in an increased $[lac^-]_B$ (Davies et al. 1986), and because Åstrand et al. (2003) do not state whether subjects were maintained isocapnic during volitional hyperpnoea it is possible that the changes in $[lac^-]_B$ were simply an artefact of the experimental design.

1.6.4 Summary

In summary, the contribution made by the respiratory muscles to lactate turnover during endurance exercise remains unclear. Although animal studies suggest that the respiratory muscles are perhaps consumers, rather than producers, of lactate, it is difficult to extrapolate these data to the exercising human. Based on human studies, it seems that even maximal exercise $\dot{V}_E$ reproduced voluntarily under resting conditions is not sufficient to cause a considerable increase in $[lac^-]_B$, so long as breathing and respiratory muscle recruitment patterns are tightly controlled. However, the possibility remains that these

observations reflect a considerable lactate clearance capacity under resting conditions, rather than a lack of lactate release from respiratory muscles, per se. More well-controlled and rigorously designed studies are certainly warranted to establish whether human respiratory muscles do indeed contribute to systemic lactate kinetics during endurance exercise.

1.7 Research aims

Given the uncertainty surrounding the ergogenic effects of respiratory muscle training, a primary aim of this thesis was to evaluate the effects and mechanisms of action of pressure-threshold inspiratory muscle strength training upon endurance exercise performance. In addition, owing to the dearth of empirical data from rigorously designed human studies exploring the influence of respiratory muscle work on lactate turnover during exercise, the current research also examined whether the respiratory muscles make a significant contribution to the $[lac^-]_B$ of heavy endurance exercise. The "lactate minimum" test was used in chapters 5 and 7 of this thesis to provide an objective estimate of maximal lactate steady state cycling power. Therefore, additional aims of the present research were to examine the theoretical validity and protocol dependency of the lactate minimum test.

CHAPTER 2

GENERAL METHODS

2.1 CYCLE ERGOMETERS

A validated (Palmer et al. 1996) air-braked cycle ergometer (Kingcycle Ltd, High Wycombe, Buckinghamshire, UK; Figure 2.1), which allows cyclists to use their own racing bicycle, was used to assess cycling time-trial performance. A photo-optic sensor monitored the velocity ($rev \cdot s^{-1}$) of the flywheel. The height of the bicycle was calibrated so that the slowing of the flywheel following acceleration matched a pre-determined reference power decay curve. During exercise, the computer display provided real-time visual feedback of distance cycled, cycling velocity and cadence, elapsed time, and power.

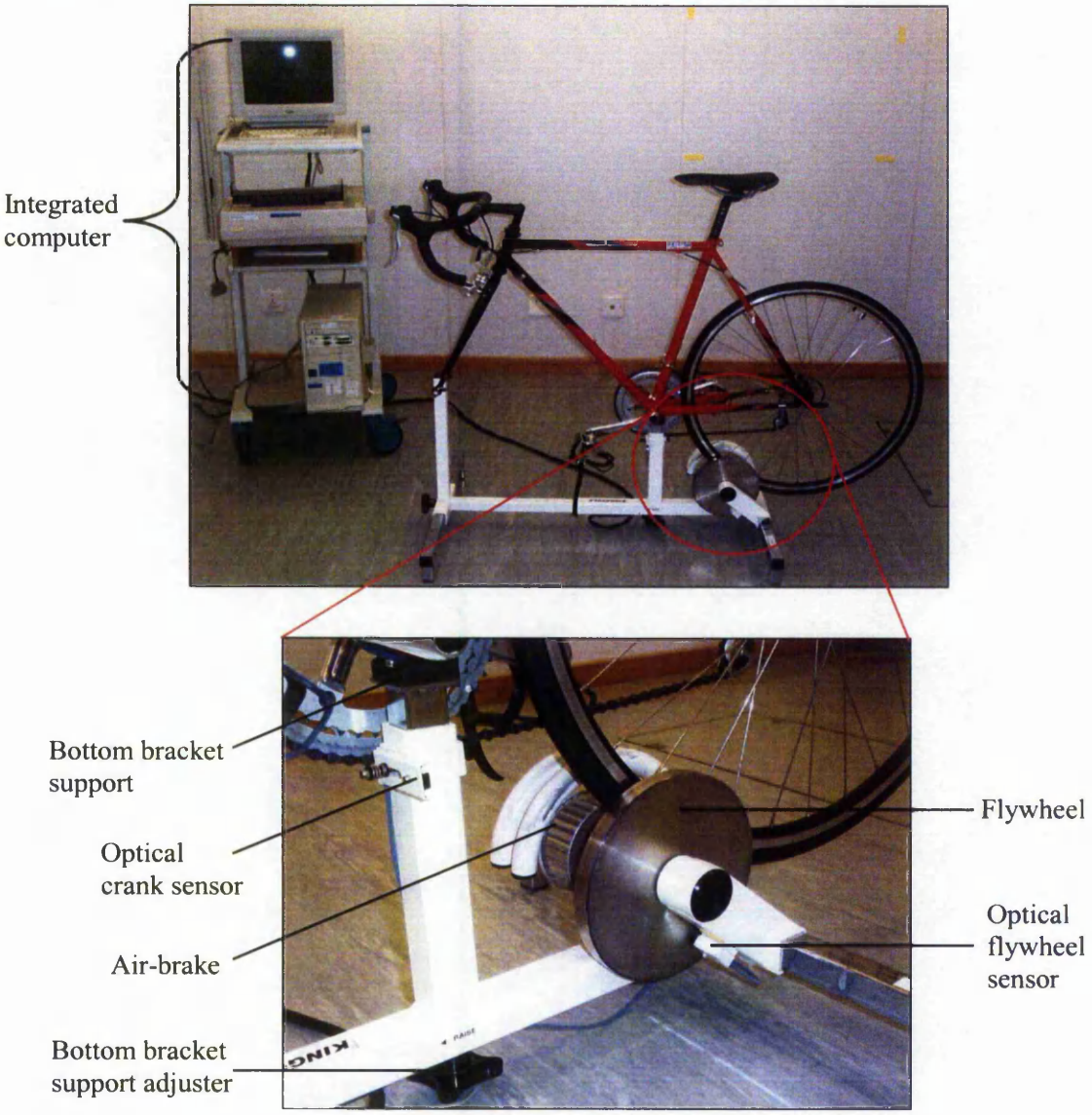

Figure 2.1 Kingcycle ergometer.

An electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) was used for exercise trials requiring constant power cycling. The height and horizontal displacement of the seat and handlebars were adjusted to suit each exercising subject, and time-trial handlebars were fitted to maximise subject comfort and to resemble actual cycling experience. When applicable, subjects were advised to use their own racing pedals and shoes.

2.2 PULMOLAB EX670

The PulmoLab EX670 (Morgan Medical, Kent, UK) is an on-line breath-by-breath respiratory system that uses mass spectrometry for gas analysis, and a turbine flowmeter for ventilatory flow rate measurement. The mass spectrometer was calibrated using certified gases (BOC Gases, Guilford, UK). The turbine flowmeter inserts into a pickup ring (Figure 2.2) that detects optical pulses produced by a rotating airscrew. Flow calibration was performed using a 3 litre syringe. The flowmeter and pick-up ring were fixed into the mouth of a facemask (model 7940, Hans Rudolph, Kansas City, Missouri). Respired gases were continuously sampled at the mouth of the facemask through a sidestream capillary tube at a rate approximating 30 $ml \cdot min^{-1}$.

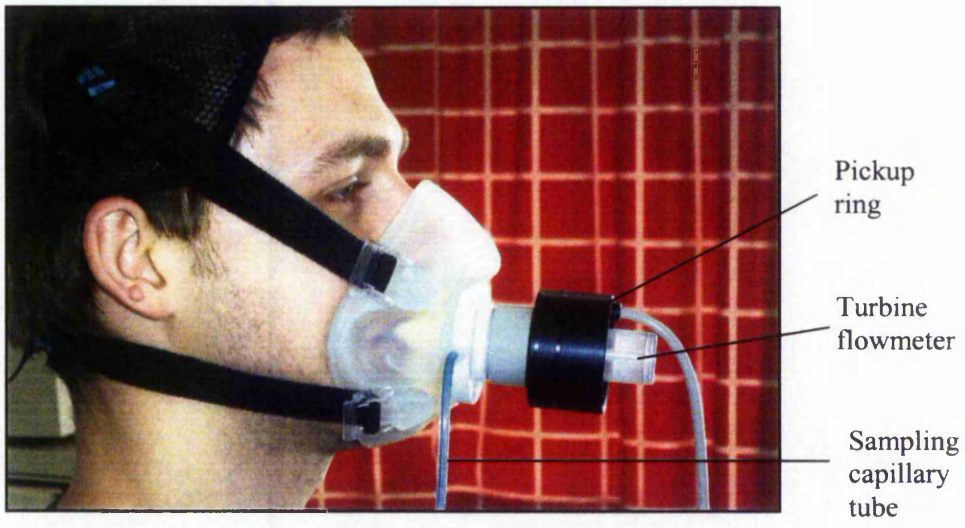

Figure 2.2 Side view of EX670 facial respiratory apparatus.

2.3 DOUGLAS BAG TECHNIQUE

Subjects wore a facemask (described above) connected to a low-resistance (1.2 and 3.0 cmH_2O at 3.3 and 6.7 $l \cdot s^{-1}$ flow rates, respectively), two-way, non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) with low dead space (100.5 ml). Expired air was collected for an accurately timed duration of 40-60 s. Concentrations of oxygen and carbon dioxide were determined by sampling through paramagnetic and infrared transducers, respectively (Servomex Series 1400, Crowborough, UK), which were calibrated using certified gases (BOC Gases, Guilford, UK). Sample volume was determined using a dry gas meter (Harvard Ltd., Edenbridge, UK). Metabolic calculations were derived from McArdle et al. (1996), and ventilation and gas volumes were corrected from ATPS to BTPS and STPD, respectively.

2.4 AGREEMENT BETWEEN EX670 AND DOUGLAS BAG METHODS

Because the Douglas bag method perhaps represents the "gold standard" of respiratory measurement, the degree of agreement between measurements made using Douglas bag and EX670 methods was evaluated to establish the accuracy of the latter. The cycle ergometer used in these experiments (Excalibur Sport) is described in section 2.1. Exercise was performed on four separate occasions at four constant cycling powers (100, 140, 180, and 220 W). To ensure steady-state conditions, measurements at each power were made following 4 min of exercise (Wasserman et al. 1967). Two samples of expired air were initially collected into Douglas bags for a timed duration of 1.5-2 min. Subsequently, expired air was sampled and averaged over a 3 min period using the EX670. Finally, two further samples of expired air were collected into Douglas bags. Values derived from all Douglas bag samples at each cycling power were averaged and compared to values derived using the EX670. Because the subject was required to switch promptly

from one measurement method to the other, a flanged mouthpiece replaced the facemask for these experiments. Figure 2.3 shows plots of $\dot{V}_E$, $\dot{V}O_2$, and $\dot{V}CO_2$ measurements taken from Douglas bag and EX670 methods on four separate occasions (each data series represents a different date). Also shown in each chart is the line of identity and the associated correlation coefficient (r) between the two methods. In all cases r was significant ($P < 0.01$), thus indicating a close association between respiratory measurements taken with Douglas bag and EX670 methods.

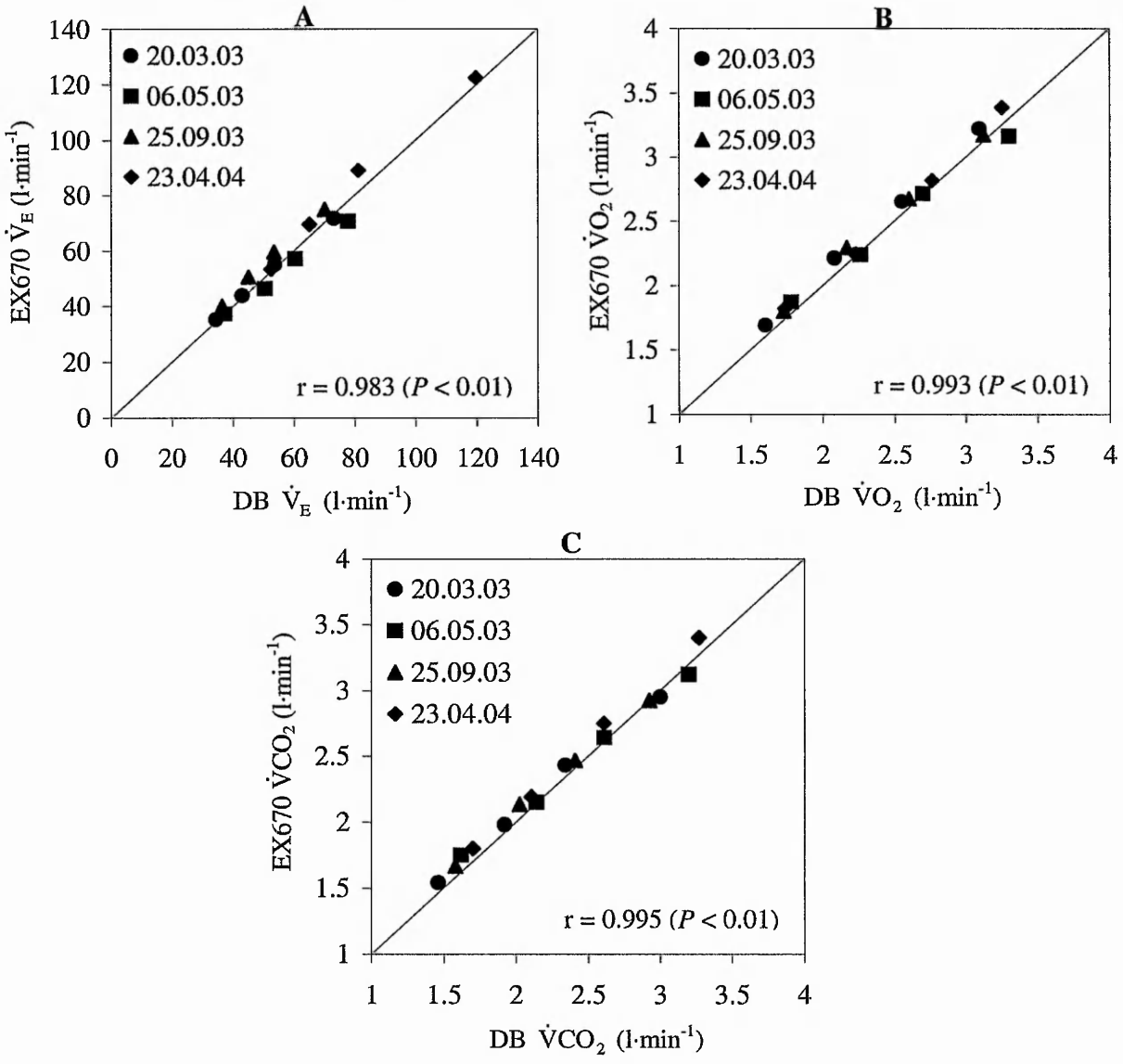

Figure 2.3 Comparisons of minute ventilation ($\dot{V}_E$) (A), pulmonary oxygen uptake ($\dot{V}O_2$) (B), and carbon dioxide production ($\dot{V}CO_2$) (C) derived using EX670 and Douglas bag (DB) methods.

Figures 2.4, 2.5, and 2.6 illustrate Bland and Altman plots (Bland and Altman 1986), which show the difference between measurements made using Douglas bag and EX670 methods against their mean. Also shown on each chart are the mean difference (bias) and the 95% limits of agreement. A 95% confidence interval (reported in the legend of each chart) for each mean and limits of agreement was also calculated to determine the uncertainty associated with each sample estimate. The narrow limits of agreement and confidence intervals for each sample estimate indicate good agreement between Douglas bag and EX670 methods of respiratory measurement. These results thus demonstrate the EX670 to be a valid and reliable system for the measurement of respiratory parameters during exercise at a range of intensities.

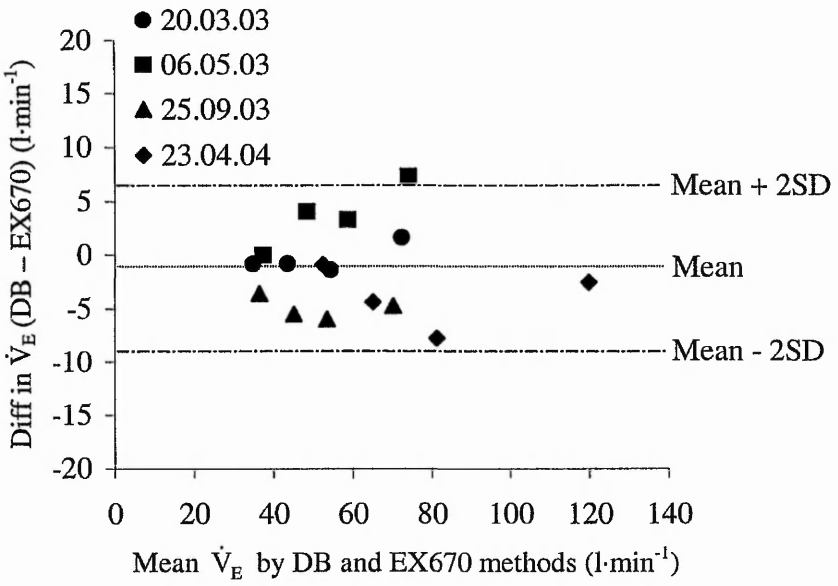

Figure 2.4 Difference in minute ventilation ($\dot{V}_E$) against mean for Douglas bag (DB) and EX670 methods. 95% confidence interval for the bias, lower limit of agreement, and upper limit of agreement is –3.5 to 0.7, –15.9 to –3.0, and 0.2 to 13.1 l·min^{-1}, respectively.

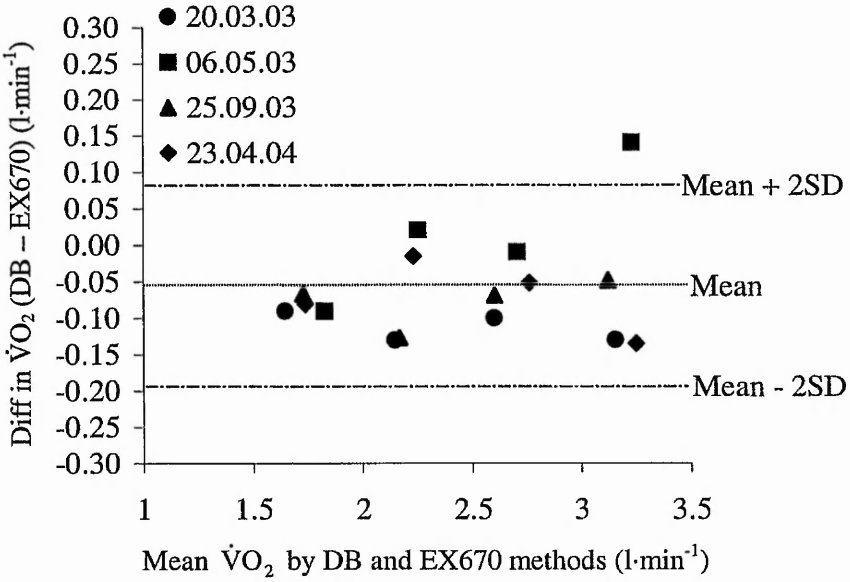

Figure 2.5 Difference in pulmonary oxygen uptake ($\dot{V}O_2$) against mean for Douglas bag (DB) and EX670 methods. 95% confidence interval for the bias, lower limit of agreement, and upper limit of agreement is –0.10 to –0.02, –0.27 to –0.14, and 0.02 to 0.14 l·min^{-1}, respectively.

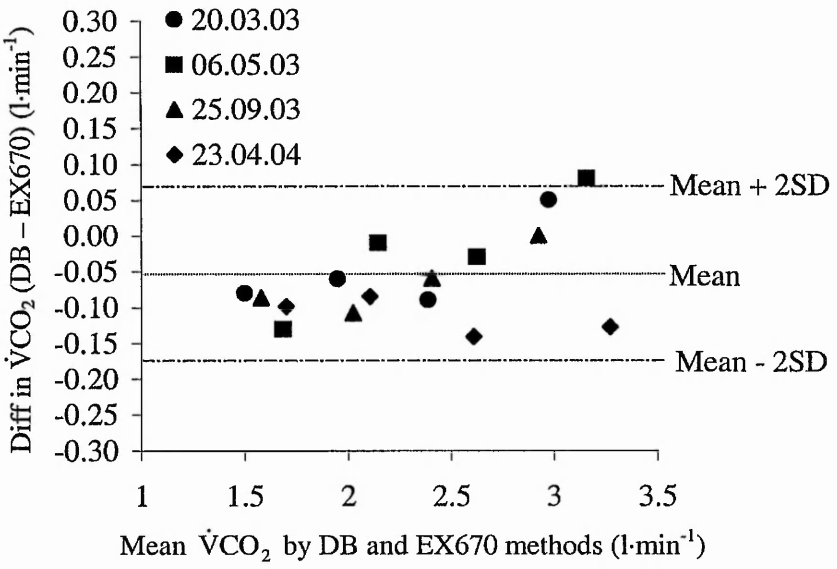

Figure 2.6 Difference in carbon dioxide production ($\dot{V}CO_2$) against mean for Douglas bag (DB) and EX670 methods. 95% confidence interval for the bias, lower limit of agreement, and upper limit of agreement is –0.10 to –0.03, –0.25 to –0.13, and 0.01 to 0.13 l·min^{-1}, respectively.

2.5 PULMONARY FUNCTION AND RESPIRATORY PRESSURES

Pulmonary function was assessed using a pneumotachograph spirometer (Compact II, Vitalograph, Buckingham, UK). Flow calibration was performed using a 1 litre syringe.

Measurements were made for vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FVC/FEV_1, peak expiratory and inspiratory flow (PEF and PIF, respectively), and maximal voluntary ventilation over 12 s (MVV_{12}). Each test was repeated 3 times and the highest result was used for subsequent analysis (Cotes 1993; Gething et al. 2004b).

A hand-held mouth pressure meter (Morgan Medical Ltd., Kent, UK) was used to measure maximal inspiratory and expiratory mouth pressures (MIP and MEP, respectively). The mouthpiece assembly incorporated a 1 mm orifice to prevent glottic closure during MIP manoeuvres and to reduce the use of buccal muscles during MEP manoeuvres. Inspiratory and expiratory manoeuvres were initiated from RV and TLC, respectively, thus controlling the initial length of the respiratory muscles. For MIP measurements subjects were instructed to exhale slowly to RV, thus eliminating variability in lung volumes and dynamic compression of the airways. Subjects inhaled (MIP) or exhaled (MEP) maximally under verbal encouragement through a flanged mouthpiece (Morgan Medical Ltd., Kent, UK) for at least 1 s. Repeat measurements separated by 30-60 s were taken until consistent values (within 5 cmH_2O of each other) were produced (Volianitis et al. 2001), and the highest was used for subsequent analysis.

2.6 Agreement between two methods of respiratory pressure measurement

To determine the accuracy of the Morgan mouth pressure meter, its agreement with another pressure transducer (model TSD104A, BIOPAC Systems, Inc. CA) that was calibrated using a water manometer was assessed. The TSD104A and the Morgan pressure meter were connected to a flanged mouthpiece so that they were simultaneously subjected to changes in mouth pressure. Ten inspiratory manoeuvres were then performed in order to generate a range of inspiratory pressures. Figure 2.7 shows inspiratory pressure measured

using the TSD104A and the Morgan pressure meter. The r (correlation coefficient) between the two methods was significant ($P < 0.01$), thus indicating a close association between respiratory pressure measurements taken using the two methods.

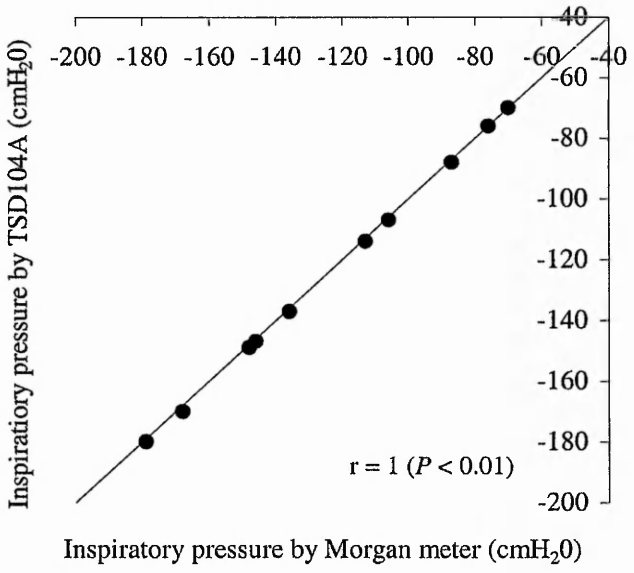

Figure 2.7 Comparison of inspiratory mouth pressure measured using the TSD104A and Morgan pressure meter.

Figure 2.8 illustrates a Bland and Altman plot that shows the difference between measurements made using the Morgan pressure meter and TSD104A methods against their mean. Also shown are the mean bias and the 95% limits of agreement. A 95% confidence interval (see figure legend) for each mean and limit of agreement was calculated to determine the uncertainty associated with each sample estimate. The narrow limits of agreement and confidence intervals for each sample estimate indicate good agreement between the two methods of mouth pressure measurement. These data suggest that the Morgan meter provides valid and accurate measurements of inspiratory mouth pressure.

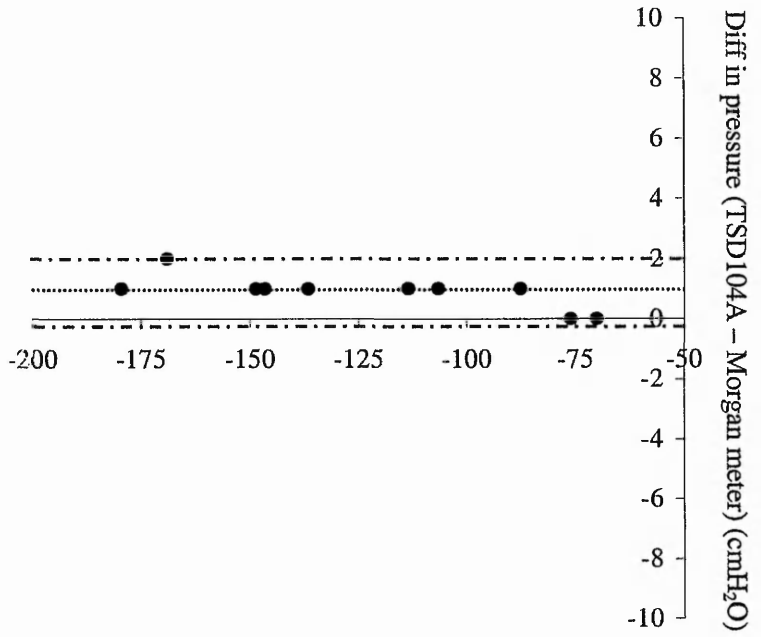

Figure 2.8 Difference in inspiratory mouth pressure against mean for TSD104A and Morgan meter methods. 95% confidence interval for the bias, lower limit of agreement, and upper limit of agreement is 0.5 to 1.3, -0.9 to 0.5, and 1.3 to 2.7 cmH_2O, respectively.

2.7 BLOOD SAMPLING AND ANALYSIS

Arterialised venous blood was sampled from a superficial dorsal hand vein via an indwelling cannula. This method provides an accurate determination of arterial PCO_2 ($PaCO_2$), pH, and $[lac^-]_B$, although PaO_2 values are slightly underestimated (Forster et al. 1972). However, although SaO_2 is dependent on PaO_2, in accord with the O_2-haemoglobin dissociation curve, the lower estimate of PaO_2 is still located on the upper "shoulder" of this relationship, thus the change is insufficient to have significant consequence for SaO_2.

Arterialised-venous blood was sampled via an indwelling 20-gauge Teflon venous cannula (Becton Dickinson UK Ltd, Oxford) positioned in a superficial dorsal hand vein and connected to a 3-way stopcock (Becton Dickinson UK Ltd, Oxford). Arterialisation was ensured by immersing the hand in water at around 40°C for 8-10 min prior to cannulation and by warming the hand during exercise using an infrared lamp (Philips,

infraphil HP3614) to between 40-43°C (skin temperature was monitored using a thermocouple). Cannulation took place with the subject supine and following cleaning of the skin using a medi-swab (70% isopropyl alcohol). Cannula and stopcock patency was maintained through 0.9% sodium chloride intravenous infusion from a 5 ml syringe. Prior to sampling, residual fluids within the cannula and stopcock were withdrawn into a 1 ml syringe. Following the removal of the cannula medical gauze was firmly applied to the punctured area for at least 10 min to prevent further leakage and haematoma development.

An automated analyser (P-GM7 MicroStat, Analox Instruments, London, UK; Figure 2.9) was used to measure $[lac^-]_B$ following calibration using an 8 $mmol \cdot l^{-1}$ lactate standard; prior to analysis blood was stored on ice in microtubes containing a heparin/fluoride/nitrite mixture (Analox Instruments, London, UK). Note that although in chapter 7 blood was also sampled from an antecubital vein via an indwelling cannula, cannulation and blood analysis procedures were as described above.

Blood-gas measurements, which included the determination of $PaCO_2$, pH, and SaO_2, were made immediately using an ABL 520 blood-gas analyser (Radiometer, Copenhagen, Denmark; Figure 2.10) after drawing a blood sample into a 2 ml pre-heperinised syringe (PICO 50, Radiometer, Copenhagen, Denmark). Values were corrected for in vivo temperature changes during exercise as measured using a rectal temperature probe (1000 Series Squirrel, Grant Instruments, Cambridge, UK) inserted 10 cm beyond the sphincter ani. The ABL 520 performed automatic calibrations at 2 h intervals. Additionally, four levels of quality control solutions (QUALICHECK 3+, Radiometer, Copenhagen, Denmark) were also introduced into the analyser to ensure test accuracy and to detect systematic analytical deviations.

Figure 2.9 P-GM7 Microstat. (From www.analox.com; accessed 23.03.2005).

Figure 2.10 ABL520 blood-gas analyser.

2.8 EFFECT OF STORAGE DURATION ON BLOOD LACTATE CONCENTRATION

During some studies blood samples for $[lac^-]_B$ determination would be stored on ice for up to approximately 90 min, thus the effect of 110 min storage duration on $[lac^-]_B$ was investigated. Two male subjects performed, on separate days, a total of 6 high-intensity cycling exercise trials. A different $[lac^-]_B$ was elicited in each trial through cycling at different power outputs. Approximately 3-5 min post-exercise a 5 ml blood sample was drawn and 0.2 ml was transferred into each of 10 storage tubes (see above), which were

subsequently placed on ice until measurement. Analysis took place following 20 min of storage and every 10 min thereafter. The results from the analysis are presented in Figure 2.11.

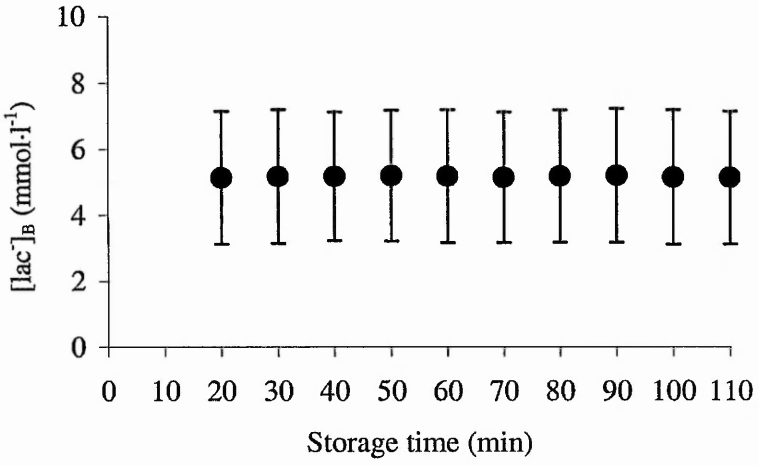

Figure 2.11 Effect of storage duration on blood lactate concentration ($[lac^-]_B$). Mean (SD).

The following equation calculated whether the slope of a regression line plotted through the $[lac^-]_B$ data was significantly different to zero:

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

where b = slope of the line, SD_x = SD of x (i.e. time), SEE = standard error of the estimate. The slope of the regression line was not significantly different to zero. The coefficient of variation was calculated at 0.5%. Therefore, when employing the methods described above $[lac^-]_B$ remains stable when samples are stored on ice for at least 110 min.

2.9 INSPIRATORY MUSCLE TRAINING

Training was performed using an inspiratory pressure-threshold device (POWERbreathe®, IMT Technologies Ltd., Birmingham, UK; Figure 2.12), which

requires continuous application of inspiratory pressure for an inspiratory valve to remain open and thus permit inspiratory flow. Inspiratory resistance is provided via a load-calibrated spring, whereas expiration is unimpeded (Caine and McConnell 2000).

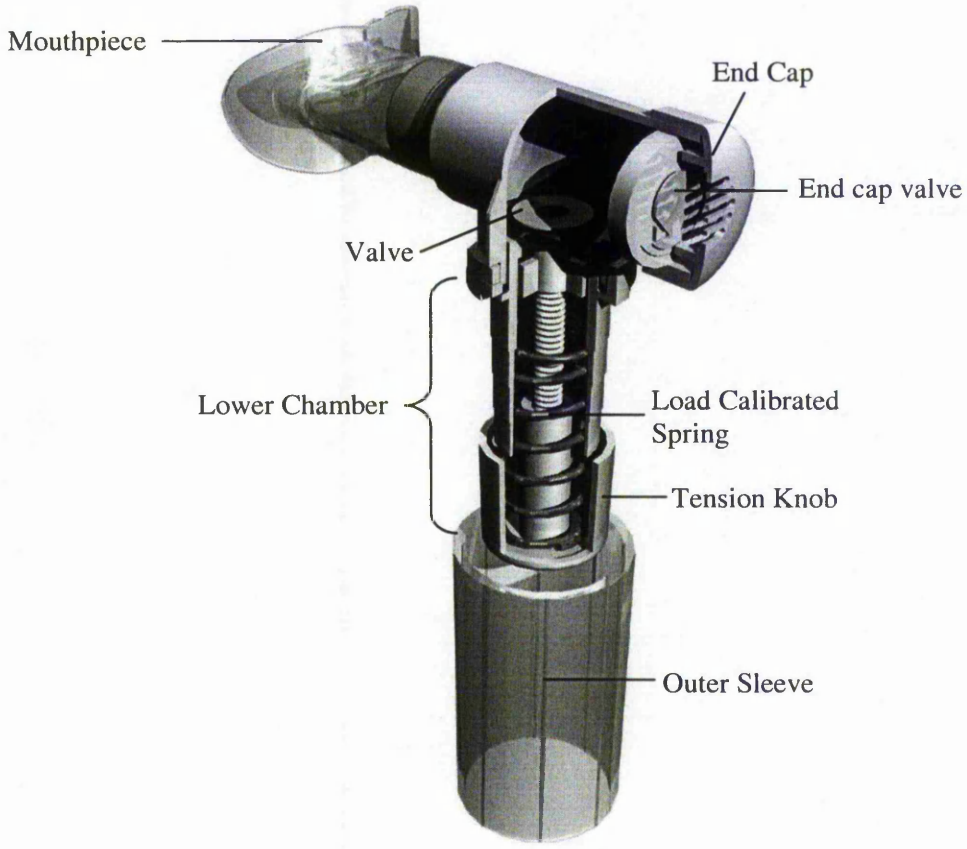

Figure 2.12 POWERbreathe® showing internal structures. (From www.powerbreathe.com; accessed 07.05.2003).

Subjects performed 30 dynamic inspiratory efforts twice daily for 6 weeks. The inspiratory load was initially set at 50% baseline MIP. Thereafter, subjects were instructed to periodically increase the resistance to a level that would allow them to only just complete 30 manoeuvres. Subjects were instructed to initiate each inspiratory effort from RV and to inspire maximally up to the limit of thoracic expansion. To avoid hyperventilation and therefore hypocapnia due to the increased V_T, subjects were coached

to adopt a reduced f_R. This protocol has proven effective in eliciting an adaptive response (Romer et al. 2002a; Volianitis et al. 2001; McConnell and Sharpe 2005).

2.10 Limb and respiratory ratings of perceived exertion

Ratings of perceived exertion (RPE) for limb and respiratory effort was assessed via the Borg 6-20 category-rating scale (Borg 1998) and the modified Borg scale (Borg 1982), respectively. Both scales were mounted onto a magnetic board that faced the subject throughout exercise, and measurements were taken via the subject moving a magnetic counter to the desired integer. Subjects were familiarised with both scales and were encouraged to provide a differential response relative to limb and respiratory effort.

CHAPTER 3

PART A – THE LACTATE MINIMUM TEST: VALIDATION AND METHODOLOGICAL CONSIDERATIONS

3.1 INTRODUCTION

In 1979 Davis and Gass examined temporal changes in $[lac^-]_B$ during an incremental exercise that commenced, due to a prior exercise bout, with elevated $[lac^-]_B$. They found that with increasing exercise intensity there was an initial decline in $[lac^-]_B$, reflecting net lactate clearance, followed by a progressive increase, reflecting net lactate appearance. The $[lac^-]_B$ profile was thus U-shaped, and Davis and Gass (1979) proposed that their observations reflected the patterns of change that would be observed during constant-load exercise and that they therefore had "predictive value for steady-state work".

The findings of Davis and Gass (1979) provided the foundations upon which Tegtbur et al. (1993) devised the lactate minimum test. The lactate minimum test protocol comprises 3 consecutive phases: (I) a lactate elevation phase comprising high-intensity exercise; (II) a short (7-8 min) recovery phase sufficient to allow $[lac^-]_B$ to peak; and (III) an incremental exercise phase in which $[lac^-]_B$ is determined at the end of each stage increment. Tegtbur et al. (1993) defined the nadir of the U-shaped $[lac^-]_B$ vs. work-rate curve of the incremental phase as the lactate minimum work-rate, or the point where lactate production and catabolism are in equilibrium. Therefore, the lactate minimum work-rate provides an objective estimate of maximal lactate steady state (MLSS), which can be defined as the highest exercise intensity and $[lac^-]_B$ that can be maintained over time without a continual blood lactate accumulation (Billat et al. 2003) (i.e. $\Delta[lac^-]_B/\Delta t = 0$, where t denotes time). MLSS therefore discriminates sustainable exercise intensities, in which exercise duration is primarily limited by the amount of available stored energy, and non-sustainable exercise intensities, in which exercise duration is primarily limited by a disturbance in cellular homeostasis (Billat et al. 2003). Accordingly, knowledge of MLSS has proven useful in the design of training programs (Jones and Carter 2000) and when planning appropriate race pace strategies for endurance events (Fukuba and Whipp 1999).

The practical advantages of the lactate minimum test are twofold. Firstly, MLSS can be estimated from a single laboratory visit, thus avoiding the laborious process of performing several constant-load tests on separate days. Secondly, the results are not influenced by changes in muscle glycogen content (Tegtbur et al. 1993). The validity of the lactate minimum test as a predictor of MLSS is evidenced in studies showing a close agreement between lactate minimum and MLSS work-rates (Bacon and Kern 1999; Jones and Doust 1998; Tegtbur et al. 1993). However, despite its practical advantages, minimal attention has been focused towards the theoretical basis of the test. A key assumption of the lactate minimum test, on which its theoretical validity hinges, is that the measured $[lac^-]_B$ during the incremental phase reflects the metabolic demands of the corresponding work-rate (Jones and Doust 1998). If this assumption holds, then using different ramp protocols for the incremental phase should not affect $\Delta[lac^-]_B/\Delta t$ at equivalent work-rates, nor the lactate minimum work-rate. However, Carter et al. (1999b) demonstrated a positive relationship between the starting speed of the incremental phase and the lactate minimum running speed, thus suggesting that lactate kinetics during the incremental phase may be critically dependent upon the specifics of the test protocol. However, because Carter et al. (1999b) determined $[lac^-]_B$ only at the end of each stage of the incremental phase, the issue of whether $\Delta[lac^-]_B/\Delta t$ at equivalent work-rates was influenced by the starting speed could not be resolved.

Therefore, the aim of series 1 in this study was to examine the effects of incremental phase protocol on $\Delta[lac^-]_B/\Delta t$ at equivalent power outputs and the lactate minimum cycling power. It was hypothesised that starting the incremental phase at a higher power output would shift the lactate minimum to a higher power output and modify $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase.

During exercise tests in which the upper body is actively engaged in the exercise (e.g. running, rowing, swimming), test interruptions are necessary to permit blood sampling from, for example, a fingertip, earlobe, or superficial arm/hand vein. However, such interruptions can, perhaps unsurprisingly, affect lactate turnover. For example, intra-stage rest intervals shift blood lactate transition thresholds during incremental exercise to higher work-rates (Heck et al. 1985), and during a series of 30 min constant power cycling tests to establish MLSS, interruptions of just 30 s every 5 min are sufficient to reduce $[lac^-]_B$ and result in a significant overestimate of MLSS power (Beneke et al. 2003). In the original study of Tegtbur et al. (1993) in which running was the exercise modality, blood sampling was performed during a 30 s test interruption that separated each stage of the incremental phase. Although Carter et al. (2000; 1999a; 1999b) have acknowledged (but not studied) the possibility that test interruptions may influence lactate kinetics during the incremental phase by keeping intra-stage rest intervals as short as possible (10-15 s), other studies using running (Bacon and Kern 1999; Denadai and Higino 2004; Simões et al. 1999), rowing (Craven et al. 1997), swimming (Ribeiro et al. 2003), and cycling (Caine et al. 1997; Kinch et al. 1997) lactate minimum protocols have included intra-stage rest intervals of 30-60 s to permit blood sampling, which may have influenced lactate kinetics during the incremental phase and the subsequent determination of the lactate minimum power.

Therefore, the aim of series 2 in this study was to compare two lactate minimum test protocols in which the incremental phase was either continuous or discontinuous (1 min interval between each stage increment), and assess the effects on $\Delta[lac^-]_B/\Delta t$ during each stage increment and the lactate minimum cycling power. It was hypothesised that a rest interval during each stage increment of the incremental phase would lower $[lac^-]_B$ and subsequently shift the lactate minimum to a higher power output. Note that although test

interruptions are not necessary in cycling lactate minimum tests, this exercise modality was chosen because of the ease with which blood sampling can be performed.

3.2 METHODS

3.2.1 PARTICIPANTS

Following local ethics committee approval and written informed consent, 16 non-smoking recreationally active male subjects were recruited for the study. Subjects were instructed not to partake in strenuous exercise the day preceding an exercise test. On test days, subjects were asked not to ingest caffeine, and refrain from consuming any substance of nutritional value during the 2 h prior to testing. Tests for each subject were separated by at least 48 h, but no more than 1 wk, and were performed at a similar time of day so as to minimise diurnal fluctuation effects.

3.2.2 EXPERIMENTAL DESIGN

Subjects were equally divided into two groups, which were assigned to either series 1 or 2. All tests were performed on an electromagnetically-braked cycle ergometer (see section 2.1). Subjects in each series performed two lactate minimum tests. In series 1 the ramp increments during the incremental phase of the lactate minimum tests were identical, but the starting power was varied. In series 2 subjects performed two lactate minimum tests incorporating either a continuous or discontinuous incremental phase protocol.

3.2.3 MEASUREMENTS

Respiratory variables were measured during exercise using an on-line breath-by-breath respiratory system (see section 2.2). Arterialised venous blood was sampled from a dorsal hand vein for the determination of $[lac^-]_B$, as described in section 2.6.

3.2.4 Series 1 – Effects of incremental phase protocol

The age, height, and body mass of the 8 subjects participating in series 1 were 23.6 (4.5) years, 179.4 (4.4) cm, and 79.6 (8.1) kg, respectively. Each subject performed two lactate minimum tests, each comprising 3 consecutive phases: (I) a lactate elevation phase consisting of a maximal incremental $\dot{V}O_2$ peak test; (II) an 8 min recovery phase at 60 W; and (III) a submaximal incremental phase consisting of 5 consecutive 4 min cycling exercise stages. For one of the lactate minimum tests the incremental phase was performed at intensities of 40, 45, 50, 55, and 60% of the $\dot{W}$ max achieved during the lactate elevation phase. For the remaining lactate minimum test the incremental phase was performed at 45, 50, 55, 60, and 65% $\dot{W}$ max. Hereafter these tests are referred to as $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$, respectively. To avoid any influence of test order, half of the subjects performed $LM_{40\text{-}60}$ first, whereas the other half performed $LM_{45\text{-}65}$ first. During the lactate elevation phase of the initial lactate minimum test, cycling power was increased every 15 s by a constant increment that was chosen for each subject in order to elicit exercise intolerance in approximately 10 min. The lactate elevation phase was terminated when, despite verbal encouragement, cycling cadence could not be maintained above 60 rpm. The power output at which exercise intolerance ensued defined $\dot{W}$ max, and the highest $\dot{V}O_2$ value averaged over any 30 s period defined $\dot{V}O_2$ peak ($\dot{V}O_2$ was measured during the lactate elevation phase only). The ramp protocol during the lactate elevation phase of the initial lactate minimum test was repeated during the second lactate minimum test, and subjects were encouraged to reach the same $\dot{W}$ max, at which point the lactate elevation phase was terminated. Blood samples for $[lac^-]_B$ determination were taken following the first minute of the recovery phase and every minute thereafter.

3.2.5 SERIES 2 – EFFECTS OF TEST INTERRUPTION

The age, height, and body mass of the 8 subjects participating in series 2 were 24.5 (5.8) years, 177.3 (6.0) cm, and 77.5 (7.0) kg, respectively. The lactate minimum test protocols performed in series 2 were identical to that described for $LM_{45\text{-}65}$, except that during one of the tests a 1 min test interruption separated each stage of the incremental phase. Hereafter, lactate minimum tests performed with and without test interruption are referred to as $LM_{discontinuous}$ and $LM_{continuous}$, respectively. To avoid any influence of test order half of the subjects performed $LM_{discontinuous}$ first, whereas the other half performed $LM_{continuous}$ first. $\dot{V}O_2$ peak was determined during the lactate elevation phase of the first lactate minimum test. During the second lactate minimum test subjects were again encouraged to reach the same $\dot{W}$ max during the lactate elevation phase as that achieved in the initial lactate minimum test. The power outputs performed during the incremental phase were thus identical in both lactate minimum tests. Blood samples for the determination of $[lac^-]_B$ were taken at the end of the recovery phase and every minute thereafter.

3.2.6 DATA MODELLING: THEORETICAL CONSIDERATIONS

The lactate minimum work-rate is typically established from the nadir of a spline or polynomial function fitted to the $[lac^-]_B$ vs. work-rate data. When used simultaneously, these methods produce identical values for the lactate minimum work-rate (Smith et al. 2002). Thus to establish the lactate minimum power in the present study $[lac^-]_B$ at the end of each exercise stage of the incremental phase was plotted against the corresponding cycling power, and the nadir of a 2^{nd} order polynomial curve fitted to the data was calculated through differentiation of the quadratic equation. There are, however, fundamental concerns associated with this interpretation of the test data. Firstly, the lactate

minimum work-rate may reside between two consecutive $[lac^-]_B$ sample points, with the latter being lower than the former. In this scenario, lactate clearance appears to exceed lactate appearance beyond the lactate minimum work-rate, and thus presumably the MLSS work-rate, which is not consistent with the theoretical basis of the test. Secondly, Tegtbur et al. (1993) and subsequent authors have defined the lactate minimum work-rate as the point at which $\Delta[lac^-]_B/\Delta w = 0$ (where w denotes work-rate). It can be readily appreciated that $\Delta[lac^-]_B/\Delta w = 0$ (the lactate minimum) does not define MLSS work-rate, at which $\Delta[lac^-]_B/\Delta t = 0$. Therefore, it is theoretically more correct to examine intra-stage changes in $[lac^-]_B$ (i.e. $\Delta[lac^-]_B/\Delta t$) and estimate MLSS as the work-rate at which $\Delta[lac^-]_B/\Delta t = 0$.

Therefore, the present study introduces the concept of examining intra-stage changes in $[lac^-]_B$. Specifically, $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase was taken as the gradient of a linear regression line fitted through the plot of $[lac^-]_B$ against time, and MLSS power was estimated as that at which $\Delta[lac^-]_B/\Delta t = 0$. Note that for each stage $\Delta[lac^-]_B/\Delta t$ was always established from 5 $[lac^-]_B$ sample times (i.e. during continuous lactate minimum tests the final $[lac^-]_B$ of one stage became the first $[lac^-]_B$ of the subsequent stage).

3.2.7 Statistical analyses

Comparisons between tests in each series were made using paired t-tests. Relationships between variables were calculated using Pearson's product moment correlation coefficient (r). All results are presented as mean (1 SD) unless otherwise indicated. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the 12.0 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA).

3.3 RESULTS

3.3.1 SERIES 1

$\dot{W}$ max and $\dot{V}O_2$ peak during the lactate elevation phase of the first lactate minimum test were 344 (56) W and 3.67 (0.45) $l \cdot min^{-1}$, respectively. During the second lactate minimum test, all subjects reached the same $\dot{W}$ max as that achieved in the initial lactate minimum test. The powers corresponding to 40, 45, 50, 55, 60, and 65% $\dot{W}$ max were 138 (22), 155 (25), 172 (28), 189 (31), 206 (33), and 224 (36) W, respectively.

When $[lac^-]_B$ at the end of each stage of the incremental phase was plotted against cycling power, all subjects demonstrated a U-shaped $[lac^-]_B$ profile during both $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$ (Figure 3.1). However, the lactate minimum power during $LM_{40\text{-}60}$ [175 (29) W, 50.9 (0.9) % $\dot{W}$ max] was significantly ($P < 0.01$) lower than that observed in $LM_{45\text{-}65}$ [184 (30) W, 53.5 (1.2) % $\dot{W}$ max]. Similarly, the $[lac^-]_B$ at the lactate minimum during $LM_{40\text{-}60}$ [4.3 (1.4) $mmol \cdot l^{-1}$] was significantly ($P < 0.01$) lower than that observed in $LM_{45\text{-}65}$ [5.1 (1.5) $mmol \cdot l^{-1}$]. Interestingly, the transition from 50 to 55% $\dot{W}$ max was characterised by a decline in $[lac^-]_B$ during $LM_{40\text{-}60}$, but an increase during $LM_{45\text{-}65}$.

Temporal changes in $[lac^-]_B$ during the recovery and incremental phases of $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$ are shown in Figure 3.2. Changes in $[lac^-]_B$ during the recovery phase were similar between tests. $[lac^-]_B$ peaked at 8.4 (0.9) and 8.4 (1.3) $mmol \cdot l^{-1}$ following 2 min of recovery in $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$ tests, respectively, and thereafter declined for the remainder of the recovery phase at a rate of 0.18 (0.09) and 0.15 (0.15) $mmol \cdot l^{-1} \cdot min^{-1}$, respectively. During the incremental phase significant differences in $[lac^-]_B$ were observed between tests during and subsequent to the second exercise stage (i.e. 45 and 50% $\dot{W}$ max in $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$ tests, respectively).

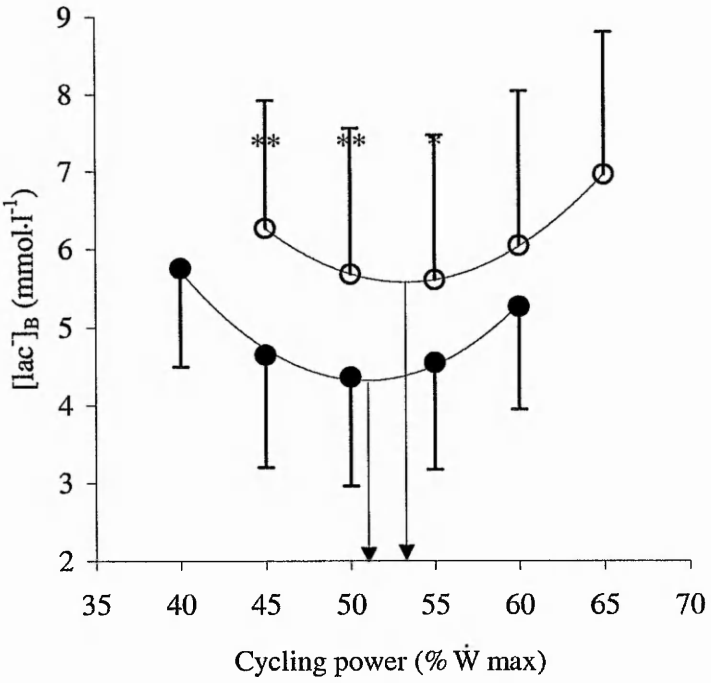

Figure 3.1 Relationship between relative cycling power and blood lactate concentration ($[lac^-]_B$) at the end of each stage of the incremental phase in $LM_{40\text{-}60}$ (•) and $LM_{45\text{-}65}$ (○). Values are mean (SD). Arrows denote lactate minimum. *Significant difference between tests ($P < 0.05$). **Significant difference between tests ($P < 0.01$).

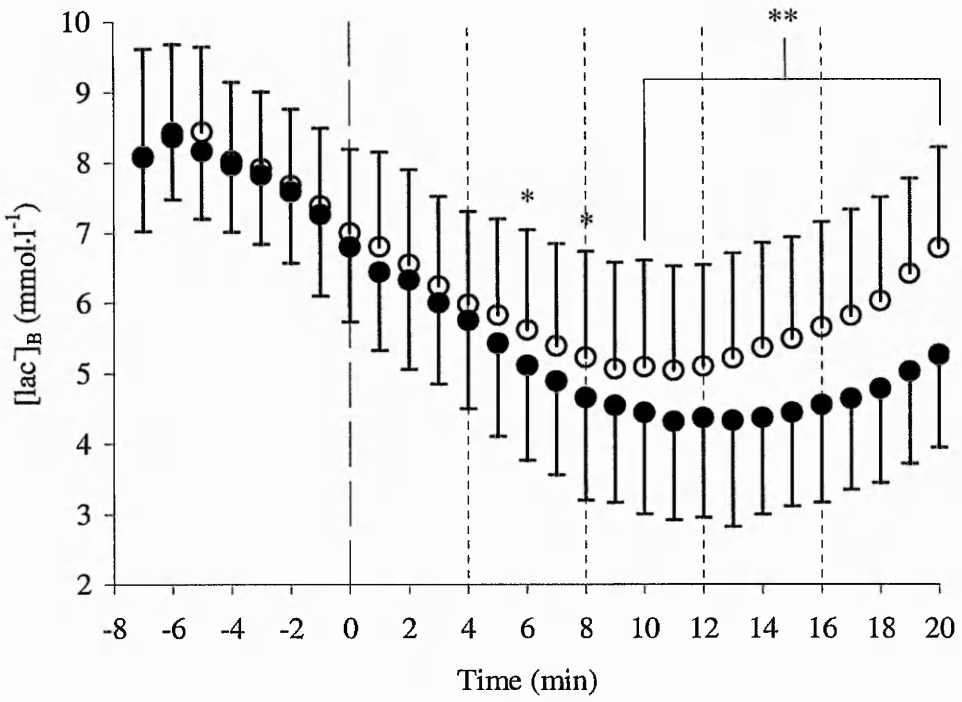

Figure 3.2 Temporal changes in blood lactate concentration ($[lac^-]_B$) during recovery and incremental phases of $LM_{40\text{-}60}$ (•) and $LM_{45\text{-}65}$ (○). Dashed vertical line at time zero represents transition from recovery to incremental phase. All other dashed vertical lines represent the end and start of consecutive stages. *Significant difference between tests ($P < 0.05$). **Significant difference between tests at all times from 10-20 min, inclusive ($P < 0.01$).

Figure 3.3 shows changes in $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase in $LM_{40\text{-}60}$ and $LM_{45\text{-}65}$. The power at which $\Delta[lac^-]_B/\Delta t = 0$ during $LM_{40\text{-}60}$ [183 (30) W, 53.3 (1.0) % $\dot{W}$ max] was significantly ($P < 0.05$) lower than that observed during $LM_{45\text{-}65}$ [190 (33) W, 55.9 (0.9) % $\dot{W}$ max]. Furthermore, when compared at equivalent power outputs $\Delta[lac^-]_B/\Delta t$ was significantly ($P < 0.01$) greater during $LM_{40\text{-}60}$ compared to $LM_{45\text{-}65}$ at 50 and 55% $\dot{W}$ max. Interestingly, at 55% $\dot{W}$ max $\Delta[lac^-]_B/\Delta t$ was positive in $LM_{40\text{-}60}$ but negative in $LM_{45\text{-}65}$.

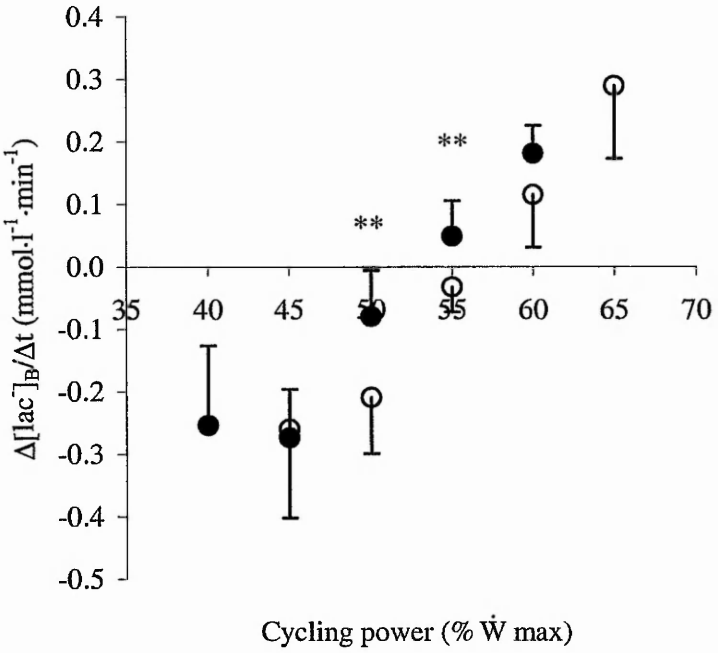

Figure 3.3 Rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during each stage of the incremental phase in $LM_{40\text{-}60}$ (•) and $LM_{45\text{-}65}$ (○).**Significant difference between tests ($P < 0.01$).

Relationships between $\Delta[lac^-]_B/\Delta t$ during $LM_{40\text{-}60}$ and $\Delta[lac^-]_B/\Delta t$ during $LM_{45\text{-}65}$ at intensities of 45-60% $\dot{W}$ max are shown in Figure 3.4. At 45% $\dot{W}$ max, $\Delta[lac^-]_B/\Delta t$ during $LM_{40\text{-}60}$ was significantly ($P < 0.05$) correlated ($r = 0.72$) with $\Delta[lac^-]_B/\Delta t$ during $LM_{45\text{-}65}$.

Conversely, for exercise at 50, 55, and 60% $\dot{W}$ max, there was no correlation between $\Delta[lac^-]_B/\Delta t$ during $LM_{40\text{-}60}$ and $\Delta[lac^-]_B/\Delta t$ during $LM_{45\text{-}65}$.

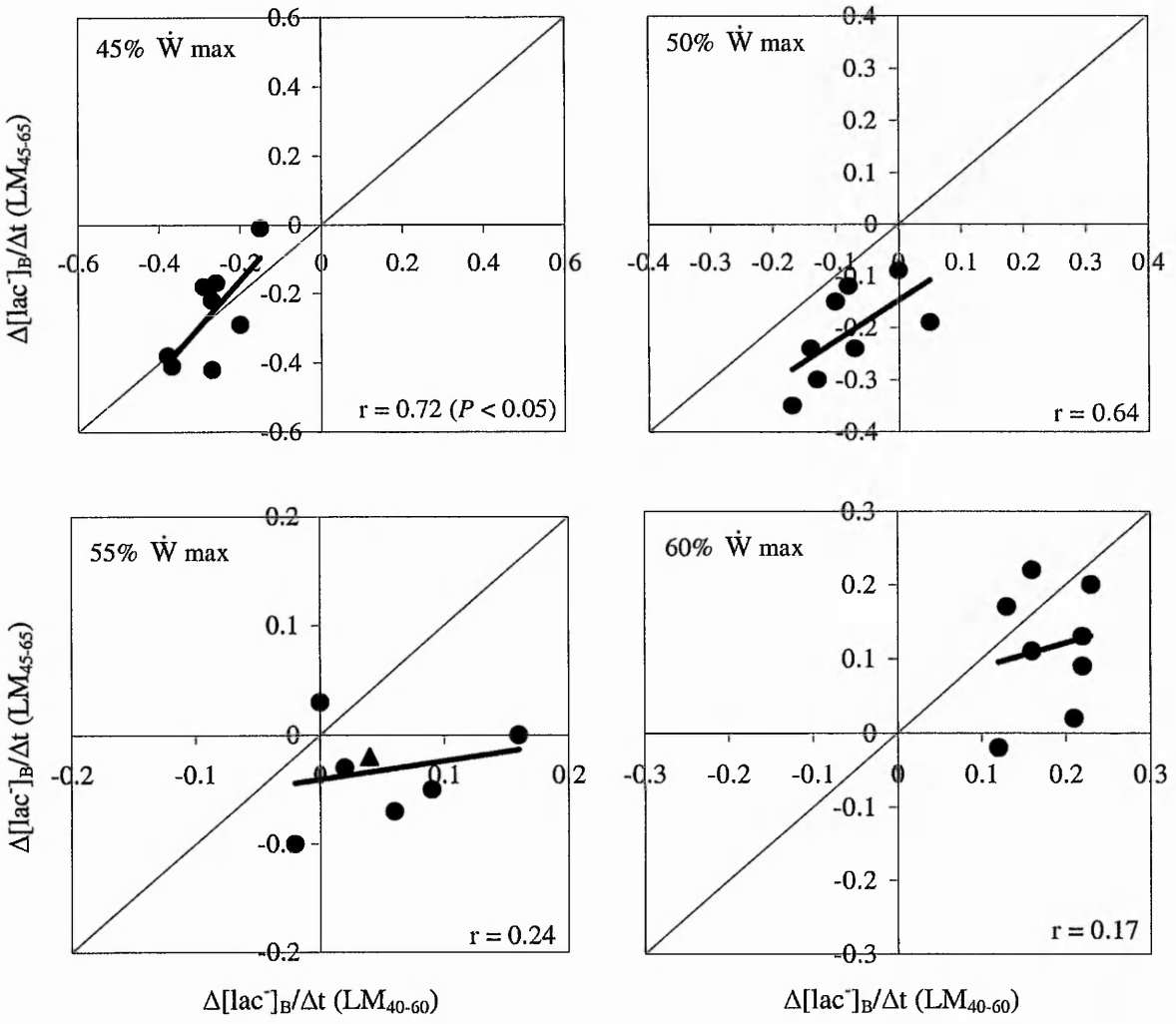

Figure 3.4 Relationship between rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during $LM_{40\text{-}60}$ and $\Delta[lac^-]_B/\Delta t$ during $LM_{45\text{-}65}$ for exercise at 45, 50, 55, and 60% $\dot{W}$ max. Each data point represents an individual subject. ▲ in 55% $\dot{W}$ max represents 2 subjects with identical values for $\Delta[lac^-]_B/\Delta t$. Line of identity is shown.

3.3.2 SERIES 2

$\dot{W}$ max and $\dot{V}O_2$ peak during the lactate elevation phase of the first lactate minimum test were 363 (49) W and 3.64 (0.49) $l \cdot min^{-1}$, respectively. Thus cycling powers corresponding to 45, 50, 55, 60, and 65% $\dot{W}$ max were 164 (22), 181 (24), 200

(27), 218 (29), and 236 (32) W, respectively. During the second lactate minimum test, all subjects reached the same $\dot{W}$ max as that achieved in the initial lactate minimum test. When $[lac^-]_B$ at the end of each stage of the incremental phase was plotted against cycling power, all subjects demonstrated a U-shaped $[lac^-]_B$ profile during both $LM_{discontinuous}$ and $LM_{continuous}$ (Figure 3.5). The lactate minimum power in $LM_{discontinuous}$ [200 (26) W, 55.3 (1.1) % $\dot{W}$ max] was significantly ($P < 0.01$) greater than that observed in $LM_{continuous}$ [192 (25) W, 52.9 (1.9) % $\dot{W}$ max). Conversely, the $[lac^-]_B$ at the lactate minimum during $LM_{discontinuous}$ [4.0 (1.1) $mmol \cdot l^{-1}$] was significantly ($P < 0.05$) lower that that observed during $LM_{continuous}$ [4.9 (0.7) $mmol \cdot l^{-1}$].

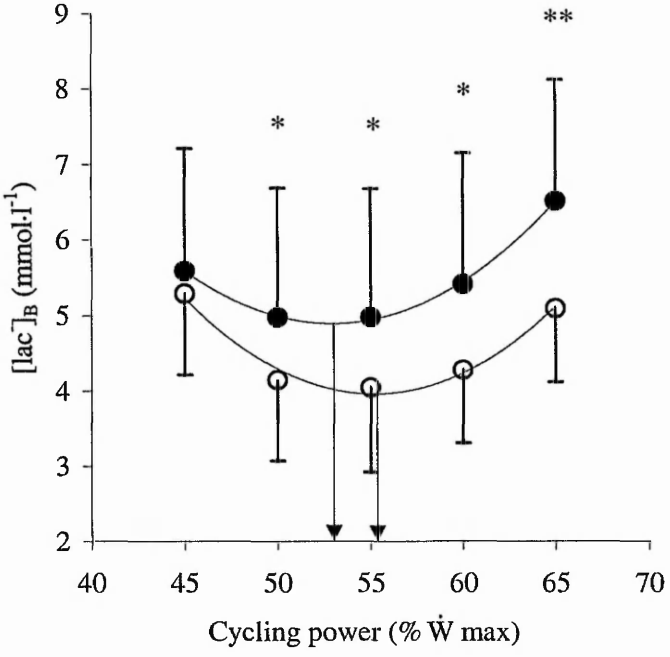

Figure 3.5 Relationship between relative cycling power and blood lactate concentration ($[lac^-]_B$) at the end of each stage of the incremental phase in $LM_{continuous}$ (•) and $LM_{discontinuous}$ (○). Values are mean (SD). Arrows denote lactate minimum. *Significant difference between tests ($P < 0.05$). **Significant difference between tests ($P < 0.01$).

Temporal changes in $[lac^-]_B$ during the incremental phase of $LM_{continuous}$ and $LM_{discontinuous}$ are shown in Figure 3.6. There was a clear affect of test interruption on the

$[lac^-]_B$ profile of the incremental phase. Firstly, compared to $LM_{continuous}$ $[lac^-]_B$ declined to lower values during $LM_{discontinuous}$. Furthermore, exercise at 55, 60, and 65% $\dot{W}$ max during $LM_{discontinuous}$ was characterised by a decline in $[lac^-]_B$ during the initial 2 min of exercise, followed by a continuous increase for the remainder of that stage. This pattern of change was not observed during $LM_{continuous}$.

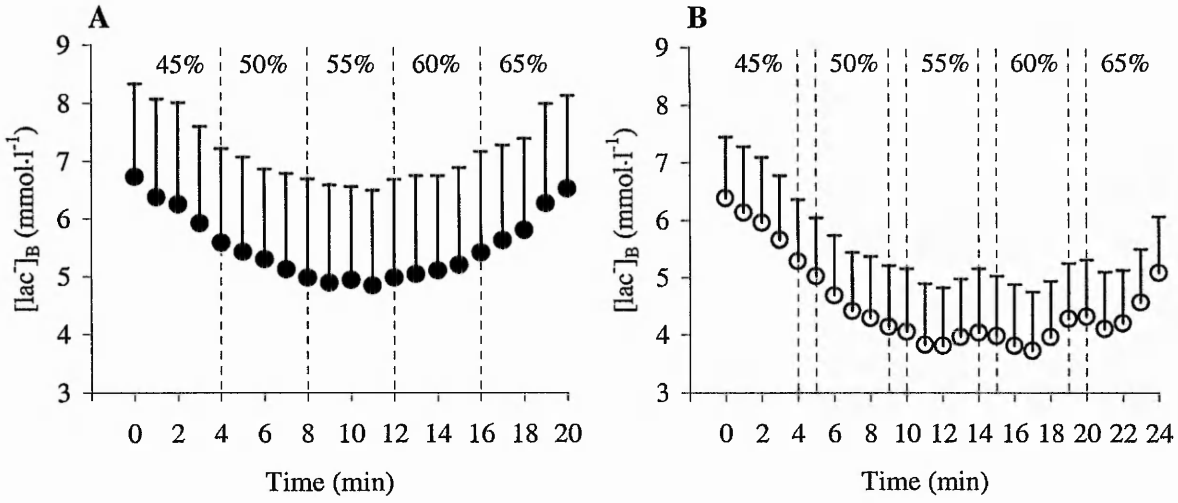

Figure 3.6 Temporal changes in blood lactate concentration ($[lac^-]_B$) during the incremental phase of $LM_{continuous}$ (A) and $LM_{discontinuous}$ (B). Values are mean (SD). Dashed vertical lines in A represent end and start of consecutive stages. Dashed vertical lines in B represent 1 min rest interval. Values at the top of each chart represent % $\dot{W}$ max.

Figure 3.7 shows changes in $\Delta[lac^-]_B/\Delta t$ during $LM_{continuous}$ and $LM_{discontinuous}$. $\Delta[lac^-]_B/\Delta t$ during each stage was similar between tests, although $\Delta[lac^-]_B/\Delta t$ at 50% $\dot{W}$ max was significantly ($P < 0.05$) lower (i.e. more negative) during $LM_{discontinuous}$ compared to $LM_{continuous}$ [-0.22 (0.07) vs. -0.15 (0.07) $mmol \cdot l^{-1} \cdot min^{-1}$]. The power at which $\Delta[lac^-]_B/\Delta t = 0$ in $LM_{continuous}$ [201 (26) W] was not significantly different from that observed in $LM_{discontinuous}$ [204 (25) W]. However, despite the group mean similarities in $\Delta[lac^-]_B/\Delta t$, there was no correlation between $\Delta[lac^-]_B/\Delta t$ during $LM_{continuous}$ and $\Delta[lac^-]_B/\Delta t$ during $LM_{discontinuous}$ at neither 45 (r = 0.14), 50 (r = 0.40), 55 (r = 0.15), 60 (r = 0.42), nor 65% (r = 0.10) $\dot{W}$ max. The relationship between $\Delta[lac^-]_B/\Delta t$ during $LM_{continuous}$ and

$\Delta[lac^-]_B/\Delta t$ during $LM_{discontinuous}$ for exercise at 55% $\dot{W}$ max is shown graphically in Figure 3.8.

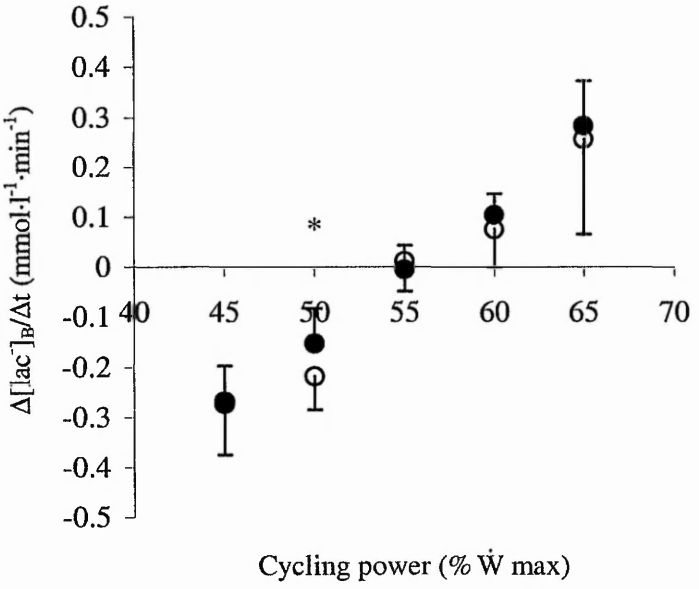

Figure 3.7 Rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during each stage of the incremental phase in $LM_{continuous}$ (•) and $LM_{discontinuous}$ (○).Values are mean (SD). *Significant difference between tests ($P < 0.05$).

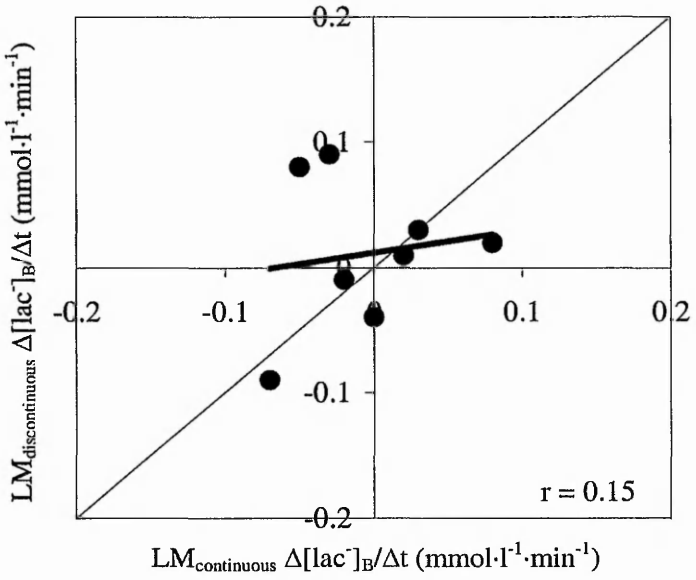

Figure 3.8 Relationship between rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during $LM_{continuous}$ and $\Delta[lac^-]_B/\Delta t$ during $LM_{discontinuous}$ for exercise at 55% $\dot{W}$ max. Each data point represents an individual subject. Line of identity is shown.

3.4 DISCUSSION

3.4.1 MAIN FINDINGS

The primary aim of this study was to investigate to what extent the lactate minimum test is protocol dependent. The main findings were that both temporal changes in $[lac^-]_B$ during the incremental phase and the lactate minimum power were significantly influenced by both small differences in incremental phase power outputs, and the inclusion of a 1 min test interruption between each stage of the incremental phase.

3.4.2 SERIES 1

The lactate minimum test represents one of numerous single-test protocols routinely used by exercise physiologists to obtain a working estimate of MLSS (Billat et al. 2003). The use of a single test to establish MLSS is attractive because it circumvents the usual laborious process of performing a series of fixed work-rate tests on separate days. The lactate minimum test has thus proven popular in Europe because of the ease and objectivity with which the lactate minimum work-rate can be determined, and also because the test is not affected by muscle glycogen content (Tegtbur et al. 1993). The premise of the test is that the early stages of the incremental phase reflect sub-MLSS work-rates, thus lactate clearance exceeds lactate appearance ($\Delta[lac^-]_B/\Delta t$ is negative). Conversely, the latter stages of the incremental phase reflect work-rates above MLSS, thus lactate appearance exceeds lactate clearance ($\Delta[lac^-]_B/\Delta t$ becomes positive). Theoretically, the lactate minimum therefore represents a point of equilibrium between lactate appearance and clearance (i.e. MLSS) (Tegtbur et al. 1993). However, although several studies have shown a close agreement between lactate minimum and MLSS work-rates (Bacon and Kern 1999; Jones and Doust 1998; Tegtbur et al. 1993), the findings of series 1 suggest that this may have been fortuitously related to the choice of incremental phase ramp protocol. Similar

conclusions were made by Carter et al. (1999b), who also demonstrated a positive relationship between the starting speed of the incremental phase and the lactate minimum speed.

If the lactate minimum work-rate is to provide a valid measure of MLSS, then the measured $[lac^-]_B$ at any exercise intensity should reflect the metabolic demands of that exercise intensity (Jones and Doust 1998). Thus irrespective of whether different incremental phase ramp protocols are used on separate occasions, $\Delta[lac^-]_B/\Delta t$ should be similar at equivalent work-rates if, as originally proposed by Davis and Gass (1979), the results are to have "predictive power for steady state work". However, the findings of series 1 cast significant doubt over this inherent assumption, which thus leads to the conclusion that the theoretical basis of the lactate minimum test is somewhat flawed. Moreover, the findings of the present study strengthen the argument of a fortuitous agreement between lactate minimum and MLSS work-rates (Carter et al. 1999b).

The reasoning of Carter et al. (1999b) may explain, in part, why the lactate minimum power was higher, and $\Delta[lac^-]_B/\Delta t$ 50 and 55% $\dot{W}$ max lower (i.e. more negative), during $LM_{45\text{-}65}$ compared to $LM_{40\text{-}60}$. These authors argue that the $[lac^-]_B$ measured during the incremental phase not only reflects the metabolic demand of the exercise intensity just completed, but that it is also mediated temporally by the blood lactate recovery kinetics from the lactate elevation phase. At any given time during the incremental phase the exercise intensity was higher during $LM_{45\text{-}65}$ compared to $LM_{40\text{-}60}$, which probably resulted in less perfusion and recruitment of lactate consuming oxidative muscle fibres in locomotor muscles, and less perfusion of lactate consuming visceral organs such as the liver and kidney. In addition, at any given time during the incremental phase glycolytic flux within locomotor muscle was presumably greater during $LM_{45\text{-}65}$ compared to $LM_{40\text{-}60}$, thus resulting in greater lactate production. It is thus possible that the

increase in lactate minimum power when the incremental phase is performed at higher intensities is partly explained by a higher $[lac^-]_B$, which promotes continued lactate clearance even when MLSS is reached/surpassed (Carter et al. 1999b).

3.4.3 SERIES 2

In series 2 of this study, the lactate minimum cycling power was increased by 9 ± 6 W when a 1 min test interruption separated each stage of the incremental phase. These findings thus add further credence to the notion that the lactate minimum work-rate is partly dependent upon specifics of the test protocol.

This is the first study to address the influence of test interruption during the incremental phase on temporal changes in $[lac^-]_B$ and the lactate minimum power. Although the lactate minimum power was increased by, on average, only 9 W during $LM_{discontinuous}$, the range of increase was 19 – 2 W, thus in some subjects the effect of test interruption was quite marked. These novel findings therefore suggest that the level of physiological exertion, and thus endurance training status, may be misinterpreted when brief interruptions to permit blood sampling are included during the incremental phase of the lactate minimum test. This assertion is consistent with previous studies also showing a significant influence of test interruption on $[lac^-]_B$ responses to exercise. For example, Beneke et al. (2003) established MLSS power at 278 W when using continuous constant power exercise trials of 30 min; however, when test interruptions of 30 and 90 s were included every 5 min during exercise MLSS power was increased to 300 and 310 W, respectively. Similarly, the work-rate corresponding to the 4 $mmol \cdot l^{-1}$ onset of blood lactate accumulation during incremental exercise has been shown to increase when test interruptions of 30-90 s separate each stage increment (Heck et al. 1985). It is therefore crucial that exercise physiologists and coaches understand the consequences of test

interruption when assessing the exercise capacity of an athlete, since this may lead to an erroneous assessment of the endurance training status of the athlete and subsequently an inappropriate prescription of training exercise intensity.

As stated by Beneke et al. (2003), during a test interruption the glycolytic rate of the previously exercised muscles is reduced while $\dot{V}O_2$ remains elevated. This would result in less lactate production and efflux from muscle, but continued whole-body lactate clearance. This assertion is supported by the data in Figure 3.6 (panel B), which shows that beyond the lactate minimum point the effect of test interruption is manifest by an initial decline in $[lac^-]_B$ during the first 2 min of the subsequent exercise stage. As a consequence, there was less time available for $[lac^-]_B$ to increase to the same value as that observed during $LM_{continuous}$, thus resulting in a rightward shift of the nadir of the $[lac^-]_B$ vs. power curve.

Although the lactate minimum power was increased when test interruptions were included during the incremental phase, the power output at which $\Delta[lac^-]_B/\Delta t = 0$ was not different between $LM_{continuous}$ and $LM_{discontinuous}$. Note, however, that the group mean similarities in $\Delta[lac^-]_B/\Delta t$ during incremental phase were quite fortuitous, since there was no correlation between $\Delta[lac^-]_B/\Delta t$ during the incremental phase of $LM_{continuous}$ and $LM_{discontinuous}$. These data further strengthen the argument that intra-stage rest intervals during the incremental phase impart a significant influence on $\Delta[lac^-]_B/\Delta t$. However, it is also interesting to highlight that there was no correlation between $\Delta[lac^-]_B/\Delta t$ at 45% $\dot{W}$ max during $LM_{continuous}$ and $LM_{discontinuous}$, even though both trials entailed identical lactate elevation and recovery phases. This raises the possibility that even when identical lactate minimum test protocols are performed on separate days, temporal changes in $[lac^-]_B$ during the incremental phase may still show little agreement.

3.4.4 CONCLUSION

In conclusion, this study has shown temporal changes in $[lac^-]_B$ during the incremental phase of the lactate minimum test, and the lactate minimum cycling power, to be dependent on two specifics of the test protocol: (I) the starting power of the incremental phase; and (II) the inclusion of a 1 min test interruption between each stage of the incremental phase. These findings suggest that the lactate minimum test is protocol dependent, and are also indicative of a flaw in the theoretical basis of the test. However, despite these findings the lactate minimum test has still been shown to provide a reasonable working estimate of MLSS (see Part B below; Jones and Doust 1998).

PART B – THE LACTATE MINIMUM TEST: PRACTICAL VALIDITY, THEORETICAL SHORTCOMINGS, AND PROTOCOL DEPENDENCY

3.5 INTRODUCTION

The temporal patterns of change in $[lac^-]_B$ during constant power exercise have been well described (Wasserman et al. 1986). The changes in $[lac^-]_B$ over time ($\Delta[lac^-]_B/\Delta t$, where t denotes time) depend on the power being performed relative to maximal lactate steady state (MLSS), which can be defined as the highest power and $[lac^-]_B$ at which $\Delta[lac^-]_B/\Delta t$ is zero (Billat et al. 2003; McConnell and Sharpe 2005). Above intensities at which $[lac^-]_B$ remains unchanged from rest, but below MLSS, there tends to be a transient rise in $[lac^-]_B$ followed by a progressive decrease over time ($\Delta[lac^-]_B/\Delta t$ is negative) whereas during exercise above MLSS $[lac^-]_B$ increases inexorably ($\Delta[lac^-]_B/\Delta t$ is positive) throughout exercise (Roston et al. 1987; Tegtbur et al. 1993).

In 1979 Davis and Gass introduced the innovation of examining $\Delta[lac^-]_B/\Delta t$ during an incremental exercise test which commenced with hyperlactaemia. They found that with increasing intensity there was an initial decline in $[lac^-]_B$, reflecting net lactate clearance, followed by a progressive increase, reflecting net lactate appearance. The $[lac^-]_B$ profile was thus U-shaped, and Davis and Gass (1979) suggested that their observations reflected the patterns of change observed during constant power exercise (outlined above) and that they therefore had "predictive value for steady-state work".

The findings of Davis and Gass (1979) provided the foundations upon which Tegtbur et al. (1993) subsequently devised the lactate minimum test. The lactate minimum test protocol comprises 3 consecutive phases: (I) a lactate elevation phase comprising high-intensity exercise; (II) a short (7-8 min) recovery phase to allow $[lac^-]_B$ to peak; and (III) a submaximal, incremental exercise phase in which $[lac^-]_B$ is determined at the end of each stage increment. Tegtbur et al. (1993) proposed that the nadir of the U-shaped $[lac^-]_B$ profile of the incremental phase (i.e. the "lactate minimum"), represented the aerobic/anaerobic transition, or the highest power at which there is equilibrium between

lactate production and catabolism (i.e. MLSS). Among a plethora of protocols, each with their own inherent limitations (reviewed in Carter et al. 1999b), the lactate minimum test thus offers a swift and objective estimate of MLSS, which is also not influenced by muscle glycogen content (Tegtbur et al. 1993). Accordingly, the lactate minimum test has proven popular in the physiological assessment of athletes throughout Europe (Carter et al. 1999b).

It is somewhat surprising, however, particularly when one considers the purpose of the lactate minimum test, that few studies have compared the lactate minimum power with the independently determined MLSS power. Indeed, only Jones and Doust (1998) have made this comparison, and found the lactate minimum running speed to significantly underestimate MLSS by, on average, 0.8 $km \cdot h^{-1}$. It is unclear, however, how these findings relate to other modes of exercise, such as leg cycling, in which the agreement between lactate minimum and MLSS powers has, to our knowledge, never been characterised.

Therefore, the aim of series 1 of this study was to determine the validity of the lactate minimum test for the determination of MLSS cycling power.

Following its introduction attention has focused on methodological aspects of the lactate minimum test, such as how the lactate minimum power is affected by different lactate elevation and incremental phase protocols (Caine et al. 1997; Carter et al. 1999b; Ribeiro et al. 2003; Smith et al. 2002). Conversely, and perhaps surprisingly, the theory central to the lactate minimum test has received scanty attention, thus key assumptions of the test remain unexplored. For example, several authors have stated that if the lactate minimum test is to provide a valid estimate of MLSS then the measured $[lac^-]_B$ at any exercise intensity of the incremental phase must reflect the metabolic demands of that exercise intensity (Carter et al. 1999b; Jones and Doust 1998). Thus by virtue, $\Delta[lac^-]_B/\Delta t$ during the incremental phase should reflect $\Delta[lac^-]_B/\Delta t$ during constant power exercise.

Indeed, this was the premise upon which Davis and Gass (1979) built their original observations. However, it appears that the relationship between $\Delta[lac^-]_B/\Delta t$ and power during any protocol involving incremental exercise has never been characterised.

Therefore, the aim of series 2 in this study was to examined whether $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase reflected $\Delta[lac^-]_B/\Delta t$ during constant power exercise.

As previously discussed, a tenet central to the lactate minimum test is that $\Delta[lac^-]_B/\Delta t$ during the incremental phase reflects $\Delta[lac^-]_B/\Delta t$ observed during constant power exercise. However, it seems unlikely that $\Delta[lac^-]_B/\Delta t$ during the incremental phase would be unaffected by the maximal exercise performed in the lactate elevation phase, as suggested by Carter et al. (1999b). This contention is entirely consistent with literature showing changes in the metabolic and cardiorespiratory response to exercise preceded by a high-intensity exercise bout (reviewed in Jones et al. 2003). In particular, evidence suggests that prior exercise may "prime" the oxidative metabolic pathway and attenuate lactate production and release from active muscle during subsequent exercise (Bangsbo et al. 2001; Campbell-O'Sullivan et al. 2002).

Therefore, series 3 of this study tested the hypothesis that the $[lac^-]_B$ profile and lactate minimum power of the incremental phase are influenced by the lactate elevation phase. Two lactate minimum protocols were compared: the first protocol comprised leg cycling throughout all phases of the test. The second protocol mimicked the first protocol except that the lactate elevation phase was performed using maximal arm-cranking exercise. Different muscle groups were chosen to perform the lactate elevation phases because the priming effect that prior exercise imparts on subsequent exercise is either absent or considerably blunted when the priming exercise bout is performed using muscle groups remote to those employed in the second exercise bout (Fukuba et al. 2002; Koppo

et al. 2003), thus this experimental design would maximise any potential effect of the lactate elevation phase on the $[lac^-]_B$ response to the incremental phase.

3.6 METHODS

3.6.1 PARTICIPANTS

Following local ethics committee approval and written informed consent, 25 non-smoking, recreationally active male subjects were recruited for the study. Subjects were instructed not to partake in strenuous exercise during the 24 h preceding an exercise test. On test days, subjects were asked to refrain from consuming caffeine, and were instructed to report the laboratory at least 2 h post-prandial. Successive tests for each subject were separated by at least 48 h, but no more that 1 week, and were performed at a similar time of day so to minimise diurnal fluctuation effects.

3.6.2 EXPERIMENTAL DESIGN

Three series were performed in this study. The order of the experiments was:

Series 1: agreement between lactate minimum and MLSS cycling powers.

Series 2: comparison of $[lac^-]_B$ response to the lactate minimum test and constant power cycling.

Series 3: effect of lactate elevation phase protocol.

Series 1 and 2 were performed on an electromagnetically-braked cycle ergometer (see section 2.1). Series 3 was also performed on the cycle ergometer, with the exception that the lactate elevation phase was performed using maximal arm-cranking exercise (Angio, Lode, Groningen, The Netherlands).

3.6.3 MEASUREMENTS

In series 1 and 3 arterialised venous blood for the determination of $[lac^-]_B$ was sampled from a superficial dorsal hand vein via an indwelling cannula, as described in section 2.6. Blood sampling and analysis procedures in series 2 were also identical to that described in section 2.6, except that blood was sampled from an antecubital vein.

An on-line breath-by-breath respiratory system (see section 2.2) was used to measure all respiratory variables during the lactate elevation phase of series 1 only.

3.6.4 SERIES 1 – AGREEMENT BETWEEN LACTATE MINIMUM AND MLSS POWERS

Seven subjects participated in series 1. The age, height, and body mass of the subjects were 27.1 (5.6) years, 167.2 (6.4) cm, and 78.3 (8.0) kg, respectively. Subjects initially performed a lactate minimum test based on the method described by Tegtbur et al. (1993). The exercise protocol comprised 3 consecutive phases: (I) a lactate elevation phase comprising maximal, incremental exercise; (II) an 8 min recovery phase at 60 W to allow $[lac^-]_B$ to peak; and (III) a submaximal incremental phase consisting of 5 consecutive 4 min cycling exercise stages at intensities of 45, 50, 55, 60, and 65% of the $\dot{W}$ max achieved during the lactate elevation phase. During the lactate elevation phase cycling power was increased every 15 s by a constant increment that was chosen for each subject in order to elicit exercise intolerance in approximately 10 min. The lactate elevation phase was terminated when, despite verbal encouragement, cycling cadence could not be maintained above 60 rpm. The final completed workload defined $\dot{W}$ max and, for series 1, the highest $\dot{V}O_2$ recorded over any 30 s period defined $\dot{V}O_2$ peak. Blood samples for $[lac^-]_B$ determination were taken at the end of the recovery phase and every minute thereafter.

The MLSS power was subsequently resolved through each subject performing at least two 30 min constant power trials, as described in section 5.2.4.

3.6.5 Series 2 – Comparison of the blood lactate concentration response to the lactate minimum test and constant power cycling

Eight subjects participated in series 2. The age, height, and body mass of the subjects were 23.4 (5.2) years, 180.4 (6.4) cm, and 79.9 (5.5) kg, respectively. Subjects initially performed a lactate minimum test as described in series 1. Subsequently, subjects performed, in random order, five 22 min cycling tests on separate days with venous blood for the determination of $[lac^-]_B$ being sampled every 2 min from 0-22 min, inclusive. Because each stage of the lactate minimum incremental phase commences with elevated $[lac^-]_B$ and systemic lactate kinetics are not only dependent on work-rate, but also the $[lac^-]_B$ (Smith et al. 2002), the initial 12 min of each constant power trial comprised cycling at 60% $\dot{W}$ max in order to elevate $[lac^-]_B$ to approximately 4-5 $mmol \cdot l^{-1}$. Subsequently, power was adjusted to one of the five power outputs performed during the incremental phase of the lactate minimum test, and exercise was continued for a further 10 min.

3.6.6 Series 3 – Effect of lactate elevation phase protocol

Ten subjects participated in series 3. The age, height, and body mass of the subjects were 23.1 (5.4) years, 178.8 (6.9) cm, and 78.2 (7.5) kg, respectively. Subjects performed two lactate minimum tests. All phases of the first lactate minimum test (following the protocol described in series 1) were performed using leg cycling ergometry. Hereafter, this test is referred to as LM_{LEG}. On a separate day, subjects mimicked LM_{LEG}, with the exception that the lactate elevation phase comprised maximal incremental arm-cranking exercise. Hereafter, this test is referred to as LM_{ARM}. The arm-cranking ergometer was securely mounted on a table and subjects remained seated throughout exercise. The centre of the arm-crank shaft was aligned to shoulder level and subjects were positioned so that slight flexion at the elbow joint was observed when the hand was at its most distal point

during cycling of the ergometer. Subjects were encouraged to keep their lower limbs stationary during exercise. Following 15 s unloaded exercise, power was increased every 15 s by either 4 or 5 W. Subjects were instructed and verbally encouraged to exercise to the limit of tolerance during the lactate elevation phase. Criteria for terminating the lactate elevation phase was similar to that described in series 1, except that due to the lower cadence adopted in arm-cranking compared to cycling, exercise was terminated if subjects could not maintain cadence above 40 rpm despite verbal encouragement. On completion of the lactate elevation phase subjects immediately transferred to the cycle ergometer and repeated the recovery and incremental phases (using identical cycling powers) performed in LM_{LEG}. For this reason, LM_{LEG} always preceded LM_{ARM}. Blood sampling procedures were as described in series 1.

3.6.7 DATA ANALYSES

The lactate minimum power and $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase were determined as described in section 2.10.2.6. $\Delta[lac^-]_B/\Delta t$ during constant power exercise was taken as the gradient of a linear regression line fitted through the plot of $[lac^-]_B$ against time over 14-22 min, inclusive. Subsequently, $\Delta[lac^-]_B/\Delta t$ was plotted against the corresponding cycling power and MLSS was estimated as that at which $\Delta[lac^-]_B/\Delta t = 0$. Comparisons of MLSS estimates within each series were made using one-way ANOVA for repeated measures followed by Tukey's HSD test when appropriate. All other comparisons were made using paired t-tests. Pearson product-moment correlation coefficients (r) were computed to assess the relationship between variables. All results are presented as mean (1 SD) unless otherwise indicated. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the 12.0 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA).

3.7 RESULTS

3.7.1 SERIES 1

$\dot{W}$ max and $\dot{V}O_2$ peak were 367 (44) W and 3.93 (0.47) l·min^{-1}, respectively. The lactate minimum power [199 (23) W, 53.0 (1.4) % $\dot{W}$ max] was significantly ($P < 0.05$) lower than MLSS power [210 (25) W, 56.1 (3.5) % $\dot{W}$ max] (Figure 3.9), although the two were significantly ($P < 0.01$) correlated (r = 0.91). The lactate minimum $[lac^-]_B$ [4.8 (2.0) mmol·l^{-1}] was not significantly different from that between16-30 min of exercise at MLSS [3.7 (0.8) mmol·l^{-1}]; however, these values were not correlated (r = 0.49). The power at which $\Delta[lac^-]_B/\Delta t = 0$ [208 (26) W, 55.3 (1.0) % $\dot{W}$ max] was not significantly different from MLSS power, with which it was significantly ($P < 0.01$) correlated (r = 0.87).

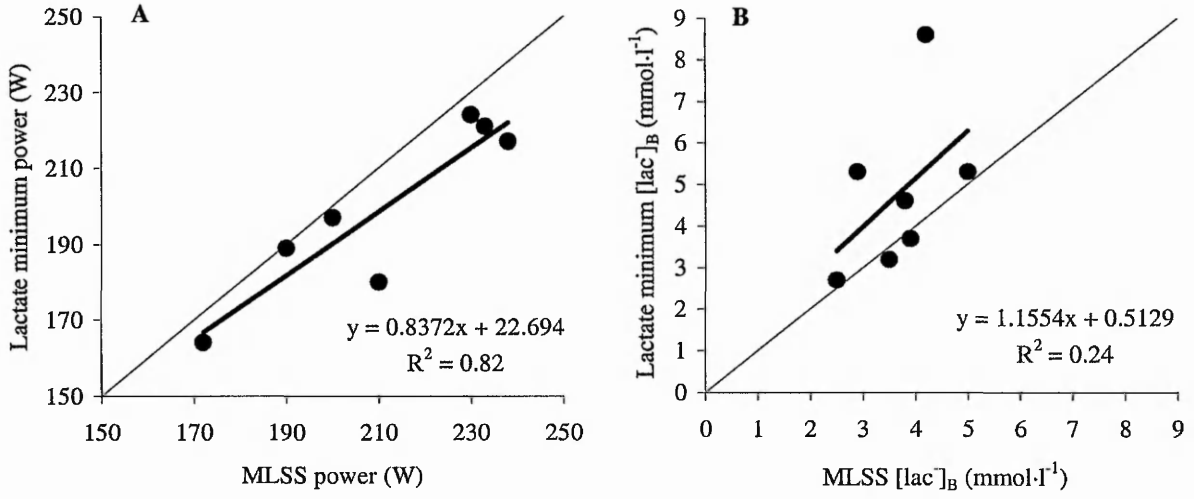

Figure 3.9 The relationship between lactate minimum and maximal lactate steady-state (MLSS) cycling powers (A), and the relationship between lactate minimum and MLSS blood lactate concentration ($[lac^-]_B$) (B). Line of identity is shown.

3.7.2 SERIES 2

$\dot{W}$ max was 363 (42) W, thus cycling powers corresponding to 45, 50, 55, 60, and 65% $\dot{W}$ max were 164 (19), 181 (21), 200 (23), 218 (25), and 236 (27) W, respectively. The lactate minimum cycling power was 192 (23) W [52.8 (1.2) % $\dot{W}$ max].

Changes in $[lac^-]_B$ during the incremental phase and during constant power exercise are shown in Figures 3.10 and 3.11, respectively. The $[lac^-]_B$ at 14 min during constant power exercise at 50% $\dot{W}$ max [4.8 (1.0) mmol·l^{-1}] was significantly ($P < 0.05$) lower than that observed at the commencement of 50% $\dot{W}$ max during the incremental phase [5.5 (1.2) mmol·l^{-1}]. There were no such differences at the other 4 intensities.

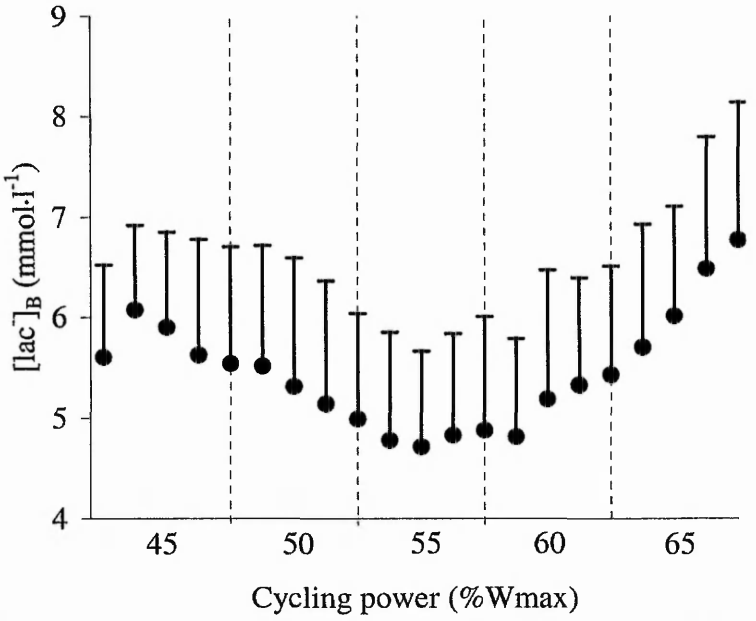

Figure 3.10 Changes in blood lactate concentration ($[lac^-]_B$) during the incremental phase. Dashed vertical lines represent the end and start of consecutive exercise stages. Values are mean (SD).

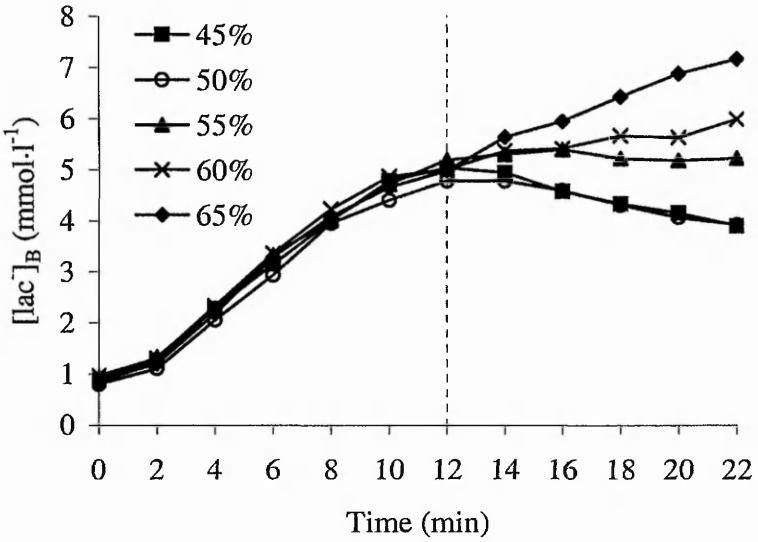

Figure 3.11 Changes in blood lactate concentration ($[lac^-]_B$) during constant power exercise. Note that for all trials the initial 12 min of exercise was performed at 60% $\dot{W}$ max: dashed vertical line represents the transition in cycling power. Values are means, but for clarity error bars are not shown.

Changes in $\Delta[lac^-]_B/\Delta t$ during the incremental phase and during constant power exercise are shown in Figure 3.12. Compared to constant power exercise, $\Delta[lac^-]_B/\Delta t$ was significantly greater during the incremental phase at 60 [0.16 (0.06) vs. 0.07 (0.08) mmol·l^{-1}·min^{-1}, $P < 0.05$] and 65% $\dot{W}$ max [0.35 (0.10) vs. 0.20 (0.08) mmol·l^{-1}·min^{-1}, $P < 0.01$].

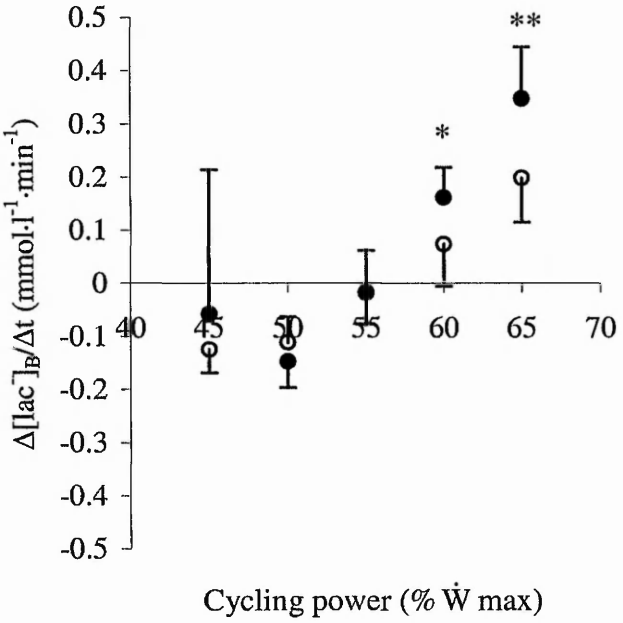

Figure 3.12 Rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during the incremental phase (•) and constant power (○) exercise. *Significant difference between trials ($P < 0.05$). **Significant difference between trials ($P < 0.01$). Values are mean (SD).

Figure 3.13 shows plots of $\Delta[lac^-]_B/\Delta t$ for each subject during the incremental phase against $\Delta[lac^-]_B/\Delta t$ during constant power exercise at each % $\dot{W}$ max. There was no correlation between $\Delta[lac^-]_B/\Delta t$ during the incremental phase and $\Delta[lac^-]_B/\Delta t$ during constant power exercise. Correlation coefficients between $\Delta[lac^-]_B/\Delta t$ recorded during the incremental phase and constant power exercise were 0.07, 0.002, -0.04, -0.14, and 0.63 for exercise at 45, 50, 55, 60, and 65% $\dot{W}$ max, respectively.

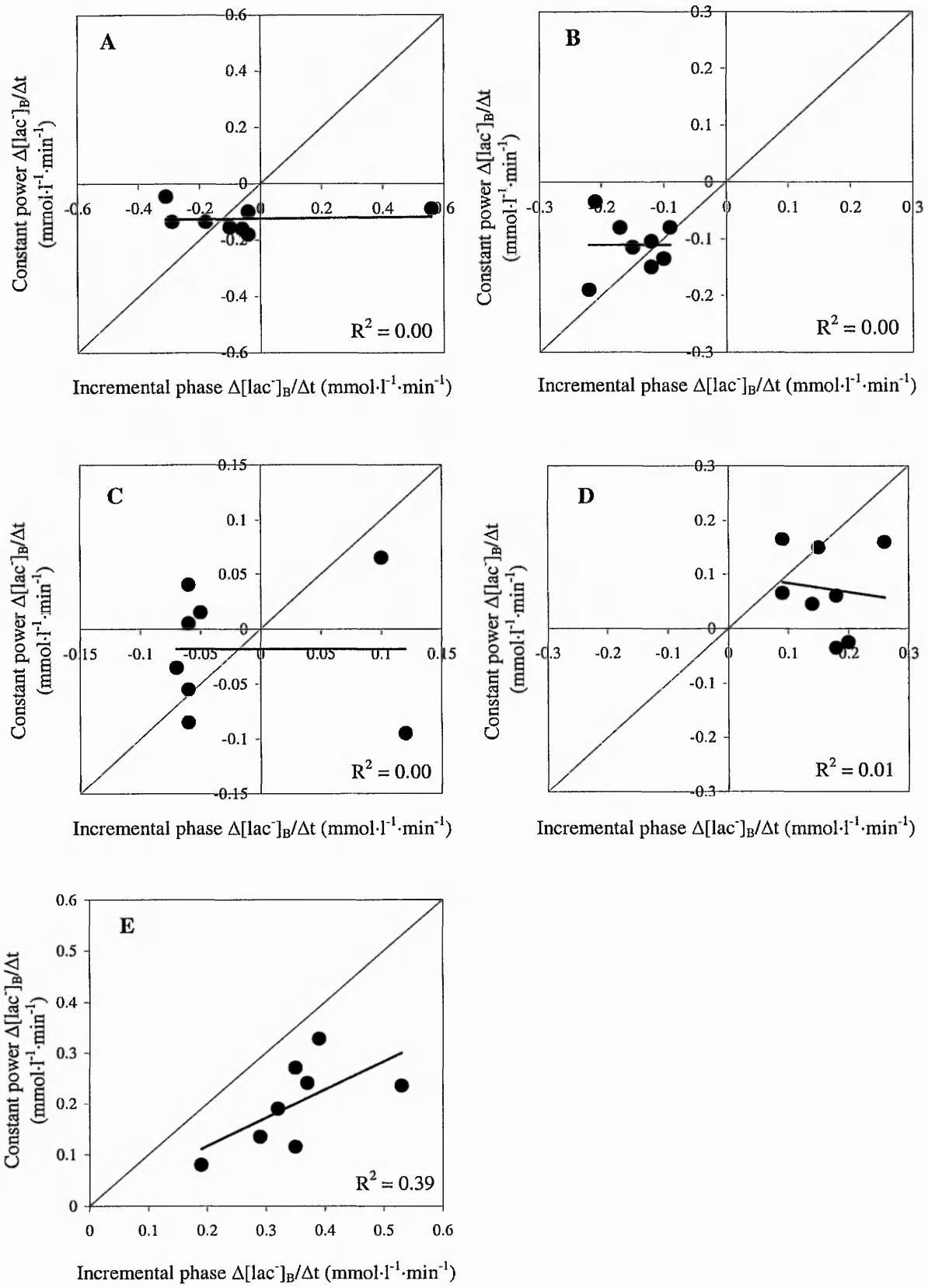

Figure 3.13 Relationship between rates of change of blood lactate concentration ($\Delta[lac^-]_B/\Delta t$) during the incremental phase and constant power exercise. A, B, C, D, and E represent 45, 50, 55, 60, and 65% $\dot{W}$ max, respectively. Line of identity is shown on each chart.

3.7.3 SERIES 3

Time to exercise intolerance during the lactate elevation phase of LM_{ARM} [8.98 (1.60) min] was not significantly different from that in LM_{LEG} [9.46 (0.75) min]. The $\dot{W}$ max attained during the lactate elevation phase of LM_{ARM} [158 (42) W] was significantly ($P < 0.01$) lower than that achieved during LM_{LEG} [316 (43) W].

All subjects demonstrated a U-shaped $[lac^-]_B$ vs. power profile during LM_{LEG} (Figure 3.14) and the lactate minimum power was 167 (21) W [53.1 (2.8) % $\dot{W}$ max]. In contrast a U-shaped $[lac^-]_B$ profile was not observed in 7 of the 10 subjects during LM_{ARM}, thus a lactate minimum cycling power was not calculable in these subjects.

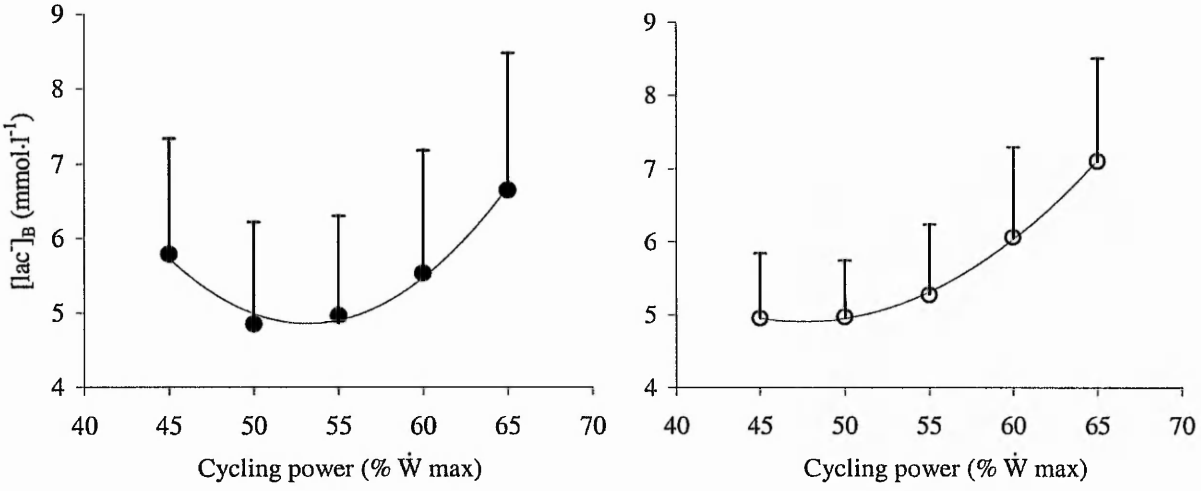

Figure 3.14 Relationship between relative cycling power and blood lactate concentration ($[lac^-]_B$) at the end of each exercise stage of the incremental phase in LM_{LEG} (•) and LM_{ARM} (○). Values are mean (SD).

Temporal changes in $[lac^-]_B$ during the incremental phase of LM_{LEG} and LM_{ARM} tests are shown in Figure 3.15. During LM_{LEG} $[lac^-]_B$ progressively declined at 45 and 50% $\dot{W}$ max, reached a nadir of 4.8 (1.4) mmol·l^{-1} after 1 min at 55% $\dot{W}$ max, and then progressively increased for the remainder of the test. Conversely, a marked decline in $[lac^-]_B$ was not observed during the early stages of LM_{ARM}: an initial 0.3 (0.2) mmol·l^{-1} decline following 1 min at 45% $\dot{W}$ max was succeeded by a relatively stable $[lac^-]_B$ of

approximately 5 mmol·l^{-1} up to the start of 55% $\dot{W}$ max where a progressive increase, similar to that demonstrated in LM_{LEG}, was observed for the remainder of the test. Thus there appeared to be a point of inflection in the $[lac^-]_B$ response to the incremental phase of LM_{ARM}, which occurred at a similar time to the nadir of the $[lac^-]_B$ vs. power curve in LM_{LEG}. Compared to LM_{ARM}, $[lac^-]_B$ was significantly ($P < 0.05$) higher during LM_{LEG} at the start of the incremental phase and during the first 3 min of exercise at 45% $\dot{W}$ max. Thereafter, no significant differences were observed between LM_{LEG} and LM_{ARM} tests.

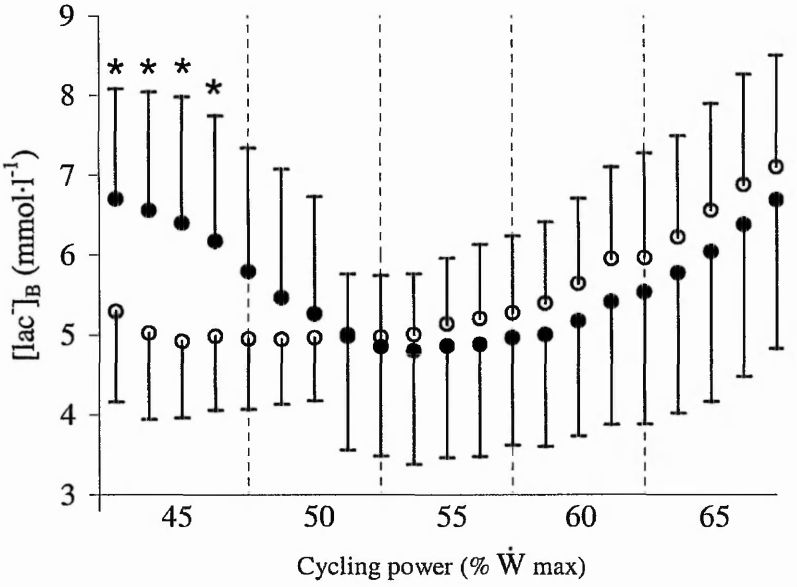

Figure 3.15 Changes in blood lactate concentration ($[lac^-]_B$) during the incremental phase of LM_{LEG} (•) and LM_{ARM} (○). Dashed vertical lines represent the end and start of consecutive exercise stages. *Significant difference between tests ($P < 0.05$).

Figure 3.16 shows changes in $\Delta[lac^-]_B/\Delta t$ during LM_{LEG} and LM_{ARM} tests. The calculated power at which $\Delta[lac^-]_B/\Delta t = 0$ in LM_{LEG} [173 (21) W, 55% (3) $\dot{W}$ max] was significantly ($P < 0.05$) greater than that in LM_{ARM} [153 (23) W, 49% (3) $\dot{W}$ max]. For the LM_{LEG} test the lactate minimum power was significantly ($P < 0.01$) lower than that at which $\Delta[lac^-]_B/\Delta t = 0$. Significant ($P < 0.05$) differences in $\Delta[lac^-]_B/\Delta t$ were observed between LM_{LEG} and LM_{ARM} tests at 45 [LM_{LEG} vs. LM_{ARM}: -0.22 (0.12) vs. –0.07 (0.11)

mmol·l^{-1}·min^{-1}] 50 [-0.23 (0.08) vs. 0.01 (0.13) mmol·l^{-1}·min^{-1}], and 60% [0.16 (0.09) vs. 0.21 (0.09) mmol·l^{-1}·min^{-1}] $\dot{W}$ max.

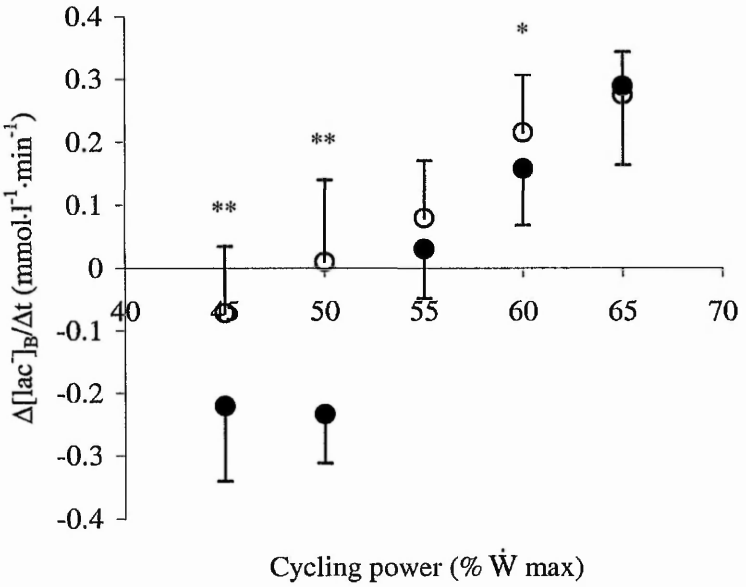

Figure 3.16 $\Delta[lac^-]_B/\Delta t$ during each stage of the incremental phase of LM_{LEG} (•) and LM_{ARM} (○). *Significant difference between tests ($P < 0.05$). **Significant difference between tests ($P < 0.01$).

3.8 Discussion

There were three primary findings from the present study: (I) the lactate minimum power provided a significant underestimate of MLSS power; (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 $[lac^-]_B$ profile of the incremental phase is dependent on whether the lactate elevation phase is performed using like or remote muscle groups.

The lactate minimum test was originally devised by Tegtbur et al. (1993) to provide an objective, working estimate of MLSS - now considered the "gold standard" measure of endurance exercise capacity (Billat et al. 2003; Jones and Carter 2000; Jones and Doust 1998). The validity of the lactate minimum test to predict MLSS has received empirical support from studies demonstrating steady-state $[lac^-]_B$ during prolonged exercise at the

lactate minimum running speed, but a continuous increase at speeds 0.13-0.2 $m \cdot s^{-1}$ above lactate minimum speed (Bacon and Kern 1999; Tegtbur et al. 1993). However, when Jones and Doust (1998) determined MLSS in a truly independent fashion, the lactate minimum running speed significantly underestimated MLSS speed by, on average, 0.8 $km \cdot h^{-1}$. It should be noted that Jones and Doust (1998) defined MLSS as the highest speed at which there was no more than a 1.0 $mmol \cdot l^{-1}$ increase in $[lac^-]_B$ between 10-30 min of constant velocity exercise. The MLSS criterion used in the present study is more rigorous, which along with the difference in exercise modalities precludes a direct comparison of the studies. However it is tenable to speculate that had Jones and Doust (1998) adopted a more stringent MLSS criteria then the discrepancy with the lactate minimum speed may have been reduced. Another possibility, however, is that if the lactate elevation phase causes fatigue of active muscle fibres, then increased recruitment of lactate producing type II fibres during the incremental phase may result in premature lactate production, and perhaps reduced lactate clearance, and thus an earlier turnpoint on the $[lac^-]_B$ vs. power curve (Carter et al. 1999b). That $\Delta[lac^-]_B/\Delta t$ was greater during the latter stages of the incremental phase compared to that observed during constant power exercise (series 2) provides some support for this contention. However, even if altered muscle fibre recruitment contributes to the discrepancy between lactate minimum and MLSS powers, a practical solution to this quandary is not readily forthcoming, since series 3 shows that when the lactate elevation phase is performed using remote muscle groups (thus "sparing" the leg locomotor muscles) a U-shaped $[lac^-]_B$ profile is not observed during the incremental phase.

In the present study the lactate minimum power underestimated MLSS power by 12 (10) W. Investigators must decide whether such a difference is acceptable and whether the lactate minimum test represents a valid tool for MLSS determination (taken on balance

with the accuracy/reliability of other protocols available for MLSS determination). The usefulness of the test in an applied setting is, however, compromised by the observation that it is not sensitive to training-induced changes in MLSS (Carter et al. 1999a).

It is interesting to note here that when the data from all 25 subjects was pooled, the lactate minimum power was 184 (26) W, or 53.0% (2.0) $\dot{W}$ max. Thus the difference between lactate minimum power and 53.0% $\dot{W}$ max was, on average, only 4 W. This suggests that for a moderately-trained population (like that used in the present study) 53.0% $\dot{W}$ max achieved during an incremental exercise test performed in accordance with the lactate elevation phase protocol described in this study, could represent a reasonable estimate of MLSS power. In support, the difference between 53.0% $\dot{W}$ max and MLSS power in series 1 was 11 (12) W, which is almost identical to the 12 (10) W difference actually noted between lactate minimum and MLSS powers. The practical advantages of this are that MLSS power may be estimated in a moderately trained population from a short (around 10 min) incremental exercise test without using invasive and sometimes expensive blood sampling procedures. However, note that this method is not recommended for monitoring training-induced changes in MLSS, which may increase independent of changes in $\dot{W}$ max (Jones and Carter 2000).

The findings of series 2 provide a possible explanation for the discrepancy between lactate minimum and MLSS powers; specifically, $\Delta[lac^-]_B/\Delta t$ during the incremental phase did not correlate with $\Delta[lac^-]_B/\Delta t$ during constant power exercise. This finding calls into question the original proposition made by Davis and Gass (1979) that $\Delta[lac^-]_B/\Delta t$ during the incremental phase has "predictive value for steady state work". Since this proposition formed the basis of Tegtbur et al.'s (1993) work these findings also cast doubt upon the theoretical underpinnings of the lactate minimum test. Carter et al. (1999b) previously acknowledged the possibility that changes in $[lac^-]_B$ during the incremental phase did not

solely reflect the metabolic demands of the corresponding work-rate; they showed that when different initial speeds were used for the incremental phase, the time taken to reach the lactate minimum speed remained unchanged, but the lactate minimum speed increased linearly with the starting speed. These data, along with those reported in series 2 of this study, imply that the theoretical basis of the lactate minimum test is fundamentally flawed and leads to the conclusion that any agreement between lactate minimum and MLSS powers may simply reflect a fortuitous artefact of the protocol design.

The lack of correlation between $\Delta[lac^-]_B/\Delta t$ during the incremental phase of the lactate minimum test and constant power exercise may be explained, in part, by the findings of series 3, in which the $[lac^-]_B$ profile of the incremental phase was dependent upon the exercise performed during the lactate elevation phase. More specifically, the findings of series 3 imply that the purpose of the lactate elevation phase is not exclusively to increase $[lac^-]_B$ (thus, in retrospect the expression "lactate elevation phase" may be somewhat misleading). Rather, the lactate elevation phase has other physiological consequences that are essential if a U-shaped $[lac^-]_B$ profile is to be observed during the incremental phase. This conclusion is based on the observation that when hyperlactaemia was induced using different muscle groups (arm cranking) the characteristic U-shaped curve was not observed during the leg cycling incremental phase. Therefore, what is referred to as the "lactate elevation phase" in this study has considerable consequences for $\Delta[lac^-]_B/\Delta t$ during the incremental phase of the lactate minimum test.

A mechanism by which the lactate elevation phase affects lactate kinetics during the subsequent incremental phase may reside in the influence that prior exercise imparts upon mitochondrial inertia. This phenomenon describes the oxygen-independent delay in ATP synthesis through oxidative pathways at the onset of exercise (Jones et al. 2003), and is usually manifest by a delayed increase in $\dot{V}O_2$ (Burnley et al. 2002; Koppo et al. 2003)

and a transient increase in leg lactate production and efflux into the blood (Wasserman et al. 1986). It is well established that prior bouts of heavy exercise reduce mitochondrial inertia during subsequent exercise (reviewed in Jones et al. 2003), thus it is likely that the lactate elevation phase of LM_{LEG} 'primed' the oxidative metabolic pathway prior to the incremental phase. This may have occurred through an increased availability of acetyl-groups for the tricarboxylic acid cycle subsequent to an increased activation of the pyruvate dehydrogenase complex (Timmons et al. 1998). Alternatively/Additionally the lactate elevation phase may have resulted in greater motor unit recruitment during the incremental phase (Burnley et al. 2002), hyperaemia and a subsequent reduction in the mismatch between muscle blood flow and $\dot{V}O_2$, and/or greater activation of type I fibres due to fatigue of type II fibres during the lactate elevation phase (Jones et al. 2003). These phenomena would favour less lactate production and enhanced lactate clearance during the initial stages of the incremental phase in LM_{LEG}. However, the priming influence of prior exercise is probably mediated by local rather than systemic factors (Fukuba et al. 2002; Koppo et al. 2003), thus this would not have occurred, at least to the same degree, during the lactate elevation phase of LM_{ARM}. This may therefore explain, in part, the differences in $\Delta[lac^-]_B/\Delta t$ and $[lac^-]_B$ profiles during the incremental phases of LM_{LEG} and LM_{ARM}.

Regardless of the mode of lactate elevation, mitochondrial inertia may complicate lactate kinetics throughout the incremental phase. Specifically, with each increment in power output during the incremental phase, there will be a temporal delay before oxidative metabolism readjusts to meet the new metabolic demand of the exercise intensity. This is shown in Figure 3.10 where the pattern of change in $[lac^-]_B$ between the first 2 values in stage 1 is somewhat erratic and unreflective of the remainder of the stage. It is also evident in Figure 3.6 (Part A) where, because of a 1 min intra-stage interruption, the initial $[lac^-]_B$ response to the latter stages of the incremental phase clearly does not reflect the remainder

of the stage. It is thus possible that mitochondrial inertia also contributes to the lack of correlation between $\Delta[lac^-]_B/\Delta t$ in the final phase of the test and that observed during constant power exercise.

Given that the lactate elevation phase is likely to severely disrupt exercise metabolism during the incremental phase, it is perhaps unsurprising that $\Delta[lac^-]_B/\Delta t$ in the final phase of the test does not correlate with $\Delta[lac^-]_B/\Delta t$ observed during constant power, continuous exercise (series 2). However, what is perhaps more surprising is that, despite this observation, the lactate minimum power provides a reasonable estimate of MLSS power (series 1; Jones and Doust 1998).

The findings of series 3 appear contrary to those of previous studies that have shown the lactate minimum cycling power to be insensitive to different lactate elevation phase protocols. For example, Caine et al. (1997) elevated $[lac^-]_B$ using either an incremental $\dot{V}O_2$ peak test or a 30 s Wingate test, and found no difference in the lactate minimum power despite observing distinct lactate profiles during the incremental phase. Similarly, Smith et al. (2002) found no change in the lactate minimum power when $[lac^-]_B$ was elevated using either an incremental $\dot{V}O_2$ peak test, a 30 or 40 s maximal sprint protocol, or a double sprint protocol consisting of two 20 s maximal sprints separated by a 60 s active recovery (dissimilar lactate profiles were again observed during the incremental phase). Note, however, that in these studies all phases of the lactate minimum test were performed using leg cycling and thus the same muscle groups; therefore, as Smith et al. (2002) suggest, the state of the locomotor muscles prior to the incremental phase was probably similar in the different lactate elevation protocols. Conversely, the experimental paradigm used in the present study presumably resulted in dissimilar intramuscular (legs) conditions prior to the incremental phase (Fukuba et al. 2002; Koppo et al. 2003), which thus enabled a more thorough evaluation of the influence of the lactate elevation phase.

It could be argued that the lower $[lac^-]_B$ during the first 3 min of the incremental phase in LM_{ARM} compared to LM_{LEG} had implications for lactate kinetics through reducing the extra-to-intramuscular lactate concentration gradient and thus lactate uptake. However, when the same muscle groups perform all phases of the lactate minimum test, $[lac^-]_B$ declines during the early stages of the incremental phase even when the starting $[lac^-]_B$ is reduced by pre-test muscle glycogen depletion (Tegtbur et al. 1993) or alternate lactate elevation phase protocols (Caine et al. 1997; Smith et al. 2002). Note also that in several subjects exercise at a given cycling power during the incremental phase was characterised by a positive $\Delta[lac^-]_B/\Delta t$ in LM_{ARM} but a negative $\Delta[lac^-]_B/\Delta t$ in LM_{LEG}, despite greater $[lac^-]_B$ in the former.

Taken together, the findings of series 3 suggest that the customary decline in $[lac^-]_B$ observed during the initial stages of the incremental phase is perhaps causatively linked to the "priming" effects resultant of prior heavy exercise. These observations could partly explain why, as shown in series 2, $\Delta[lac^-]_B/\Delta t$ during the incremental phase fails to reflect $\Delta[lac^-]_B/\Delta t$ during constant power exercise.

In summary, this study has shown that the lactate minimum power underestimates MLSS power (series 1), possibly because lactate kinetics during the incremental phase have limited predictive power for the lactate kinetics of constant power exercise (series 2). This latter observation could be partly explained by the observation that the $[lac^-]_B$ response to the incremental phase is modified by the high-intensity exercise performed in the lactate elevation phase (series 3).

CHAPTER 4

EFFECTS OF INSPIRATORY MUSCLE TRAINING UPON CYCLING TIME-TRIAL PERFORMANCE IN CYCLISTS

4.1 INTRODUCTION

In 1976 Leith and Bradley demonstrated that the respiratory muscles are sensitive to both strength and endurance training regimens. Since this landmark study, much attention has focused on whether specific respiratory muscle training (RMT) can improve whole-body endurance exercise performance (McConnell and Romer 2004a). However, the ergogenicity of RMT remains somewhat controversial. For example, studies employing respiratory muscle endurance training via voluntary isocapnic hyperpnoea have observed significant improvements in cycling endurance at moderate (70-85% $\dot{W}$ max) but not high (90% $\dot{W}$ max or 95% $\dot{V}O_2$ peak) exercise intensities (Boutellier et al. 1992; Fairbarn et al. 1991; Markov et al. 2001; McMahon et al. 2002; Morgan et al. 1987; Spengler et al. 1999). Also, studies utilising pressure-threshold or flow resistive inspiratory muscle training (IMT) have recorded improvements in cycling endurance at 75% $\dot{V}O_2$ peak (Gething et al. 2004b) but no change in running endurance at 85% $\dot{V}O_2$ peak (Williams et al. 2002). These discrepancies are difficult to comprehend, but perhaps unsurprising given the differences between studies in many aspects of experimental design. More importantly, however, the significance of these results is somewhat undermined by the fact that these studies either lacked a true placebo (PLC) group and/or because they used a fixed work-rate performance evaluation test that is often criticised for being unreliable and lacking in external validity (Jeukendrup et al. 1996).

In light of these experimental deficiencies, recent well-controlled RMT studies have incorporated legitimate PLC groups and time-trial tests of exercise performance. Volianitis et al. (2001) demonstrated 3.5 and 1.6% improvements in the distance covered during a 6 min all-out rowing exercise following pressure-threshold IMT and PLC (IMT with negligible resistance) regimens, respectively, with changes in performance being significantly greater for the IMT group. Similarly, Romer et al. (2002a) observed 3.5 and

3.4% improvements in 20 and 40 km cycling time-trial performances, respectively, in trained cyclists following pressure threshold IMT; again, these changes exceeded those observed in a PLC (IMT with negligible resistance) group. These IMT-induced improvements in rowing and cycling time-trial performance were also accompanied by an attenuation of exercise-induced global inspiratory muscle fatigue, as quantified using maximal inspiratory mouth pressure (MIP) measurements. These observations, together with recently reported IMT-induced reductions in $[lac^-]_B$ (McConnell and Sharpe 2005; Romer et al. 2002b), $\dot{V}_E$ (Gething et al. 2004b; McConnell and Sharpe 2005), and perceived limb and respiratory effort (Gething et al. 2004b; Romer et al. 2002b; Romer et al. 2002c; Williams et al. 2002), are taken as evidence of a genuine training effect of IMT.

However, it is also suggested that PLC effects primarily account for the ergogenic influence of IMT: although Sonneti et al. (2001) demonstrated a 1.8% improvement in 8 km cycling time-trial performance following concurrent inspiratory muscle strength and endurance training in trained cyclists, this improvement failed to exceed changes in performance observed in a sham hypoxic-training PLC group. Sonetti et al. (2001) argue that the PLC design of most IMT studies (i.e. IMT with negligible resistance) fails to activate important PLC factors, whereas their sham 'altitude breather' satisfied the criteria for a legitimate PLC; that is, to be both inert and generate expectations, involvement, subjective utility, and be meaningful to the subjects.

Given the ambiguity surrounding the efficacy of RMT, the aim of this study was to re-evaluate the effects of pressure threshold IMT on cycling time-trial performance in competitive cyclists. In addition, the present study examined, for the first time, the physiological and perceptual response to time-trial exercise pre- and post-IMT. To address the issue of whether PLC effects explain the ergogenicity of IMT, an identical PLC treatment to that used by Sonetti et al. (2001) was employed. A secondary purpose of this

study was to derive an estimate of critical power [trained cyclists typically perform prolonged cycling time-trial exercise at an intensity close to CP (Harnish et al. 2001)], on which the effects of IMT are presented in chapter 4.

4.2 METHODS

4.2.1 PARTICIPANTS

Following local ethics committee approval and written informed consent, 11 male non-smoking competitive cyclists with normal resting pulmonary function were recruited for the study. Participants were assigned to either a pressure-threshold IMT group (n = 6) or a sham hypoxic training PLC group (n = 5). The age, height, and body mass of the IMT group were 30 ± 3 years, 181 ± 1.3 cm, and 76.1 ± 3.1 kg, respectively. Corresponding values for the PLC group were 29 ± 3 years, 177.9 ± 1.9 cm, and 77.9 ± 3.5 kg, respectively. For each subject the study took place during a maintenance phase of their regular training regimen. Subjects were instructed not to engage in strenuous activity the day before, or the day of, an exercise test. On test days, subjects were asked not to ingest caffeine, and to arrive at the laboratory at least 2 h post-prandial.

4.2.2 EXPERIMENTAL DESIGN

Pulmonary and inspiratory muscle function was initially assessed, and subjects subsequently performed a simulated 25 km cycling time-trial performance test. Subjects then commenced a 6 week IMT or PLC regimen. The effects of the intervention were evaluated through repeating the pre-training schedule at least 48 h after the final training session. Pre- and post-intervention trials were performed at a similar time of day so as to minimise diurnal fluctuation effects.

4.2.3 MEASUREMENTS

Pulmonary function and MIP was assessed according to the procedures outlined in section 2.5. Arterialised venous blood was sampled via an indwelling cannula for the determination of $[lac^-]_B$, as described in section 2.6. Limb and respiratory RPE were assessed during exercise according to the procedures outlined in section 2.9.

4.2.4 25 KM TIME-TRIAL PERFORMANCE

Cycling time-trial performance was evaluated as the time to cycle 25 km. Exercise was performed on an air-braked stationary cycle ergometer (Kingcycle; see section 2.1 for further details). Subjects rode their own bicycle (recommended) or, in the case of two subjects, the laboratory road bicycle (Raleigh Ventura). Subjects performed a 2 min warm-up at a self-selected work-rate and initiated the test from a rolling start. Expired air was collected in Douglas bags at 5, 10, 15 and 20 km, and analysed according to the procedures outlined in section 2.3. Regular verbal encouragement was given throughout exercise. Blood samples for $[lac^-]_B$ determination were taken at rest and every 4 min during exercise. Heart rate (HR) (Polar Accurex Plus, Kempele, Finland) and limb and respiratory RPE were determined every 4 min during exercise and at the end of the trial. Following exercise, subjects performed a 3 min cool-down at a self-selected work-rate. An assessment of MIP was made prior to exercise, following the 3 min cool-down period, and 15 min thereafter.

4.2.5 TRAINING PROTOCOLS

Subjects in the IMT group performed pressure-threshold IMT. A description of the IMT device and the training protocol employed are provided in section 2.8. The PLC group used a sham hypoxic trainer 5 $d \cdot wk^{-1}$ for 15 min. The training device was identical to that

used by the IMT group; however, the load-calibrated spring was removed and the lower chamber of the device was loosely packed with aquarium gravel. Subjects were told that the gravel was oxygen absorbent and subsequently reduced the oxygen content of inspired air, thus mimicking altitude/hypoxic exposure (Sonetti et al. 2001). Subjects were instructed to breathe normally and not to increase their normal breathing effort. This protocol has been shown to be effective in producing minimal changes in MIP (Sonetti et al. 2001). A training diary was provided to each subject, within which they recorded adherence to the prescribed training regimen, and also the mode, duration (min), and intensity (HR) of all whole-body exercise training sessions. An assessment of MIP was made every 2 weeks during the intervention.

4.2.6 Data analyses

Data collected at set time points during exercise were aligned to fixed percentages of total exercise time by assuming linear relationships between consecutive data points and interpolating between these values. Differences in baseline measurements between IMT and PLC groups were determined using t-tests for unpaired observations. Pre- and post-training results and group interactions were compared using one-way or two-way ANOVA for repeated measures. When a significant F ratio was detected Tukey's HSD post-hoc analysis was applied to determine where significant differences existed between pairs of mean values. The critical significance level was set at $P < 0.05$. Values are presented as mean ± SEM unless otherwise indicated. Statistical analyses were performed using the 11.0 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA).

4.3 RESULTS

4.3.1 TRAINING COMPLIANCE AND HABITUAL EXERCISE TRAINING

All subjects demonstrated excellent compliance with the prescribed training programme. The IMT group completed 81 ± 2 out of a possible 84 IMT sessions (97 ± 2% adherence), whereas the PLC group completed 29 ± 1 out of a possible 30 PLC sessions (95 ± 2% adherence). No marked deviations from habitual physical training strategies were observed in either group during the intervention.

4.3.2 PULMONARY AND INSPIRATORY MUSCLE FUNCTION

There were no changes in pulmonary function or MIP following PLC (Table 4.1 and Figure 4.1), and pulmonary function also remained unchanged following IMT. However, there was a significant ($P < 0.01$) 15.3 ± 3.7% increase in MIP following IMT which, as demonstrated by the significant ($P < 0.01$) interaction effect, exceeded that in the PLC group. A significant ($P < 0.01$) increase in MIP from baseline (+9.5 ± 1.7%) was observed in the IMT group following 4 weeks of training.

Table 4.1 Pulmonary and inspiratory muscle function in IMT and PLC groups. (Mean ± SEM).

	Pre-IMT[a]	Post-IMT[a]	Pre-PLC[b]	Post-PLC[b]
VC (l)	6.11 ± 0.29	6.18 ± 0.38	5.99 ± 0.47	6.17 ± 0.46
	(118 ± 5)	(119 ± 6)	(121 ± 8)	(125 ± 8)
FVC (l)	5.88 ± 0.26	5.91 ± 0.34	5.53 ± 0.42	5.78 ± 0.44
	(114 ± 4)	(114 ± 6)	(114 ± 8)	(120 ± 9)
FEV_1 (l)	5.02 ± 0.32	5.01 ± 0.37	4.66 ± 0.3	4.61 ± 0.3
	(119 ± 7)	(119 ± 8)	(112 ± 6)	(111 ± 6)
FEV_1/FVC (%)	85.7 ± 3.1	84.5 ± 3.1	84.4 ± 2.7	80.0 ± 2.2
	(107 ± 4)	(106 ± 4)	(101 ± 3)	(96 ± 3)
PEF ($l \cdot s^{-1}$)	10.7 ± 1.1	10.8 ± 1.4	10.4 ± 0.6	9.2 ± 1.0
	(101 ± 10)	(102 ± 13)	(95 ± 5)	(84 ± 9.4)
MVV_{12} ($l \cdot min^{-1}$)	207.8 ± 19.8	204.8 ± 17.7	162.8 ± 9.6	157.0 ± 14.5
	(135 ± 12)	(133 ± 11)	(105 ± 8)	(102 ± 13)
MIP (cmH_2O)	159 ± 5	183 ± 8**	168 ± 8	168 ± 7
	(145 ± 8)	(167 ± 10)	(145 ± 8)	(145 ± 8)

[a]$n = 6$, [b]$n = 5$. VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; PEF, peak expiratory flow; MVV_{12}, 12 s maximum voluntary ventilation; MIP, maximal inspiratory mouth pressure. Numbers in parentheses represent % of predicted value (Cotes 1993; Wilson et al. 1984). **Significantly different from pre-IMT ($P < 0.01$).

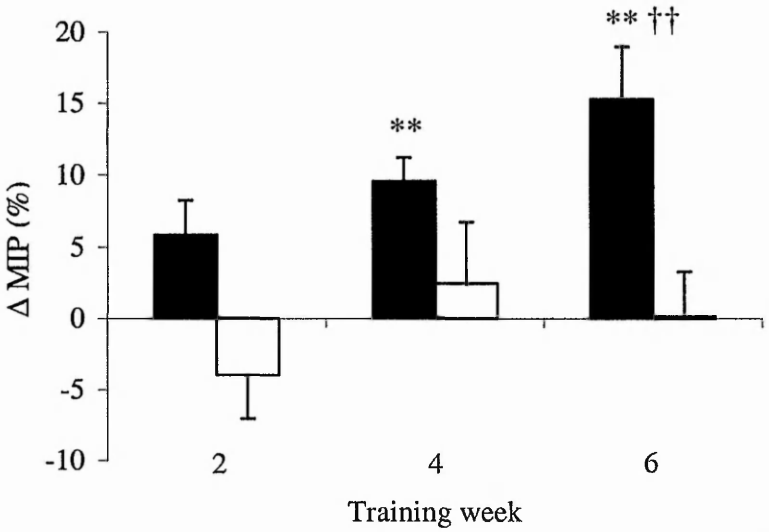

Figure 4.1 Changes in MIP during IMT (filled bars) and PLC (open bars). **Significantly different from baseline ($P < 0.01$). ††Significant interaction effect ($P < 0.01$).

4.3.3 TIME-TRIAL PERFORMANCE

Individual performance times and mean relative changes in 5 km split times are presented in Figures 4.2 and 4.3, respectively. Time-trial performance was unchanged following PLC (pre- vs. post-: 36.03 ± 0.86 vs. 36.62 ± 0.54 min, $P = 0.35$). Conversely, there was a significant ($P < 0.05$) 3.0 ± 1.1% improvement in time-trial performance following IMT (35.68 ± 1.34 vs. 34.64 ± 1.52 min). Changes in time-trial performance were also different between groups ($P < 0.05$). There were also significant ($P < 0.05$) improvements in 5 km split time at 15 and 20 km following IMT, whereas no changes were observed following PLC. Significant interaction effects were observed at both 15 ($P < 0.05$) and 20 ($P < 0.01$) km.

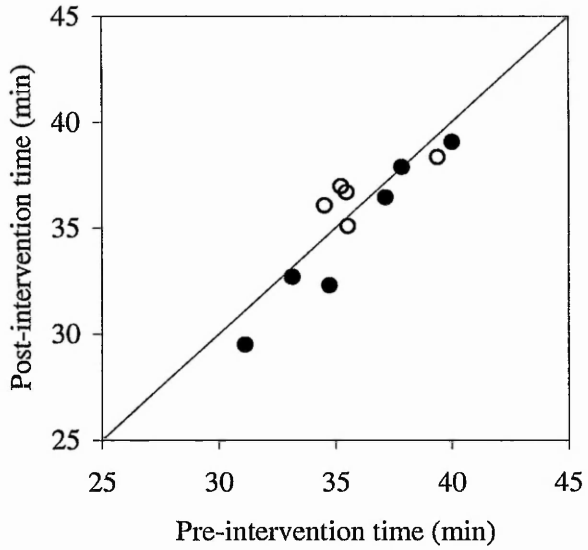

Figure 4.2 Individual performance times in IMT (•) and PLC (○) subjects. Points positioned below the line of identity indicate performance improvement post-intervention.

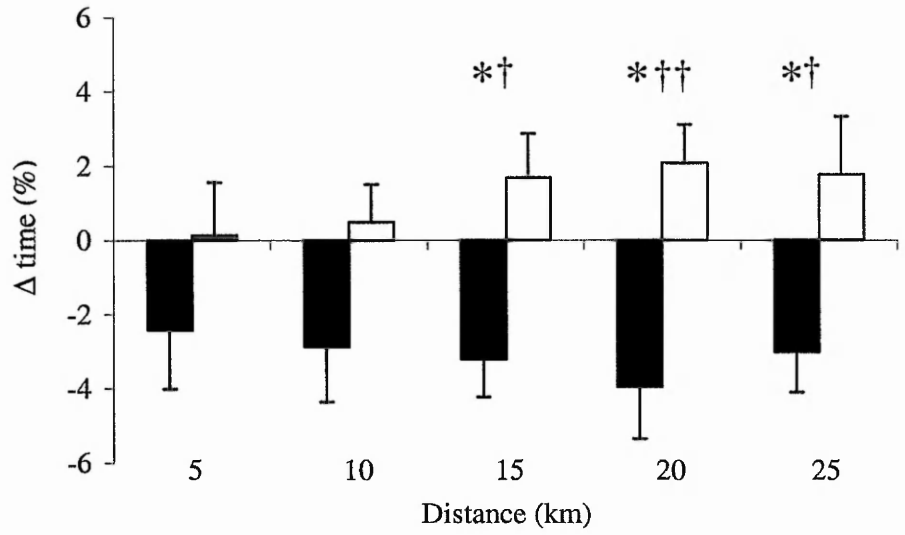

Figure 4.3 Relative changes in performance times at 5 km intervals in IMT (filled bars) and PLC (open bars) groups. *Significantly different from pre-intervention ($P < 0.05$). †Significant interaction effect ($P < 0.05$). ††Significant interaction effect ($P < 0.01$).

4.3.4 PHYSIOLOGICAL RESPONSES TO EXERCISE

Mean values for $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, and HR during time-trial exercise before and after the intervention are shown in Table 4.2. No changes were observed in $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, or RER at equal distances or when averaged across all distances following IMT or PLC. HR responses were also unchanged in both groups following the intervention.

Due to failed blood sampling, $[lac^-]_B$ was determined in only 3 PLC subjects during exercise, thus statistical analysis of $[lac^-]_B$ results was performed only on the IMT group; note, however, that the mean $[lac^-]_B$ during exercise pre- and post-PLC was quite similar (5.8 ± 0.6 vs. 5.4 ± 1.0 mmol·l^{-1}, respectively). The mean $[lac^-]_B$ during exercise was significantly ($P < 0.05$) reduced from 5.2 ± 0.7 to 4.2 ± 0.9 mmol·l^{-1} (-22.5 ± 7.6%) following IMT (Figure 4.4). Following IMT $[lac^-]_B$ was significantly ($P < 0.05$) lower at 40 (5.8 ± 0.8 vs. 4.5 ± 1.0 mmol·l^{-1}), 60 (5.2 ± 0.9 vs. 4.4 ± 0.9 mmol·l^{-1}), and 80% (5.1 ± 0.9 vs. 4.1 ± 1.0 mmol·l^{-1}) total exercise time.

Table 4.2 Mean physiological responses to time-trial exercise pre- and post-intervention in IMT and PLC groups. (Mean ± SEM).

	IMT		PLC	
	Pre	Post	Pre	Post
$\dot{V}_E$ ($l \cdot min^{-1}$)	131.9 ± 18.5	127.9 ± 17.4	120.0 ± 6.0	116.3 ± 8.9
	(73 ± 5)	(71 ± 5)	(77 ± 5)	(76 ± 9)
$\dot{V}O_2$ ($l \cdot min^{-1}$)	3.84 ± 0.37	3.91 ± 0.41	3.64 ± 0.15	3.59 ± 0.18
	(81 ± 4)	(82 ± 4)	(86 ± 3)	(85 ± 4)
$\dot{V}CO_2$ ($l \cdot min^{-1}$)	3.59 ± 0.36	3.65 ± 0.37	3.33 ± 0.12	3.33 ± 0.14
	(76 ± 3)	(77 ± 3)	(75 ± 2)	(75 ± 3)
RER	0.93 ± 0.01	0.93 ± 0.02	0.92 ± 0.02	0.93 ± 0.03
	(93 ± 3)	(93 ± 3)	(86 ± 1)	(87 ± 1)
HR ($beats \cdot min^{-1}$)	176 ± 5	173 ± 3	182 ± 3	180 ± 3

$\dot{V}_E$, minute ventilation; $\dot{V}O_2$, pulmonary oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; RER, respiratory exchange ratio; HR, heart rate. Values in parenthesis represent percentage of maximum values determined during a maximal incremental exercise test.

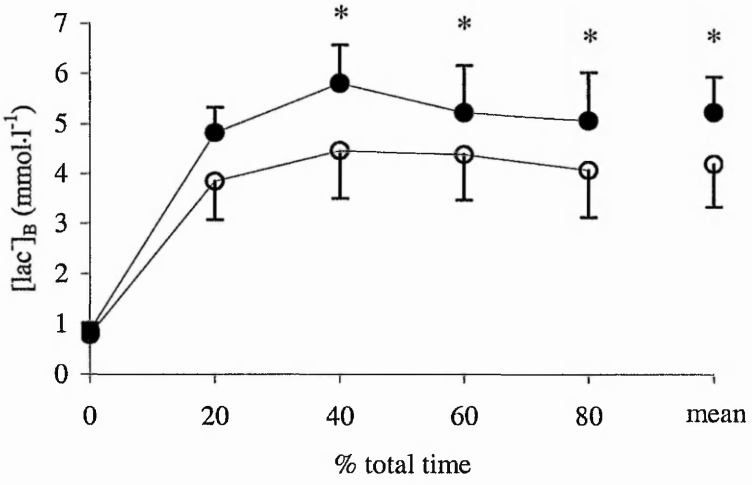

Figure 4.4 Changes in blood lactate concentration ($[lac^-]_B$) during exercise pre- (•) and post- (○) IMT. *Significantly different from pre-IMT ($P < 0.05$).

4.3.5 PERCEPTUAL RESPONSES TO EXERCISE

Respiratory RPE increased progressively during exercise pre- and post-intervention (Figure 4.5). There was a significant ($P < 0.05$) reduction in mean respiratory RPE

following IMT (6.5 ± 0.2 vs. 5.4 ± 0.4, -17.4 ± 6.3%), although the interaction effect failed to reach statistical significance ($P = 0.071$). There was a trend ($P \leq 0.085$) for respiratory RPE to be lower from 20-80% of the total exercise time, inclusive, following IMT.

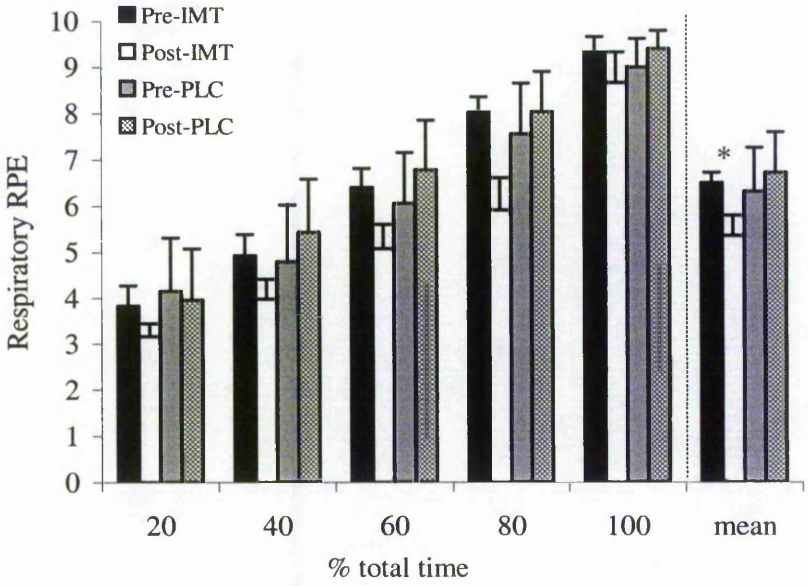

Figure 4.5 Respiratory ratings of percived exertion (RPE) during exercise in IMT and PLC groups. *Significant difference pre vs. post ($P < 0.05$).

Limb RPE also increased progressively in both groups during exercise, reaching maximal or near-maximal (i.e. 19-20) values at the end of exercise both pre- and post-intervention (Figure 4.6). There was a trend ($P = 0.056$) for mean limb RPE during exercise to be reduced following IMT (16.3 ± 0.4 vs. 15.5 ± 0.3, -4.7 ± 1.9%). Significant reductions in limb RPE were observed following IMT at 20 (13.6 ± 0.6 vs. 11.8 ± 0.6, -12.9 ± 3.0%, $P < 0.01$) and 40% (14.9 ± 0.5 vs. 13.7 ± 0.5, -8.1 ± 2.4%, $P < 0.05$) total exercise time, although the interaction effect just failed to attain statistical significance at both time points ($P = 0.062$ and 0.086 at 20 and 40% total exercise time, respectively).

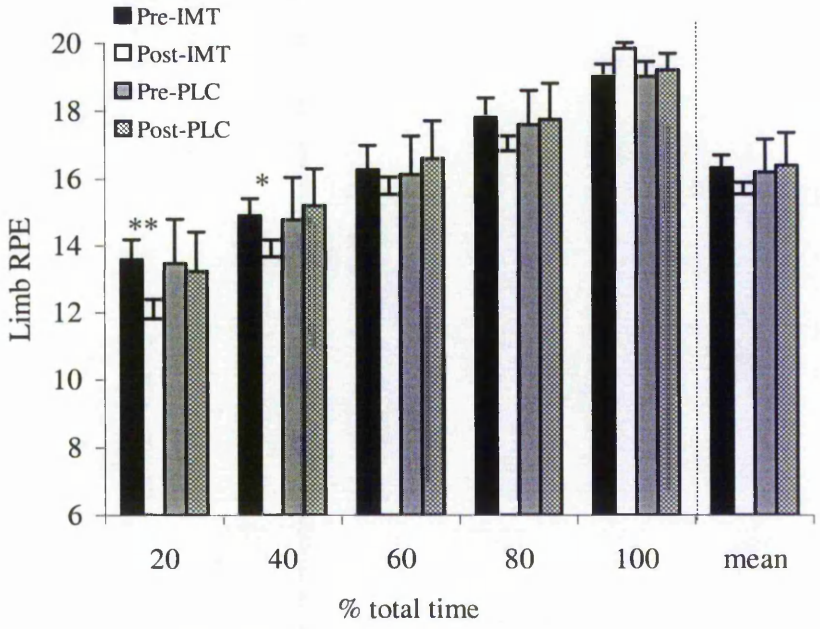

Figure 4.6 Limb ratings of percived exertion (RPE) during exercise in IMT and PLC groups. *Significant difference pre vs. post ($P < 0.05$). **Significant difference pre vs. post ($P < 0.01$).

4.3.6 INSPIRATORY MUSCLE FATIGUE

Exercise-induced changes in MIP are shown in Figure 4.7. Measurements made 3 min post-exercise prior to the intervention revealed reductions in MIP from baseline of 8.4 ± 3.9 ($P = 0.075$) and 13.5 ± 2.1% ($P < 0.05$) for IMT and PLC groups, respectively. Note, however, that a non-significant P value was attained in the IMT group primarily because one subject demonstrated a 5.7% increase in MIP post-exercise. All other IMT subjects demonstrated a reduction in MIP ranging from 6.1-19.5%. Following 15 min of recovery values were not different from baseline in either IMT or PLC groups. Following the intervention MIP measurements made 3 min post-exercise revealed significant ($P < 0.05$) reductions in MIP from baseline of 6.8 ± 1.3 and 9.0 ± 2.7% in IMT and PLC groups, respectively. Following 15 min recovery values remained 5.8 ± 1.4% below baseline in the IMT group ($P < 0.05$) but were not different from baseline in the PLC group. Exercise-induced changes in MIP following the intervention were not different from those observed

prior to the intervention in both IMT and PLC groups. Changes in MIP following exercise were not different between IMT and PLC groups pre- or post-intervention.

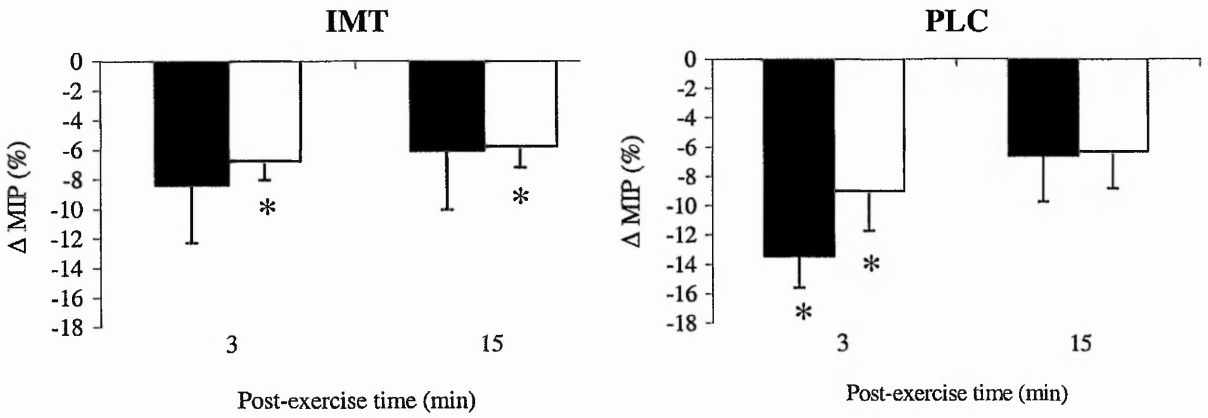

Figure 4.7 Relative changes in maximal inspiratory mouth pressure (MIP) following exercise in IMT (left panel) and PLC (right panel) groups. Filled and open bars denote pre- and post-intervention, respectively. *Significantly different from baseline ($P < 0.05$).

4.4 Discussion

The main finding of this study was that 6 weeks of pressure threshold IMT significantly improved 25 km cycling time-trial performance in competitive cyclists. In addition, these improvements were associated with reductions in $[lac^-]_B$ and the perceptual response to exercise, but not, perhaps surprisingly, a reduction in global inspiratory muscle fatigue.

The effectiveness of our IMT regimen, and the high degree of training adherence, was epitomised in the present study by the 15.3% increase in MIP. This IMT-mediated increase in global inspiratory muscle strength is almost 2-fold greater than the 8% increase reported by Sonetti et al. (2001), but is considerably less than the 25-55% increase reported in several other IMT studies (Gething et al. 2004b; Huang et al. 2003; Inbar et al. 2000; Leith and Bradley 1976; Romer et al. 2002a; Romer et al. 2002c; Volianitis et al. 2001). For a discussion of the possible factors that determine the extent with which IMT improves MIP, consult the Discussion of chapter 5.

The 3.0% improvement in 25 km cycling time-trial performance observed in the present study concurs with the findings of previous studies in which comparable improvements in cycling and rowing time-trial performances were observed in endurance athletes performing an identical IMT regimen to that employed in the present study (Romer et al. 2002b; Volianitis et al. 2001). Hopkins et al. (1999) suggest that for an enhancement in athletic performance to be deemed worthwhile, it should be at least half of the typical variation between individual performances. For time-trials lasting approximately 30-60 min competitive male cyclists have a typical variation in performance of approximately 1-3.4% (Jeukendrup et al. 1996; Palmer et al. 1996; Paton and Hopkins 2001). Therefore, the IMT-mediated change in performance observed in this study would probably constitute a worthwhile improvement.

The finding of a positive outcome with IMT is also consistent with a previous study in which strengthening of the inspiratory muscles through inspiratory flow resistive loading improved constant-load cycling endurance at 75% $\dot{V}O_2$peak (Gething et al. 2004b). Conversely, pressure-threshold IMT failed to improve running endurance at 85% $\dot{V}O_2$peak (Williams et al. 2002). The duration of the fixed work-rate running trial (approximately 19 min) in this latter study was similar to that reported in studies showing a positive ergogenic effect with RMT for leg cycling endurance at fixed intensities (McMahon et al. 2002; Spengler et al. 1999). Thus although speculative, exercise modality might influence the efficacy of RMT, with cycling performance benefiting more than running performance. This notion is consistent with the idea that the crouched position of cycling increases abdominal impedance and thus diaphragmatic work (Hill et al. 1991), which subsequently results in greater inspiratory muscle fatigue during cycling compared to running (Boussana et al. 2001). However, this paradigm is grounded on the assumption that an attenuation of inspiratory muscle fatigue is the primary mechanism by which IMT

improves endurance performance; this has yet to be proven and is not supported by the present study. An alternative explanation for the results of Williams et al. (2002) is that they utilised a fixed-intensity running test to evaluate performance, which may be less sensitive to the ergogenic effects of IMT (McConnell and Romer 2004a).

Although the present study suggests that IMT can have a positive influence upon cycling time-trial performance, the underlying mechanisms responsible remain obscure. Sonetti et al. (2001) propose that the efficacy of IMT resides primarily in PLC effects. The findings of the present study are not, however, consistent with this notion since the improvement in time-trial performance following IMT exceeded that observed in the PLC group that, like the PLC group in Sonetti et al.'s (2001) study, also performed sham hypoxic training. Thus it is likely that the changes in performance observed in this study are evidence of a real ergogenic effect with IMT.

The present study is the first to identify an approximate 1.0 $mmol \cdot l^{-1}$ reduction in $[lac^-]_B$ during cycling time-trial exercise following IMT. Although it was not possible to substantiate this observation by making comparisons with the PLC group, this finding adds credence to previous studies showing similar post-IMT reductions in $[lac^-]_B$ during incremental rowing exercise (Volianitis et al. 2001) and high-intensity, intermittent shuttle running (Romer et al. 2002c). Moreover, it is likely that the experienced cyclists in this study performed exercise at an intensity close to maximal lactate steady-state (Harnish et al. 2001), an assertion supported by the temporal changes in $[lac^-]_B$ during exercise (Figure 4.4). Therefore, the findings of this study also agree closely with those of McConnell and Sharpe (2005), who observed an approximate 1.0 $mmol \cdot l^{-1}$ reduction in the maximal lactate steady-state $[lac^-]_B$ following pressure-threshold IMT. It is thus likely that the lower $[lac^-]_B$ during time-trial exercise following IMT in the present study was a genuine consequence

of the training intervention, and evidence of a significant role of the inspiratory muscles in lactate turnover.

However, given that only systemic $[lac^-]_B$ was measured in the present study it is difficult to identify whether the reduced $[lac^-]_B$ following IMT resulted from a greater rate of lactate clearance, a reduced rate of lactate appearance, or a combination of these processes. Furthermore, we can only speculate upon whether such changes resulted from altered lactate kinetics in the inspiratory muscles, locomotor muscles, or both. Enzymatic adaptations and mitochondrial proliferation within the inspiratory muscles are not likely to result from IMT (i.e. a strength training regimen) (Tesch 1988); however, it is speculated that inspiratory muscle MCT's may increase following IMT and that this may contribute to increased lactate clearance by inspiratory muscles (McConnell and Sharpe 2005). A change in lactate kinetics in locomotor muscles could occur if IMT improved breathing efficiency and subsequently "freed" blood flow to locomotor muscles, thereby increasing the extra-to-intramuscular lactate concentration gradient and thus lactate uptake. However, mechanical unloading of the inspiratory muscles using proportional assist ventilation during maximal exercise caused no change in the arterial and femoral-venous $[lac^-]_B$, despite an increase in leg blood flow from 18.4 to 19.2 $l \cdot min^{-1}$ (Harms et al. 1997). It seems inconceivable that IMT increases limb perfusion more than that observed with mechanical unloading, thus whether increased lactate clearance by, or decreased lactate release from, locomotor muscles is responsible for an IMT-mediated reduction in $[lac^-]_B$ remains unclear. Note, however, that although an IMT-mediated reduction in $[lac^-]_B$ is intriguing and the mechanisms responsible warrant further research, a lower $[lac^-]_B$ during exercise following RMT does not always accompany an increase in exercise tolerance (Kohl et al. 1997; McMahon et al. 2002; Sonetti et al. 2001), thus a "cause-effect" relationship seems unlikely. However, since a close relationship has been shown between improved exercise

tolerance and reduced $[lac^-]_B$ following RMT (Romer et al. 2002c; Spengler et al. 1999), changes in performance might be related to other factors that change simultaneously with lactate turnover.

Exercise-induced decrements in MIP are often taken as evidence of global inspiratory muscle fatigue (Romer et al. 2002a; Volianitis et al. 2001). Note that the potential implications of such fatigue were addressed in detail in section 1.4 and are not elaborated upon here. The post-exercise reductions in MIP observed in the present study thus suggest that the force generating capacity of the inspiratory muscles was impaired following time-trial exercise. Surprisingly, however, the extent to which MIP declined following time-trial exercise was not influenced by IMT in this study. This finding contrasts the observations of Romer et al. (2002a) who demonstrated, following IMT in trained cyclists, a reduction in exercise-induced inspiratory muscle fatigue (assessed by MIP) following 20 and 40 km cycling time-trials. Note, however, that the pre-IMT fall in MIP recorded by Romer et al. (2002a) following 20 km time-trial exercise was 17-18% and values remained 10% below baseline following 30 min recovery. The severity of inspiratory muscle fatigue thus appeared much less in the present study, which might be explained by the high baseline MIP (McConnell et al. 1997): 159 ± 5 and 168 ± 8 cmH_2O in IMT and PLC groups, respectively, vs. 99-105 cmH_2O in Romer et al.'s (2002a) study. However, it remains unclear why exercise-induced decrements in MIP were not reduced following IMT in the present study, although this observation calls into question the significance of an IMT-mediated reduction in the extent with which MIP declines following exercise. One possibility is that the relatively modest improvement in MIP (compared to previous studies) was insufficient to result in a substantial reduction in inspiratory muscle fatigue. In support, it is interesting to note that the two IMT subjects demonstrating the greatest increase in MIP (28.5 and 21.0%, respectively) also

demonstrated the greatest decline in the severity of inspiratory muscle fatigue: the relative decline in MIP 3 min post-exercise was reduced from -19.5 and -18.8% to -8.4 and -5.0%, respectively. MIP manoeuvres are also highly dependent on subject motivation and aptitude, thus slight variability in MIP may have concealed any minor reduction in inspiratory muscle fatigue. It is also worthy of note that, in addition to a decline in force production, muscle fatigue can be manifest in other ways (Ker and Schultz 1996; Perret et al. 1999; Romer et al. 2002a), thus we cannot discount the possibility that exercise-induced inspiratory muscle fatigue was indeed alleviated in the present study following IMT. Indeed, some indirect support for this assertion is provided by the IMT-mediated reduction in dyspnoea (see below).

The reduction in dyspnoea during time-trial exercise following IMT in the present study corroborates the observations of previous IMT studies in which a decline in the perception of respiratory effort was recorded during incremental cycling (Romer et al. 2002b) and rowing (Volianitis et al. 2001) exercise, and also during intermittent shuttle running (Romer et al. 2002c). There thus appears to be a clear link between inspiratory muscle strength and the intensity of dyspnoea. Evidence also suggests that dyspnoea may contribute to exercise limitation. For example, when the inspiratory muscles were mechanically unloaded (using proportional assist ventilation) during high-intensity (90% $\dot{V}O_2$peak) cycling exercise, an approximate 15% increase in endurance capacity was significantly correlated with a reduction in dyspnoea (Harms et al. 2000). It is thus possible that the reduction in dyspnoea during exercise following IMT in the present study made a significant contribution to improving cycling time-trial performance. However, the precise mechanisms by which IMT attenuates dyspnoea remain obscure. According to El-Manshawi et al. (1986) dyspnoea is mediated by a conscious awareness of the outgoing motor command via corollary discharge from respiratory neurones to the sensory cortex.

Reduced dyspnoea during exercise following IMT might thus reflect a reduction in the proportion of maximum force utilised for a given inspiratory load (Kellerman et al. 2000). Alternatively, that IMT alleviates dyspnoea could also be taken as indirect evidence of reduced inspiratory muscle fatigue, since a greater motor command to fatigued inspiratory muscles would be required to achieve the same mechanical output (McConnell and Romer 2004b). This mechanism is supported by the observation that dyspnoea is exacerbated during exercise preceded by voluntary fatigue of the inspiratory muscles (Mador and Acevedo 1991). An IMT-mediated decline in dyspnoea may also occur through a reduction in the extent of functional weakening (McConnell and Romer 2004b). More specifically, during heavy endurance exercise an increase in end-expiratory lung volume due to expiratory flow limitation (Johnson et al. 1992) would shorten and thus weaken the inspiratory muscles, subsequently increasing dyspnoea (Killian et al. 1984). Although end-expiratory lung volume was not determined in this study, $\dot{V}_E$ was comparable to that previously observed in endurance-trained athletes demonstrating significant expiratory flow limitation (Johnson et al. 1992). It is thus possible that due to expiratory flow limitation subjects in the present study were somewhat "hyperinflated" during exercise. In this context, the potential benefit of IMT is that an increased rate of inspiratory pressure development (Romer and McConnell 2003) may shorten inspiratory duration and thus lengthen expiratory duration. This may preserve end-expiratory lung volume and thus the pressure-generating capacity of the inspiratory muscles, thereby attenuating respiratory effort (Killian et al. 1984).

Further to the IMT-mediated reduction in dyspnoea during time-trial exercise in the present study, there was also a decline in limb RPE, although statistical significance was only evident during the early stages of the test. Nevertheless, a decline in the overall perceptual response to exercise (limb and respiratory) following IMT in the present study

may have allowed a higher external power output to be maintained. A reduction in limb discomfort following IMT has also been reported during high-intensity intermittent shuttle running (Romer et al. 2002c), incremental cycling (Romer et al. 2002b), and constant power cycling at 75% $\dot{V}O_2$ peak (Gething et al. 2004b). That IMT attenuates limb RPE is difficult to explain, but may result from favourable changes in acid-base balance commensurate with a reduced $[lac^-]_B$ (Romer et al. 2002c). Indeed, rather than impair muscle function, acidosis may contribute to exercise intolerance by activating group III-IV nerve afferents in muscle, thus increasing muscular discomfort (Westerblad et al. 2002). However, in a previous study mechanical loading of the inspiratory muscles during heavy (90-100% $\dot{V}O_2$ peak) endurance exercise caused an increase in limb RPE in the absence of any change in $[lac^-]_B$ or pH of arterial and femoral-venous blood (Harms et al. 1997). More studies thus appear warranted to establish whether IMT does indeed modify acid-base balance during exercise.

In conclusion, this study supports recent evidence demonstrating improvements in cycling time-trial performance in competitive cyclists following IMT. In addition, the present study provides new evidence showing improvements in time-trial performance to be accompanied by an IMT-mediated reduction in both the $[lac^-]_B$ and perceptual response to exercise.

CHAPTER 5

EFFECTS OF INSPIRATORY MUSCLE TRAINING UPON THE POWER-T_{LIM} RELATIONSHIP IN CYCLISTS

5.1 INTRODUCTION

Although the ability of the respiratory muscles to adapt to specific strength and endurance training regimens is well-established (Boutellier 1998; Leith and Bradley 1976; McConnell and Romer 2004a), the effects of specific respiratory muscle training (RMT) on whole-body endurance exercise performance remain somewhat controversial (McConnell and Romer 2004a). However, the uncertainty surrounding the efficacy of RMT is perhaps explained by the disparate nature with which whole-body endurance exercise capacity has been previously assessed. More specifically, evidence is accumulating to suggest that the ergogenicity of RMT scales inversely with the intensity of the endurance exercise performance evaluation test (Boutellier 1998; McConnell and Romer 2004a). For example, whereas RMT via voluntary isocapnic hyperpnoea had no effect on high-intensity (95% $\dot{V}O_2$ peak and 90% $\dot{W}$ max) cycling endurance in trained cyclists (Fairbarn et al. 1991; Morgan et al. 1987), it did improve moderate intensity (70-85% $\dot{W}$ max) cycling endurance by 24% in sedentary subjects (Markov et al. 2001) and by approximately 30% in endurance trained athletes (McMahon et al. 2002). Similarly, whereas Sonetti et al. (2001) observed a 1.8% improvement in 8 km cycling time-trial performance following RMT in trained cyclists, Romer et al (2002b) observed greater improvements of 3.5 and 3.4% for 20 and 40 km time-trials, respectively.

Empirical evidence thus suggests that improvements in endurance exercise performance following RMT are primarily observed when the performance evaluation test is submaximal and prolonged, with performance gains becoming progressively less discernible at higher exercise intensities. We questioned whether this common attribute that attests the efficacy of RMT relates to an adjustment in the hyperbolic relationship between power and time to exercise intolerance (T_{lim}). First described by Monod and Scherrer (1965) for local muscular work, the power-T_{lim} relationship derives two

parameters, critical power (CP) and anaerobic work capacity (AWC) (Hill 1993), and is mathematically described as:

$$T_{lim} = AWC/(power - CP)$$

Theoretically, CP, which is defined by the asymptote of the power-T_{lim} curve, represents an inherent component of the aerobic energy supply system (Monod and Scherrer 1965) that characterises the highest exercise intensity at which a steady state can be maintained in $\dot{V}O_2$, $[lac^-]_B$, and blood acid-base balance (Poole et al. 1988). According to Jones and Carter (2000) the physiological underpinnings of CP thus make it, in theory, synonymous with maximal lactate steady-state, although in practice, probably due to methodological differences (Hill 1993), the former may overestimate the latter (Pringle and Jones 2002). Studies have shown a close relationship between CP and other indicators of aerobic endurance, such as cycling time-trial performance, $\dot{V}O_2$peak, and anaerobic threshold (Moritani et al. 1981; Smith et al. 1999). Conversely, AWC, which is defined by the curvature constant of the power-T_{lim} curve, is thought to represent a constant, but finite, energy store that can be utilised when exercise intensity exceeds CP (Monod and Scherrer 1965). That AWC is increased following oral creatine supplementation (Smith et al. 1998), decreased following glycogen depletion (Miura et al. 2000), and unaltered by hypoxic conditions (Monod and Scherrer 1965; Moritani et al. 1981), confirms the anaerobic nature of this parameter.

Although the mechanisms by which RMT enhances whole-body endurance exercise tolerance may involve a complex interplay between respiratory muscles, active locomotor muscles, and the brain (Wetter and Dempsey 2000), the hyperbolic nature of the power-T_{lim} relationship would predict, following even small increases in CP, substantial improvements in endurance exercise tolerance at power outputs proximal to, but above, CP, with progressively smaller improvements being observed at higher work-rates. It is

thus attractive to speculate that the inverse relationship between the ergogenicity of RMT and the intensity of the exercise performance evaluation test results from an RMT-mediated alteration in the power-T_{lim} relationship. Moreover, an RMT-mediated increase in CP could explain improvements in prolonged cycling time-trial performance (chapter 4; Romer et al. 2002b) since experienced cyclists intuitively perform prolonged (30-60 min) time-trials at an intensity close to CP (Harnish et al. 2001).

Therefore, the primary aim of the present study was to examine the effects of a 6 week pressure-threshold inspiratory muscle training (IMT) regimen on the power-T_{lim} relationship in trained cyclists. Note that, with the exception of one subject from each treatment group, all participants in this study also participated in the preceding study of chapter 4.

5.2 Methods

5.2.1 Participants

Following local ethics committee approval and written informed consent, eleven non-smoking, competitive male cyclists were recruited for the study. Throughout the study, subjects were instructed to adhere to their usual training regimen and not to engage in strenuous activity the day before, or the day of, an exercise test. On test days, subjects were asked to refrain from consuming caffeine, and were instructed not to eat or ingest any substance of nutritional value during the 2 h prior to testing. For each subject, tests were scheduled at a similar time of day so to minimise diurnal fluctuation effects.

5.2.2 Experimental design

Subjects were randomly assigned to either a pressure threshold IMT group (n = 6) or a sham hypoxic training placebo (PLC) group (n = 5). The age, height, and body mass

of the IMT and PLC groups were 31 ± 3 and 29 ± 3 years, 180.1 ± 1.5 and 177.9 ± 1.8 cm, and 74.6 ± 3.3 and 77.9 ± 3.5 kg, respectively. Initially, baseline pulmonary and inspiratory muscle function was assessed. Prior to the intervention subjects completed, on separate days, 3 constant power, T_{lim} cycling tests on an electromagnetically braked cycle ergometer (see section 2.1) to establish the power-T_{lim} relationship. Both groups then completed the prescribed training regimen for 6 weeks. An assessment of MIP was made every 2 weeks during the intervention. The battery of exercise tests was repeated in random order upon completion of the training period, starting at least 48 h after the final training session. All tests were separated by at least 48 h.

5.2.3 MEASUREMENTS

Pulmonary function and MIP was assessed according to the procedures outlined in section 2.5. Arterialised venous blood was sampled from a dorsal hand vein via an indwelling cannula for the determination of $[lac^-]_B$, as described in section 2.6. Inspiratory and expiratory flow rates were measured during exercise using a turbine flowmeter (see section 2.2 for more details). Limb and respiratory RPE was assessed during exercise according to the procedures outlined in section 2.9.

5.2.4 POWER-T_{LIM} RELATIONSHIP

The power-T_{lim} relationship was determined using 3 separate, constant power cycling tests performed under verbal encouragement to the limit of exercise tolerance. To gain further insight into whether the ergogenicity of IMT is dependent on the exercise intensity of the performance evaluation test, power outputs for the 3 constant power tests were chosen to elicit exercise intolerance within each of the following time domains: 3-10, 10-20, and 20-30 min. Hereafter, these trials are referred to as Ex1, Ex2, and Ex3,

respectively. No warm-up was performed prior to exercise. Hill et al. (1995) have shown that using varied pedal cadence during predictive trials maximises T_{lim} and produces better fits of data to the power-T_{lim} relationship. Therefore, in accord with Hill et al.'s (1995) recommendations subjects adopted a spontaneous cycling cadence during exercise, but keeping within 60-120 rpm. Note, however, that 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. A test was terminated when cycling cadence could not be maintained above 60 rpm. A 3 min cool-down at 60 W was performed following exercise. Blood samples for $[lac^-]_B$ determination were drawn every 3 min during Ex2 and Ex3 only. Limb and respiratory RPE was assessed every minute during Ex1 and every 2 min during Ex2 and Ex3. Heart rate (HR) (Accurex Plus, Polar, Kempele, Finland) was recorded every 30 s during Ex1 and every minute during Ex2 and Ex3. An assessment of MIP was made prior to exercise, following the 3 min cool-down, and 15 min thereafter.

To allow a suitable power output to be prescribed for the initial constant power exercise test, estimates of CP and AWC were made for each subject. The mean power output during the 25 km cycling time-trial performed in chapter 4 was used to estimate CP (Harnish et al. 2001), and 18 kJ was taken as an estimate of AWC, which represents that previously reported for trained cyclists (Jenkins and Quigley 1990). Following the first constant power trial, power outputs for subsequent trials were calculated based on the regression equation described by the nonlinear power-T_{lim} model.

5.2.5 Training protocols

IMT subjects performed pressure-threshold IMT. A description of the IMT device and the training protocol employed is provided in section 2.8. The PLC group used a sham

hypoxic-trainer, as described in chapter 4, section 4.2.5. A training diary was provided to each subject, within which they recorded training adherence, and also the mode, duration (min), and intensity (HR) of all whole-body exercise training sessions.

5.2.6 Statistical modelling and analyses

Statistical analyses were performed using the 11.0 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA). Parameter estimates were derived using linear and non-linear regression techniques after the data was fit to 3 mathematically equivalent models of the power-T_{lim} relationship:

(I) the non-linear power-T_{lim} model, which was mathematically described in the Introduction,

(II) the linear work-T_{lim} model where:

$$work = AWC + (CP \cdot T_{lim})$$

(III) the linear power-$1/T_{lim}$ model where:

$$power = CP + (AWC \cdot 1/T_{lim})$$

All 3 models were used as Hill (1993) suggests that the existence of differences between parameter estimates derived using the 3 models can be taken as evidence that there is systematic error in the predicting data or that the data points are outside of the range for which the power-T_{lim} relationship is hyperbolic. In other words, similarity of values between models suggests that valid parameter estimates were derived. Note, however, that for the purpose of this study criterion measures of parameter estimates were taken from the non-linear power-T_{lim} model as this appropriately designates T_{lim} as a function of power (Gaesser et al. 1995).

To account for the variability in T_{lim} between subjects and between trials, an isotime comparison approach, similar to that used by Mador and Acevedo (1991), was

used to describe changes in measurements during each predictive trial. For each trial pre- and post-intervention ventilatory parameters were averaged and compared over the first minute, over the last equivalent minute that comprised data from both trials, and over the middle minute between these limits. Comparisons for HR during Ex1 were made at 1 min, at the final equivalent minute completed in both trials, and at the middle minute between these limits. Comparisons for HR during Ex2 and Ex3 differed slightly in that the first comparison was made after 2 min. Comparisons for RPE and $[lac^-]_B$ were made between the first measurement taken, the final measurement taken at an equivalent time-point during both trials, and the measurement taken midway between these limits. If the mid-test measurement did not correspond to an actual time of measurement, a linear relationship between the two data points either side was assumed and the required value was estimated through interpolation between these points. Hereafter, the first, second, and final isotime shall be referred to as isotime 1, isotime 2, and isotime 3, respectively.

Groups were compared for baseline measurements using t-tests for unpaired observations. Pre- and post-intervention results and group interactions were compared using one-way or two-way ANOVA for repeated measures. For ANOVA analyses homogeneity of variance and homogeneity of covariance was determined using Mauchly's test of sphericity, and any violations were corrected according to the procedures outlined by Vincent (1999). A significant F ratio was followed with Tukey's HSD post-hoc analysis. Statistical significance was set at $P < 0.05$. Values are presented as mean ± SEM unless otherwise indicated.

5.3 RESULTS

5.3.1 TRAINING COMPLIANCE AND HABITUAL EXERCISE TRAINING

No marked deviations were observed from each participant's habitual training regimen during the intervention. Both groups also demonstrated excellent training

compliance, with the IMT group completing 81 ± 2 of the 84 training sessions (96% adherence), and the PLC group completing 29 ± 1 of the 30 training sessions (97% adherence).

5.3.2 PULMONARY AND INSPIRATORY MUSCLE FUNCTION

IMT and PLC groups did not differ for any baseline measure of pulmonary or inspiratory muscle function. Pulmonary function was also unchanged following both interventions (Table 5.1).

Table 5.1 Pulmonary function for IMT and PLC groups (mean ± SEM).

	Pre-IMT[a]	Post-IMT[a]	Pre-PLC[b]	Post-PLC[b]
VC (l)	6.04 ± 0.28	6.08 ± 0.36	5.98 ± 0.47	6.17 ± 0.46
	(119 ± 4)	(119 ± 6)	(121 ± 9)	(125 ± 8)
FVC (l)	5.83 ± 0.24	5.85 ± 0.32	5.53 ± 0.42	5.78 ± 0.44
	(114 ± 4)	(115 ± 5)	(114 ± 8)	(120 ± 9)
FEV_1 (l)	4.87 ± 0.33	4.79 ± 0.41	4.66 ± 0.30	4.61 ± 0.3
	(117 ± 7)	(115 ± 9)	(112 ± 6)	(111 ± 6)
FEV_1/FVC (%)	83.7 ± 3.5	81.3 ± 4.3	84.4 ± 2.66	80.0 ± 2.2
	(105 ± 4)	(102 ± 5)	(101 ± 3)	(96 ± 3)
PEF ($l \cdot s^{-1}$)	10.8 ± 1.0	10.2 ± 1.5	10.4 ± 0.6	9.2 ± 1.0
	(103 ± 9)	(97 ± 14)	(95 ± 5)	(84 ± 9.4)
MVV ($l \cdot min^{-1}$)	207.8 ± 19.8	204.8 ± 17.7	162.8 ± 9.6	157.0 ± 14.5
	(132 ± 12)	(133 ± 11)	(105 ± 8)	(102 ± 13)

[a] $n = 6$, [b] $n = 5$. VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; PEF, peak expiratory flow; MVV_{12}, 12 s maximum voluntary ventilation. Numbers in parentheses represent % of the predicted value (Cotes 1993).

Baseline MIP values in IMT and PLC groups were 157 ± 5.4 and 168 ± 8.8 cmH_2O, respectively, which represented 142.7 ± 2.3 and 150.0 ± 1.7% of predicted values (Wilson et al. 1984), respectively. No changes in MIP were observed for the PLC group

throughout the intervention (Figure 5.1). Conversely, MIP increased progressively during IMT and a significant ($P < 0.05$) 8.1 ± 1.7% increase from baseline was observed following 4 weeks. Following 6 weeks of IMT the increase in MIP from baseline was 12.6 ± 3.9% ($P < 0.01$), which exceeded that in the PLC group ($P < 0.05$).

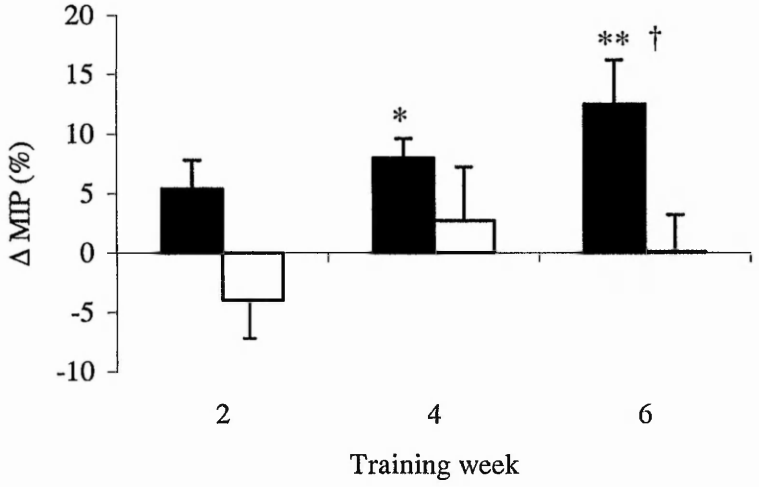

Figure 5.1 Relative changes in MIP during IMT (filled bars) and PLC (open bars) (mean ± SEM). *Significant difference from baseline ($P < 0.05$). **Significant difference from baseline ($P < 0.01$). †Significant interaction effect ($P < 0.05$).

5.3.3 POWER-T_{LIM} RELATIONSHIP

Cycling power outputs for Ex1, Ex2, and Ex3 trials in the IMT group were 341 ± 30, 297 ± 26, and 283 ± 26 W, respectively. Corresponding powers for the PLC group were 309 ± 9, 283 ± 9, and 265 ± 9 W, respectively (no significant differences between groups). For one IMT subject, the power-T_{lim} relationship was established from only 2 predictive trials (Ex1 and Ex3) because for a third trial (post-IMT) exercise intolerance was not imminent following 45 min of cycling, and diet, dehydration, temperature regulation, and motivation effects are recognised factors that influence endurance performance of this duration. Accordingly, because parameter estimates derived from different mathematical models are identical when only 2 data points are used, any bias in the results was avoided by not including this individual's data when making statistical

comparisons of parameter estimates using the 3 models. Similarly, this approach was also taken where the associated standard error of the estimate (SEE) and R^2 values are reported.

Group mean values for CP and AWC and the associated SEE and R^2 are presented in Tables 4.2 and 4.3 for IMT and PLC groups, respectively. The power-T_{lim} relationship was well described by all 3 mathematical models, and the parameter estimates derived from the models were not significantly different from one another pre- or post-intervention. Parameter estimates derived from the non-linear power-T_{lim} model were unchanged following both IMT and PLC. The effects of IMT on the power-T_{lim} relationship are also shown graphically in Figure 5.2 for a representative subject.

Table 5.2 Parameter estimates derived using 3 mathematical models pre- and post-IMT (mean ± SEM).

	Pre-IMT			Post-IMT		
	power-T_{lim}	work-T_{lim}	power-1/T_{lim}	power-T_{lim}	work-T_{lim}	power-1/T_{lim}
CP (W)	268 ± 28	271 ± 30	271 ± 30	267 ± 29	268 ± 29	269 ± 29
SEE (W)	6 ± 4	4 ± 1	3 ± 1	3 ± 1	2 ± 1	2 ± 1
AWC (kJ)	28.4 ± 2.4	25.8 ± 2.7	25.2 ± 2.8	32.3 4.3	30.5 ± 4.4	29.8 ± 4.4
SEE (kJ)	6.5 ± 3.4	3.6 ± 1.0	1.6 ± 0.2	3.2 1.3	2.3 ± 0.8	1.1 ± 0.4
R^2	0.982 ± 0.014	1.000 ± 0.000	0.995 ± 0.002	0.995 ± 0.003	1.000 ± 0.000	0.997 ± 0.002

Table 5.3 Parameter estimates derived using 3 mathematical models pre- and post-PLC (mean ± SEM).

	Pre-PLC			Post-PLC		
	power-T_{lim}	work-T_{lim}	power-1/T_{lim}	power-T_{lim}	work-T_{lim}	power-1/T_{lim}
CP (W)	240 ± 11	242 ± 11	242 ± 13	237 ± 10	239 ± ± 10	240 ± 10
SEE (W)	7 ± 3	8 ± 3	9 ± 2	4 ± 1	4 ± 1	5 ± 2
AWC (kJ)	30.9 ± 3.3	30.2 ± 4.9	30.3 ± 6.3	35.3 ± 8.3	34.1 ± 7.8	33.1 ± 7.6
SEE (kJ)	7 ± 1.9	6.5 ± 1.4	5.4 ± 0.8	3.7 ± 0.9	3.6 ± 0.9	3.1 ± 0.7
R^2	0.960 ± 0.029	0.998 ± 0.001	0.941 ± 0.034	0.990 ± 0.006	1.000 ± 0.000	0.981 ± 0.012

Group mean T_{lim} results for Ex1, Ex2, and Ex3 are presented in Figure 5.3. Cycling endurance increased by 13.9 ± 7.9, 6.7 ± 6.5, and 7.0 ± 5.5% for Ex1, Ex2, and Ex3, respectively, following IMT, although these changes were not significant. Similar improvements of 5.9 ± 10.1, 10.5 ± 17.6, and 3.4 ± 14.9% were also observed for Ex1,

Ex2, and Ex3, respectively, following PLC, although again these changes were not significant.

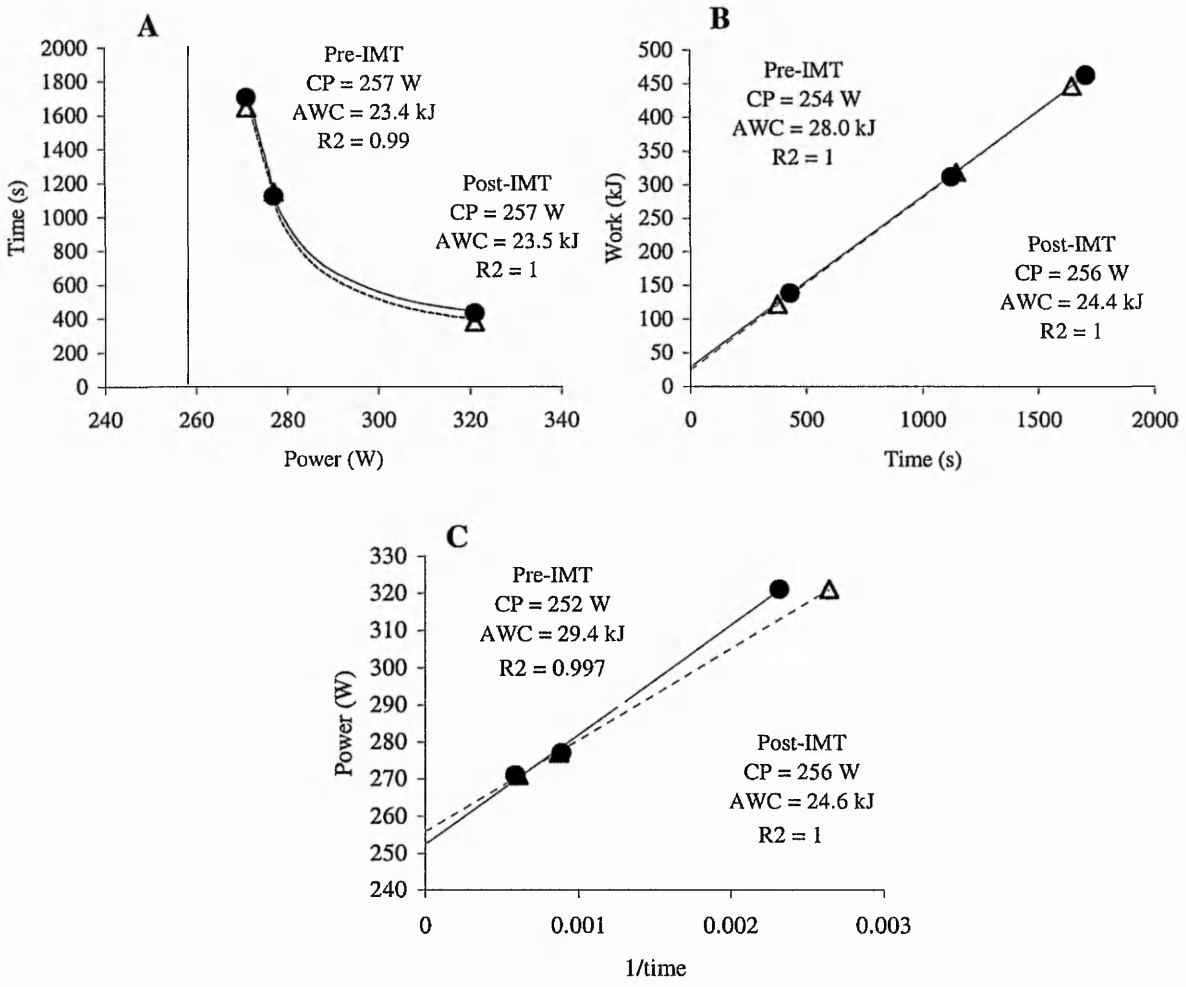

Figure 5.2 The power-T_{lim} relationship before (•) and after (Δ) IMT in a representative subject. Three mathematically equivalent models are shown: (A) non-linear power-T_{lim}; (B) linear work-T_{lim}; and (C) linear power-$1/T_{lim}$. Solid vertical line in non-linear power-T_{lim} model represents asymptote of power-T_{lim} curve.

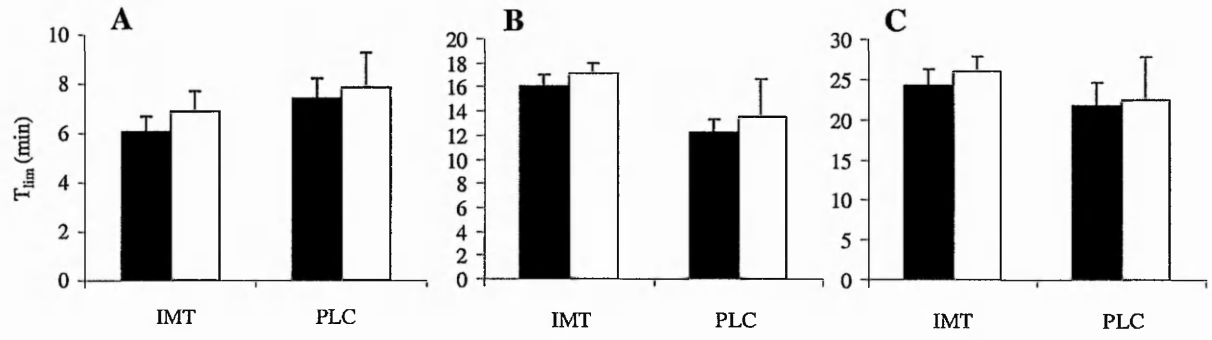

Figure 5.3 Group mean (± SEM) T_{lim} results for Ex1 (A), Ex2 (B), and Ex3 (C) in IMT and PLC groups. Closed bars, pre-intervention; open bars, post-intervention.

5.3.4 BLOOD LACTATE CONCENTRATION

Both groups demonstrated post-intervention reductions in $[lac^-]_B$ during Ex2 (Figure 5.4). Significant ($P < 0.05$) reductions were evident post-IMT at isotime 3 (7.4 ± 0.3 vs. 6.4 ± 0.3 mmol·l^{-1}, -13.5 ± 4.1%), and post-PLC at isotime 2 (6.4 ± 0.7 vs. 4.6 ± 0.5 mmol·l^{-1}, -26.6 ± 5.8%). There was a trend for mean $[lac^-]_B$ during Ex2 to be lower following both IMT (5.5 ± 0.3 vs. 4.7 ± 0.2 mmol·l^{-1}, -15.3 ± 4.8%, $P = 0.0504$) and PLC (5.7 ± 0.4 vs. 4.4 ± 0.4 mmol·l^{-1}, -19.6 ± 6.6%, $P = 0.052$). The $[lac^-]_B$ response to Ex3 was not different post-PLC. However, there was a trend for $[lac^-]_B$ to be reduced by approximately 1.0 mmol·l^{-1} at all isotimes post-IMT, although P values just failed to reach the 0.05 significance level at isotimes 1 ($P = 0.058$) and 2 ($P = 0.062$). The mean $[lac^-]_B$ during Ex3 also tended to be lower post-IMT (4.6 ± 0.6 vs. 3.7 ± 0.5 mmol·l^{-1}, -16.9 ± 7.9%, $P = 0.065$).

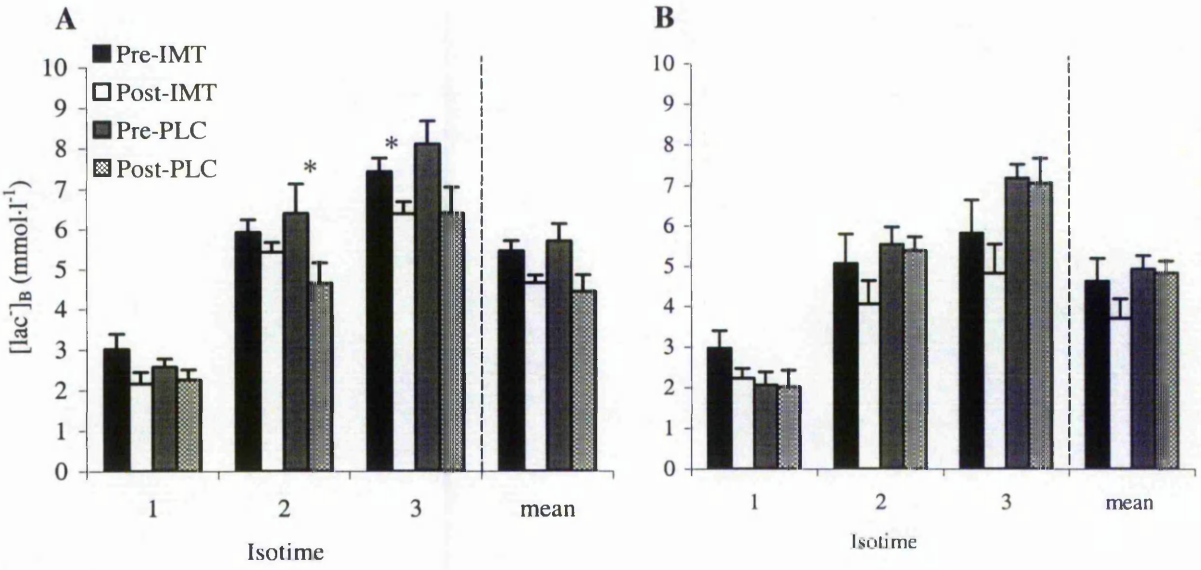

Figure 5.4 Blood lactate concentration ($[lac^-]_B$) during Ex2 (A) and Ex3 (B) in IMT and PLC groups. *Significant difference pre vs. post ($P < 0.05$).

5.3.5 Perceptual responses to exercise

The perceptual response to exercise was unchanged following PLC (Figure 5.5). Conversely, there were some alterations in both limb and respiratory RPE following IMT. A significant ($P < 0.05$) 7.1 ± 2.0% reduction in limb RPE was observed following IMT at isotime 2 during Ex1, which exceeded that in the PLC group ($P < 0.05$). Mean limb RPE (i.e. averaged across all isotimes) following IMT was also reduced by 5.7 ± 3.6% ($P < 0.05$). There were also significant ($P < 0.05$) 27.4 ± 7.1 and 17.3 ± 5.6% reductions in respiratory RPE during Ex1 at isotimes 2 and 3, respectively, and the mean respiratory RPE was also significantly ($P < 0.01$) reduced by 20.4 ± 3.9%, although the interaction effect failed to reach statistical significance ($P = 0.059$). During Ex2 there were also significant ($P < 0.05$) IMT-induced reductions in limb RPE of 10.5 ± 2.8% and 8.4 ± 1.9% at isotimes 1 and 2, respectively, although similar reductions were also observed following PLC. Mean limb RPE for Ex2 was significantly ($P < 0.01$) reduced by 7.6 ± 1.4% following IMT, although a similar non-significant 7.7 ± 4.8% reduction was also observed following PLC. No significant changes in respiratory RPE were observed during Ex2, and the perceptual response to Ex3 was also unchanged following the intervention.

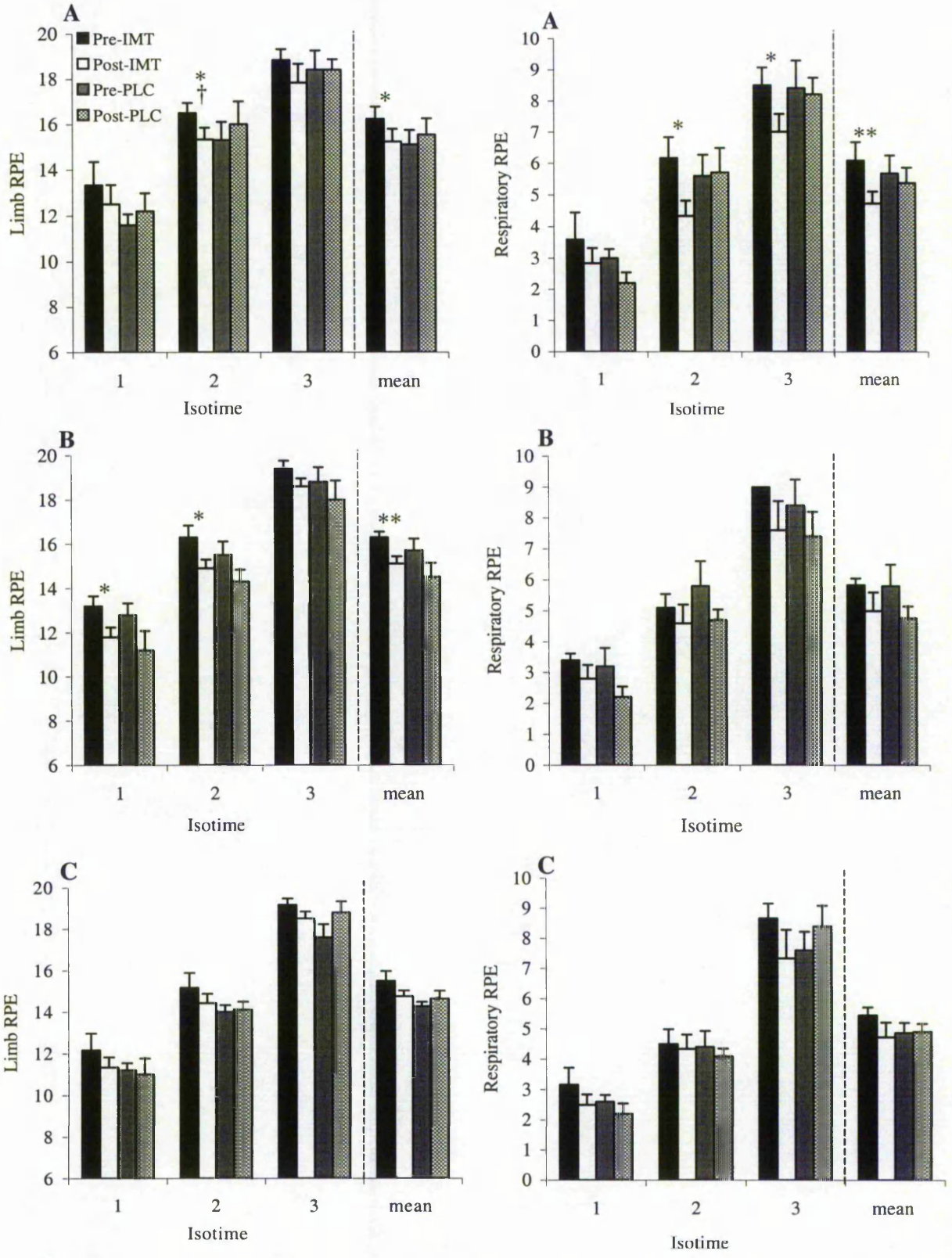

Figure 5.5 Limb (left panels) and respiratory (right panels) RPE during Ex1 (A) Ex2 (B) and Ex3 (C) in IMT and PLC groups. *Significant difference pre vs. post. ($P < 0.05$). **Significant difference pre vs. post. ($P < 0.01$). †Significant interaction effect ($P < 0.05$). Note that all IMT subjects reported a respiratory RPE score of 9 at isotime 3 during Ex2, thus accounting for the absence of an error bar.

5.3.6 VENTILATORY RESPONSES TO EXERCISE

Ventilatory responses to Ex1 and Ex2 were not different following either IMT or PLC (data not shown). However, significant changes in the ventilatory response to Ex3 were noted after IMT (Figure 5.6).

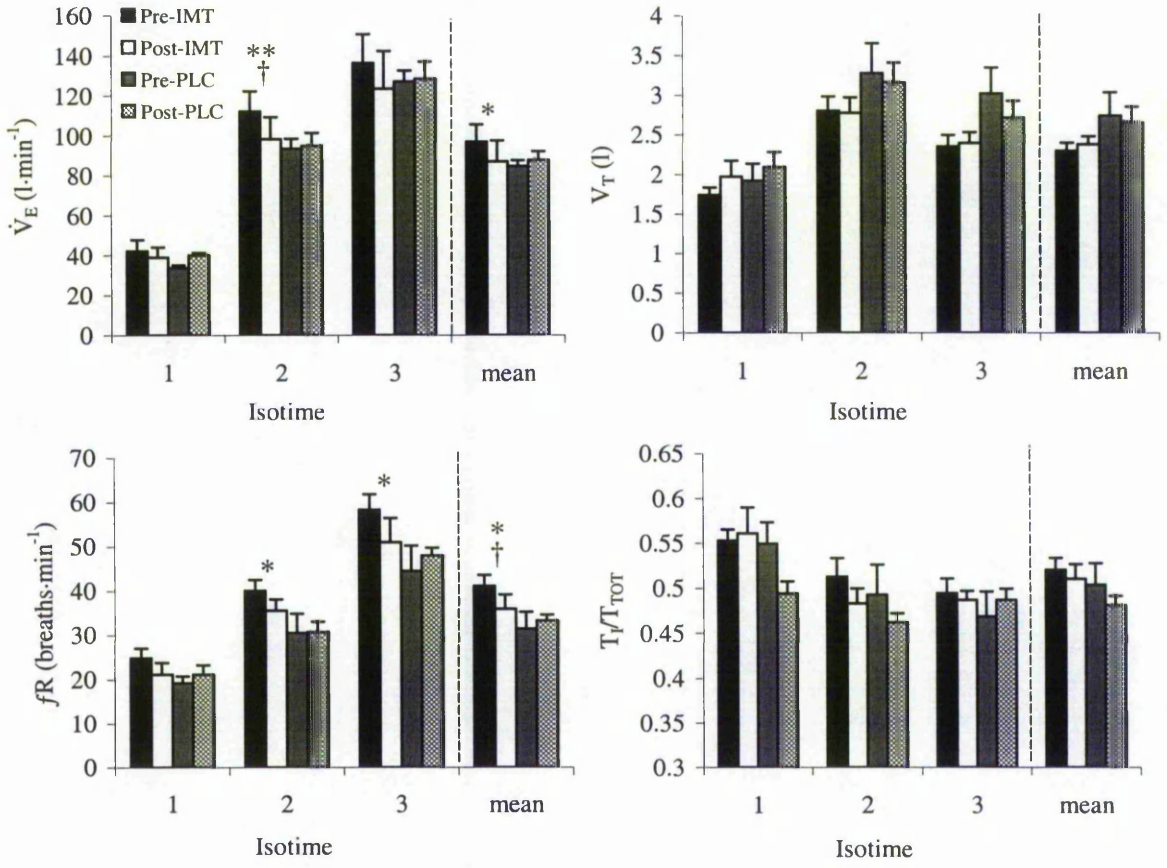

Figure 5.6 Ventilatory response to Ex3 in IMT and PLC groups. $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, respiratory frequency; T_I/T_{TOT}, inspiratory time/total breath time (duty cycle). *Significant difference pre vs. post ($P < 0.05$). **Significant different pre vs. post ($P < 0.01$). †Significant interaction effect ($P < 0.05$).

Specifically, there was a significant ($P < 0.01$) post-IMT reduction in $\dot{V}_E$ at isotime 2 (112.1 ± 10.0 vs. 98.3 ± 11.0 l·min^{-1}, -13 ± 2.8%), and the interaction effect was also significant ($P < 0.05$). Additionally, mean exercise $\dot{V}_E$ was also reduced ($P < 0.05$) post-IMT (96.9 ± 8.6 vs. 86.8 ± 10.7 l·min^{-1}, -10.7 ± 4.0%), although the interaction effect

marginally exceeding the critical significance level (P = 0.052). The attenuated $\dot{V}_E$ observed in the IMT group was primarily explained by a significant ($P < 0.05$) reduction in f_R at isotime 2 (40.1 ± 2.4 vs. 35.6 ± 2.5 breaths·min^{-1}, -11.0 ± 3.8%) and 3 (58.3 ± 3.5 vs. 50.9 ± 5.4 breaths·min^{-1}, -13.8 ± 4.8%). Mean f_R was also significantly ($P < 0.05$) attenuated post-IMT (41.1 ± 2.5 vs. 35.9 ± 3.2 breaths·min^{-1}, -13.4 ± 3.7%), and the interaction effect was significant ($P < 0.05$). Duty cycle remained unchanged following IMT.

5.3.7 INSPIRATORY MUSCLE FATIGUE

Absolute group mean values for MIP pre- and post-exercise in IMT and PLC groups are presented in Tables 5.4 and 5.5, respectively. The magnitude of inspiratory muscle fatigue following exercise, albeit somewhat moderate even prior to the intervention, was not affected by either IMT or PLC for any predictive trial. Intriguingly, exercise at the highest intensity (Ex1) had no effect on MIP in either group pre- or post-intervention. Indeed, compared to Ex1, post-exercise reductions in MIP were often greater following Ex2 and Ex3.

Table 5.4 Changes in MIP (cmH_2O) 3 and 15 min following exercise pre- and post-IMT (mean ± SEM).

	Pre-IMT			Post-IMT		
	Pre-exercise	3 min	15 min	Pre-exercise	3 min	15 min
Ex1	149.3 ± 6.8	147.3 ± 9.8	151.2 ± 9.6	171.8 ± 8.5	166.8 ± 9.6	166.5 ± 9.7
		(–1.3 ± 2.9)	(1.2 ± 2.3)		(–2.9 ± 2.4)	(–3.1 ± 1.5)
Ex2[a]	146.8 ± 5.3	134.6 ± 5.2**	137.0 ± 6.2**†	171.6 ± 10.7	155.2 ± 8.0†	162.2 ± 7.9
		(–8.3 ± 1.8)	(–6.7 ± 1.9)		(–9.6 ± 3.8)	(–5.5 ± 2.4)
Ex3	149.5 ± 7.1	141.7 ± 7.5	138.7.0 ± 8.1*†	175.7 ± 9.6	159.8 ± 10.3*†	160.8 ± 9.4
		(–5.2 ± 2.1)	(–7.2 ± 1.8)		(–9.0 ± 3.0)	(–8.4 ± 3.5)

[a]n = 5. *Significant difference from pre-exercise ($P < 0.05$). **Significant difference from pre-exercise ($P < 0.01$). †Significantly greater reduction than that observed during Ex1 at same time ($P < 0.05$).

Table 5.5 Changes in MIP (cmH_2O) 3 and 15 min following exercise pre- and post-PLC (mean ± SEM).

	Pre-PLC			Post-PLC		
	Pre-exercise	3 min	15 min	Pre-exercise	3 min	15 min
Ex1	168.2 ± 10.6	164.2 ± 12.3	163.6 ± 10.6	170.6 ± 8.9	172.0 ± 7.1	174.8 ± 4.6
		(–2.4 ± 1.9)	(–2.7 ± 2.9)		(0.8 ± 1.6)	(2.5 ± 3.8)
Ex2	167.6 ± 13.8	164.4 ± 11.2	161.2 ± 12.2	169.6 ± 5.4	156.0 ± 6.1*††	156.8 ± 6.1††
		(–1.9 ± 3.2)	(–3.8 ± 4.0)		(–8.0 ± 3.4)	(–7.5 ± 3.2)
Ex3	167.6 ± 6.6	154.0 ± 10.0	165.2 ± 10.5	171.0 ± 8.6	157.6 ± 8.1*††	163.2 ± 8.4††
		(–8.1 ± 3.4)	(–1.4 ± 3.6)		(–7.8 ± 1.9)	(–4.6 ± 2.6)

*Significantly different from pre-exercise ($P < 0.05$). ††Significantly greater reduction than that observed during Ex1 at same time ($P < 0.01$).

5.4 DISCUSSION

According to Monod and Scherrer (1965) the power-T_{lim} relationship has 2 parameters: the CP and an energetic reserve that is now commonly described as the AWC (Hill 1993). The primary finding of this study was that 6 weeks of pressure-threshold IMT did not significantly affect either of these parameters. Therefore, a change in the power-T_{lim} relationship appears an unlikely mechanism by which IMT improves endurance exercise tolerance in competitive cyclists. Theoretically, CP and maximum lactate steady state represent the same physiological phenomenon (Jones and Carter 2000), thus the present findings concur closely with those of McConnell and Sharpe (2005) who observed no change in the maximum lactate steady state cycling power following an IMT protocol identical to that employed in the present study. Although the physiological underpinnings of AWC are less well understood, variation in this parameter will clearly affect T_{lim}. However, regardless of what factors determine AWC it appears that they are insensitive to pressure-threshold IMT.

The 12.6% increase in MIP following IMT in the present study demonstrates that the training intervention was effective and moreover, the degree of training compliance was excellent. The increase in MIP does however appear modest when compared to the 28-45% increases reported in other pressure-threshold IMT studies, several of which were also

performed on endurance trained subjects (Huang et al. 2003; Romer and McConnell 2003; Romer et al. 2002a; Suzuki et al. 1993; Volianitis et al. 2001). However, the present findings concur more closely with the 8% increase observed by Sonetti et al. (2001). It should be noted that Sonetti et al. (2001) utilised a concurrent inspiratory muscle strength and endurance training regimen, which may have inhibited MIP development (Leveritt et al. 1999). However, the results of the present study, in which only IMT was performed, suggest that the degree of IMT-mediated increase in MIP may be dependent upon other factors. Since the magnitude of training-induced adaptation in a physiological system is largely dependent upon the baseline status of the system (Kraemer 2000), one possibility is that IMT-mediated improvements in MIP are partly dependent upon baseline MIP values: those studies showing large IMT-mediated increases in MIP report baseline MIP values ranging from 98-112 cmH_2O (i.e. comparable to predicted values) (Huang et al. 2003; Romer and McConnell 2003; Romer et al. 2002a; Suzuki et al. 1993; Volianitis et al. 2001), whereas baseline MIP values reported here and in Sonetti et al.'s (2001) study were 157 ± 5 and 168.5 ± 39.8 cmH_2O, respectively (both approximately 143% of predicted; Wilson et al. 1984). Although an explanation for the high baseline MIP reported in this study is not readily forthcoming, inter-subject variation in MIP is probably not explained by differences in endurance training status, height, nor body mass (Coast et al. 1990; McConnell et al. 1997; McCool et al. 1997; Robinson and Kjeldgaard 1982). However, variation in the muscularity of the inspiratory pump, a variable unaccounted for in reference equations for normal MIP values, may explain some of the difference in MIP between subjects possessing similar anthropometric characteristics (McCool et al. 1997). Interestingly, studies have also shown a physiological plateau in strength and power development in response to IMT at 6 wk (Romer and McConnell 2003; Volianitis et al. 2001), thus implying an upper limit to which MIP may improve with IMT. These

observations are consistent with the paradigm that all physiological systems have an upper limit (i.e. 'genetic ceiling') for adaptation (Kraemer 2000).

Increases in inspiratory pressures following IMT may thus scale with baseline MIP values. It may also be possible that baseline MIP values have other ramifications; Williams et al. (2002) observed no change in running endurance at 85% $\dot{V}O_2$peak following an intensive 4 week IMT regimen in a group of endurance athletes that also demonstrated a relatively high baseline MIP (approximately 145 cmH_2O, or 140% of predicted). It is thus possible that the degree of IMT-induced increase in MIP and whole-body endurance exercise performance is partly dependent upon baseline MIP values: those subjects with a relatively high baseline MIP may experience smaller training effects. However, this assertion is difficult to resolve with the observation that all IMT subjects participating in the present study experienced a significant improvement in 25 km cycling time-trial performance post-IMT (see chapter 4). These results therefore raise the possibility that the negative findings of Williams et al. (2002), and previous authors (Fairbarn et al. 1991; Morgan et al. 1987), were due to the nature of the performance evaluation test used (T_{lim} test), and that positive outcomes may have been observed had performance been evaluated using a time-trial based performance measure.

It is unclear at present why IMT appears to improve time-trial performance to a greater extent than constant-load exercise endurance. However, it is likely that the factors that determine high-intensity constant-load or incremental exercise endurance (i.e. tests that ultimately end in the attainment of $\dot{V}O_2$peak) are not entirely consistent with those that determine exercise performance in tests performed at a self-selected intensity (i.e. time-trials) (Noakes 2000). Indeed, McConnell and Romer (2004a) contend that because time-trial exercise is performed at a self-selected intensity this may allow subjects to fully express the benefits of IMT, whereas because constant-load tests will terminate when the

energy supply systems become limiting, the efficacy of IMT under these conditions may be small and difficult to measure. Therefore, the physiological factors that limit endurance performance under different conditions may be a critical facet that partly determines the ergogenic influence of IMT. It is thus crucial that the nature of the performance evaluation test is duly considered when investigating the effects of IMT on exercise performance.

That IMT caused no change in the power-T_{lim} relationship is somewhat surprising given the body of literature suggesting a positive ergogenic effect of IMT (chapter 4; reviewed in McConnell and Romer 2004a). Moreover, it is generally assumed that any change in endurance performance should be manifest by a rightward shift in the power-T_{lim} curve (Jones and Carter 2000). However, our results suggest that time-trial performance may be improved without a major shift in the power-T_{lim} curve. Similar conclusions were drawn by Paavolainen et al. (1999), who demonstrated a significant improvement in 5 km running performance, but an unchanged lactate threshold, in elite cross-country runners following an "explosive-type" leg strength training regimen. Although direct comparisons between this study and the present study are complicated by the different training interventions, the findings suggest that, rather than an increase in maximal sustainable power, other factors are capable of improving time-trial performance following different types of strength training regimens. This premise supports the opinion of Noakes (1988), who argues that improved exercise tolerance following training interventions does not, contrary to the traditional construct, necessarily result solely from adaptations that enhance whole-body oxygen utilisation.

Following the recommendations of Hill (1993) parameter estimates were established using the 3 versions of the hyperbolic model, and it was found that the power-T_{lim} relationship was well described by all 3 models. The high R^2, low SEE, and similarity of parameter estimates using the 3 models, suggests that valid estimates were also derived

(Hill 1993). Note also that CP and AWC estimates are reliable: test-retest correlation coefficients of 0.90-0.96 and 0.79-0.87, respectively, have been reported (Hill 1993). Therefore, the present findings are likely to represent a valid and true representation of the effects of IMT on the power-T_{lim} relationship in competitive cyclists. It could, however, be argued that the present study was limited by the small sample size and that this potentially reduced statistical power and possibly contributed to a type II error. However, a power calculation reveals that the data were not subject to a type II error. The standard deviation of the differences for CP values pre- to post-IMT in the present study was 4.67. If 10 W is taken to represent a meaningful change in CP post-IMT, and assuming that power = 0.80 and $P = 0.05$, then $n = 4$. Moreover, with $n = 6$ and standard deviation of the differences = 4.67, the smallest IMT-mediated change in CP that could have been detected was 6.5 W. It is thus unlikely that the present study was underpowered by the sample size of the groups. Note also that the finding that CP does not change following IMT is entirely consistent with the observation that maximal lactate steady state cycling power is also insensitive to pressure-threshold IMT (McConnell and Sharpe 2005). However, McConnell and Sharpe (2005) caution that small (<6 W), but potentially worthwhile, improvements in maximal sustainable power may result from IMT, and that this may explain part of the increase in exercise tolerance. Note, however, that McConnell and Sharpe (2005) did not assess IMT's influence on endurance exercise performance; only changes in maximal lactate steady state were examined. Nevertheless, that small increases in CP may result in impressive improvements in exercise tolerance is supported by the data of Billat et al. (2004) who observed, following whole-body endurance training, a slight 3.4% increase in maximal lactate steady state running velocity, but a substantial 50% increase in running endurance time at the pre-training maximal lactate steady state work-rate. Clearly, the distinct training interventions (whole-body endurance training vs. IMT) preclude direct comparisons being

made between the present study and that of Billat et al. (2004). However, given these observations we should perhaps not discount the possibility that IMT causes very small, perhaps immeasurable, increases in CP. Note however that because no data exist concerning the interrelationship of magnitude of CP improvement and enhanced exercise performance, the functional relevance of small changes in CP is unknown.

Exercise-induced decreases in MIP are widely considered to be indicative of inspiratory muscle fatigue. The results of the present study suggest that the inspiratory muscles working in synergy are more susceptible to decreases in strength following prolonged exercise (10-30 min) compared to short-term (3-10 min) high-intensity exercise. In contrast, the severity of diaphragm fatigue (assessed using phrenic nerve stimulation techniques) increases with the exercise intensity (Johnson et al. 1993). However, note that MIP reflects, primarily, inspiratory rib cage muscle strength (McCool et al. 1992), thus these data are indicative of greater inspiratory rib cage muscle fatigue during prolonged endurance exercise compared to short-term high-intensity exercise. This pattern of fatigue is consistent with the pattern of respiratory muscle recruitment during exercise. Specifically, as heavy endurance exercise progresses the inspiratory rib cage muscles make a progressively greater contribution to the total respiratory muscle pressure output (Johnson et al. 1993), which might promote greater fatigue of the inspiratory rib cage muscles during prolonged exercise bouts. However, perhaps surprisingly, IMT did not influence the extent to which MIP decreased following Ex2 and Ex3 trials. These findings thus contrast previous studies in which pressure-threshold IMT attenuated the magnitude with which MIP declined following heavy endurance exercise (Romer et al. 2002a; Volianitis et al. 2001). Note, however, that in the present study relative decrements in MIP following exercise were always less than 10%, and significant reductions were only recorded following Ex2 (duration = 10-20 min) and Ex3 (duration = 20-30 min). In

comparison, MIP fell by 17% in trained cyclists (baseline MIP was 105 cmH_2O) following a 20 km time-trial lasting approximately 30 min (Romer et al. 2002a). Similarly, MIP fell 11.2% in competitive female rowers (baseline MIP was 104 cmH_2O) following a 6 min all-out rowing exercise (Volianitis et al. 2001). However, baseline MIP was much higher in the present study (149 and 167 cmH_2O recorded in IMT and PLC groups respectively) and previous findings suggest that the extent of exercise-induced inspiratory muscle fatigue is inversely related to baseline MIP (McConnell et al. 1997). Thus the high baseline MIP demonstrated by subjects in the present study may explain why MIP was either maintained following Ex1 or fell only modestly following Ex2 and Ex3. However, it remains unclear why exercise-induced decrements in MIP following Ex2 and Ex3 were not attenuated following IMT, although because in chapter 4 cycling time-trial performance was improved in the absence of any reduction of inspiratory muscle fatigue, this observation is unlikely to explain why cycling endurance was unaffected in the present study. Perhaps the somewhat modest improvement in MIP following IMT in the present study was insufficient to cause a substantial attenuation of inspiratory muscle fatigue. Alternatively, the variability that sometimes accompanies volitional MIP manoeuvres may have masked any slight reduction of inspiratory muscle fatigue following IMT.

Several studies have demonstrated an approximate 1 $mmol \cdot l^{-1}$ reduction in $[lac^-]_B$ during exercise following IMT (McConnell and Sharpe 2005; Romer et al. 2002c; Volianitis et al. 2001), and some speculate that a commensurate alteration in acid-base balance contributes to reducing the perceptual response to exercise (Romer et al. 2002c). Consistent with previous studies, $[lac^-]_B$ tended to be lower by approximately 1 $mmol \cdot l^{-1}$ following IMT in the present study, although because similar reductions were also observed during Ex2 following PLC, these observations remain inconclusive. Moreover, these reductions were not consistently accompanied by a reduced perceptual response to

exercise, or an improvement in exercise tolerance. Thus although an IMT-mediated reduction in $[lac^-]_B$ is intriguing, and the mechanisms responsible warrant further study, the functional relevance of this change remains unknown.

The present study confirms previous observations that IMT attenuates $\dot{V}_E$ during submaximal, constant power cycling exercise (Gething et al. 2004b; McConnell and Sharpe 2005). The reduced $\dot{V}_E$ during Ex3 resulted from a reduced f_R. Tachypnoea is often observed during exercise preceded by voluntary fatigue of the inspiratory muscles through inspiratory threshold loading (Mador and Acevedo 1991), a task that preferentially fatigues the inspiratory rib cage muscles (McCool et al. 1992). IMT may thus attenuate f_R through reducing inspiratory rib cage muscle fatigue, although this was not reflected in the MIP data. Changes in breathing pattern may also result from changes in the locomotor muscles. Studies have shown increased $\dot{V}_E$ and f_R during whole-body exercise performed with either fatigued or weakened (through curare administration) locomotor muscles (Asmussen et al. 1965; Spengler et al. 2000). The mechanism for this might be linked to an increased central command from the cerebral cortex to locomotor muscles, which results in greater collateral excitation of the respiratory centre in the brain stem (Asmussen et al. 1965; Spengler et al. 2000). Therefore, if IMT "freed" blood flow to locomotor muscles, then this may mediate a decline in $\dot{V}_E$ by alleviating locomotor muscle fatigue. Although this mechanism remains highly speculative, note that mechanical unloading of the inspiratory muscles during heavy cycling exercise results in an increase in leg blood flow (Harms et al. 1997) and an alleviation of quadriceps muscle fatigue (Romer et al. 2004). However, it is important to reiterate that although IMT reduced $\dot{V}_E$ during Ex3, the perceptual response to exercise and endurance exercise performance were not significantly changed, although as discussed previously, the nature of the performance evaluation test

(T_{lim} test vs. time-trial) may be critical to whether or not IMT-induced adaptations translate into an increase in exercise performance.

Thus far, attention has been primarily focused on whether IMT increases CP, and it has been highlighted that very small changes in CP would be very difficult to measure. Because AWC is very sensitive to even small errors in T_{lim} (Vandewalle et al. 1997) small changes in this parameter would be even more difficult to detect. However, given that AWC is thought to be equivalent to a finite energy store comprised of a phosphagen pool, an anaerobic glycolytic component, and oxygen bound to myoglobin (Miura et al. 2000; Monod and Scherrer 1965; Vandewalle et al. 1997), it seems unlikely that IMT would influence AWC. In support, Romer et al. (2002c) demonstrated no change in repetitive sprint performance, predominantly dependent on the phosphocreatine and glycolytic systems, following pressure-threshold IMT.

In conclusion, the present study provides novel evidence showing that improvements in endurance exercise tolerance following IMT do not result from an alteration in the power-T_{lim} relationship. In addition, the results of the present study together with those reported in chapter 4 suggest that the efficacy of IMT partly depends on the specifics of the performance evaluation test. Additional studies are warranted to clarify whether baseline inspiratory muscle strength does indeed influence the extent to which MIP increases during IMT.

CHAPTER 6

EFFECTS OF MAXIMAL VOLITIONAL HYPERPNOEA DURING EXERCISE AT MAXIMAL LACTATE STEADY STATE

6.1 INTRODUCTION

Values of $\dot{V}_E$ up to 250 $l \cdot min^{-1}$ have been recorded during maximal exercise (Secher 1993): this equates to a power output of around 50 W from the respiratory muscles (Milic-Emili 1991). Our understanding of the physiological consequences of such breathing efforts has increased over recent years. For example, around 15% of both the pulmonary $\dot{V}O_2$ and cardiac output ($\dot{Q}$) is consumed by the respiratory muscles during maximal exercise (Aaron et al. 1992; Harms et al. 1998b), and diaphragmatic fatigue is consistently observed following exhaustive exercise at intensities exceeding 85% $\dot{V}O_2$peak (Babcock et al. 1998; Babcock et al. 1995; Johnson et al. 1993). A respiratory muscle metaboreflex phenomenon has also been described (Dempsey et al. 2002; Harms et al. 1997; Rodman et al. 2003), which is considered to be responsible for the steal of locomotor muscle perfusion by the respiratory muscles during fatiguing ventilatory work.

Furthermore, studies of the responses of human beings to respiratory muscle training (RMT) have demonstrated a consistent reduction in $[lac^-]_B$ at identical exercise intensities post-RMT (reviewed in McConnell and Romer 2004a; McConnell and Sharpe 2005).

Despite these latter observations, it is at present unclear whether the work of breathing significantly affects $[lac^-]_B$ during heavy endurance exercise. Studies performed on exercising rats suggest that, rather than being major producers of lactate, the respiratory muscles (diaphragm and intercostals) may engage in lactate consumption during heavy exercise (Fregosi and Dempsey 1986). However, inter-species differences in respiratory muscle fiber types (Edwards and Faulkner 1985) and breathing mechanics during exercise (Bramble and Carrier 1993) make it difficult to extrapolate these observations to the exercising human being. In human beings, respiratory muscle work does not appear to significantly affect $[lac^-]_B$ during heavy endurance exercise. This viewpoint is based

primarily upon the results of studies showing either no change in $[lac^-]_B$, or only a modest 0.5 $mmol \cdot l^{-1}$ increase when levels of $\dot{V}_E$ observed during maximal exercise are reproduced voluntarily at rest (Babcock et al. 1995; Martin et al. 1984), or when maximal voluntary isocapnic hyperpnoea was superimposed on exercise at the anaerobic threshold (Engelen et al. 1995). Similarly, various loaded breathing protocols that stress the inspiratory and expiratory muscles cause little or no change in $[lac^-]_B$ when performed at rest (Eldridge 1966; Freedman et al. 1983).

However, these studies were performed at rest or at exercise intensities at which there was capacity for tissues, such as active and resting skeletal muscle, the kidney, and the liver (Brooks 1986), to clear lactate produced by other metabolically active tissues (i.e. the respiratory muscles). Thus, the possibility exists that respiratory muscle work may have resulted in additional lactate efflux, but that concurrent clearance by other tissues resulted in either minimal or no change in $[lac^-]_B$.

It was reasoned that increasing the work of breathing whilst simultaneously exercising at maximum lactate steady state (MLSS) would resolve the uncertainty relating to the influence of respiratory muscle work upon $[lac^-]_B$. It is known that MLSS represents the highest exercise intensity and $[lac^-]_B$ that can be maintained over time without a continual blood lactate accumulation (Billat et al. 2003). In other words, during constant-load exercise at or below MLSS there is an initial transient increase in $[lac^-]_B$, after which the rate of lactate appearance in the blood is matched, or surpassed, by the rate of lactate removal from the blood. However, beyond MLSS, greater rates of lactate appearance compared to lactate clearance result in a continual increase in $[lac^-]_B$. Thus, due to a limited capacity to match further lactate appearance with an equal rate of lactate clearance during exercise at MLSS, further lactate efflux caused by additional respiratory muscle work should be manifest by an increase in $[lac^-]_B$.

Accordingly, the purpose of the present study was to evaluate the influence of maximal voluntary hyperpnoea on $[lac^-]_B$ whilst cycling at MLSS.

6.2 METHODS

6.2.1 PARTICIPANTS

Following local ethics committee approval and written informed consent, 7 non-smoking, physically active male subjects were recruited. The age, height, and body mass of the subjects were 25.6 ± 1.8 years, 179.2 ± 2.4 cm, and 80.3 ± 2.9 kg, respectively. Subjects were instructed not to partake in strenuous exercise the day before an exercise test. On test days, subjects were asked to refrain from consuming caffeine, and were instructed not to eat or ingest any substance of nutritional value during the 2 h prior to testing. Tests for each subject were performed at a similar time of day so to minimise diurnal fluctuation effects.

6.2.2 EXPERIMENTAL DESIGN

Initially, pulmonary and respiratory muscle function tests were performed. On subsequent testing days separated by at least 48 h subjects sequentially performed a battery of exercise tests on an electromagnetically-braked cycle ergometer (see section 2.1). A "lactate minimum" test (Tegtbur et al. 1993) was initially performed to establish an estimate of MLSS power, which was then resolved using at least two 30 min constant power tests. Thereafter, subjects performed a 30 min control trial at MLSS. On a further occasion (experimental trial) the control trial was mimicked except that from 20-28 min maximal isocapnic volitional hyperpnoea was superimposed on exercise. Subjects immobilised their arms during exercise by resting their forearms on time-trial handlebars. A 24 h diet record was completed prior to the control trial and this was then replicated in

the 24 h prior to the experimental trial.

6.2.3 MEASUREMENTS

Pulmonary function and maximal inspiratory and expiratory mouth pressures (MIP and MEP, respectively) were assessed according to the procedures outlined in section 2.5. In accord with the procedures outlined in section 2.6 arterialised venous blood was drawn from a superficial dorsal hand vein via an indwelling cannula for the determination of $[lac^-]_B$ and blood-gas variables. Actual plasma bicarbonate concentration ($[HCO_3^-]$) was calculated from $PaCO_2$ and pH values using the Henderson-Hasselbalch equation:

$$pH = pK + \log\frac{[HCO_3^-]}{0.03 \times PaCO_2}$$

where pK is the negative log of the dissociation constant of carbonic acid and has the value 6.1. Actual $[HCO_3^-]$ was subsequently incorporated into the Siggaard-Anderson equation to calculate base excess of the extracellular fluid (BE_{ECF}), with the assumption that the haemoglobin concentration of the extracellular fluid = 5 $g \cdot dl^{-1}$ (Siggaard-Andersen and Fogh-Andersen 1995):

$$BE_{ECF} = 0.93 \times \left([HCO_3^-] - 24.4 + 14.83 \times (pH - 7.40)\right)$$

In addition, during the period of volitional hyperpnoea (times 22-28 min, inclusive) metabolic and respiratory components of any acid-base derangements were separated by calculating the standard $[HCO_3^-]$. The mean $PaCO_2$ of minutes 20 and 30 during the experimental trial was taken as the control $PaCO_2$ in this calculation. The standard $[HCO_3^-]$ was subsequently incorporated into the Henderson-Hasselbalch equation to partition changes in pH due to metabolic and respiratory acidosis.

Respiratory variables were measured during exercise using an on-line breath-by-breath respiratory system (see section 2.2). Note, however, that during control and

experimental trials a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) was connected distal to the turbine flowmeter.

6.2.4 Determination of MLSS power

An estimate of MLSS power was initially made using a "lactate minimum" test based on the method described by Tegtbur et al. (1993). The exercise protocol comprised 3 consecutive phases: (I) a lactate elevation phase consisting of a maximal, incremental $\dot{V}O_2$ peak test; (II) a recovery phase consisting of 8 min of cycling at 60 W to maximise $[lac^-]_B$; and (III) an incremental phase consisting of 5 consecutive 4 min cycling exercise stages at intensities of 45, 50, 55, 60, and 65% of the $\dot{W}$ max achieved during the lactate elevation phase. During the lactate elevation phase cycling power was increased every 15 s by a constant increment that was chosen for each subject in order to elicit exercise intolerance in approximately 10 min. The lactate elevation phase was terminated when, despite verbal encouragement, cycling cadence could not be maintained above 60 rpm. The power output at which exercise intolerance ensued defined $\dot{W}$ max, and the highest $\dot{V}O_2$ and $\dot{V}_E$ recorded in any 30 s period defined $\dot{V}O_2$ peak and maximal exercise $\dot{V}_E$, which hereafter, is referred to as $\dot{V}_E$ max. During the incremental phase $[lac^-]_B$ was determined during the final seconds of each stage, and values were then plotted against the corresponding cycling power. The nadir (i.e. estimated MLSS power) of a 2nd order polynomial curve fitted to the plot of $[lac^-]_B$ against cycling power was calculated through differentiation of the quadratic equation.

The MLSS power was subsequently resolved through each subject performing at least two constant power trials of 30 min duration (preceded by a 3 min warm-up at 50% of the prescribed power). $[lac^-]_B$ was determined every 2 min between 16-30 min inclusive. MLSS power was defined as the highest power output at which a significant, positive

gradient of a linear regression line fitted through the plot of $[lac^-]_B$ against time was not observed (see section 2.7 for calculation formula). The critical significance level for this analysis was set at $P < 0.10$. Subsequent trials were performed at a power 2.5% above or below the initial power until MLSS was verified.

6.2.5 CONTROL TRIAL

Once MLSS power was established a 30 min control trial at this intensity was performed. Exercise was preceded by a 3 min warm-up period at 50% MLSS power. $[lac^-]_B$ was determined every 2 min during exercise. Blood-gas variables were determined every 4 min from 2-18 min inclusive, and then every 2 min thereafter. Respiratory variables were averaged over the final minute of every 2 min interval. Heart rate (HR) was recorded continuously during exercise by using short range-range telemetry (Polar Accurex Plus, Polar, Kempele, Finland). A non-invasive estimate of arteriovenous oxygen content difference [C(a-vDO$_2$)], $\dot{Q}$, and stroke volume (SV) was made from the $\dot{V}O_2$ peak achieved in the lactate elevation phase of the lactate minimum test, and the measured $\dot{V}O_2$ and HR during exercise (Stringer et al. 1997).

6.2.6 EXPERIMENTAL TRIAL

The experimental trial mimicked the control trial except for the following differences. Following 19.5 min of exercise a 1.5 m length of tubing was connected to the inspiratory line of the two-way valve. Inserted halfway along this tubing was a small-bore tube that was connected, via a CO_2 rotameter (flow range = 0.6-4.4 l·min^{-1}), to a 100% CO_2 certified gas cylinder (BOC Gases, Guilford, UK). Following 20 min of exercise subjects were instructed and verbally encouraged to volitionally elevate $\dot{V}_E$ to the highest sustainable level for a duration of 8 min. To maintain isocapnia during volitional

hyperpnoea, end-tidal CO_2 ($ETCO_2$) was held constant by adjusting the flow of CO_2 into the inspiratory circuit. Subjects were given real-time visual feedback of $\dot{V}_E$, and were encouraged to maintain this at a maximal level. Subjects returned to spontaneous breathing for the remaining 2 min of exercise.

6.2.7 Statistical analyses

Statistical analyses were performed independently over two time periods: 12-20 min (steady-state period) and 20-30 min (intervention period). A repeated measures factorial ANOVA was applied to establish differences between control and experimental trials in the magnitude of change over time in all dependent variables. When a significant *F* ratio was detected, differences between trials at equal times were determined using paired t-tests or one-way ANOVA for repeated measures, and differences over time within each trial were determined using a one-way ANOVA for repeated measures. Differences in breathing pattern between that associated with $\dot{V}_E$ max and that demonstrated at each measurement time during volitional hyperpnoea were established using a one-way ANOVA for repeated measures. One-way ANOVA analyses were followed by Tukey's HSD post-hoc analysis. Pearson's product moment correlation coefficient (r) was calculated to determine the degree of association between selected variables. The critical significance level was set at $P < 0.05$. Results are presented as means ± standard error of the mean (SEM). Statistical analyses were performed using the 12.0 release version of SPPS for Windows (SPSS Inc., Chicago, IL, USA).

6.3 Results

Resting pulmonary and respiratory muscle function are presented in Table 6.1. $\dot{W}$ max and $\dot{V}O_2$ peak during the lactate elevation phase of the lactate minimum test were

367 ± 16 W and 3.87 ± 0.16 l·min^{-1}, respectively. The MLSS cycling power was 207 ± 8 W (range = 233 – 172). Hereafter, unless otherwise indicated, all results pertain to control and experimental trials.

Table 6.1 Resting pulmonary and respiratory muscle function (mean ± SEM).

Variable	Measurement
FVC (l)	5.57 ± 0.18 (109 ± 2)
FEV_1 (l)	4.71 ± 0.12 (111 ± 1)
PEF (l·s^{-1})	10.6 ± 0.2 (102 ± 4)
MVV_{12} (l·min^{-1})	180.0 ± 9.8 (117 ± 6)
MIP (cmH_2O)	168 ± 5 (146 ± 5)
MEP (cmH_2O)	179 ± 10 (114 ± 6)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; MVV_{12}, 12 s maximum voluntary ventilation; MIP, maximal inspiratory mouth pressure; MEP, maximal expiratory mouth pressure. Numbers in parentheses represent % of predicted values (Cotes 1993; Wilson et al. 1984).

6.3.1 BREATHING PATTERN: CONTROL VS. EXPERIMENTAL TRIAL

Breathing pattern responses are shown in Figure 6.1. During the steady state period (12-20 min) no significant differences in breathing pattern were observed between trials. However, as expected, volitional hyperpnoea resulted in $\dot{V}_E$ being significantly ($P < 0.01$) higher in the experimental trial at all times from 22-28 min, inclusive. The mean $\dot{V}_E$ during volitional hyperpnoea (169.6 ± 7.1 l·min^{-1}) was not different ($P = 0.48$) from $\dot{V}_E$max (171.9 ± 6.8 l·min^{-1}). The increase in $\dot{V}_E$ during volitional hyperpnoea was primarily achieved via an increase in f_R, which, compared to the control trial, was significantly ($P < 0.01$) higher at all times from 22-28 min, inclusive. The pattern of change in V_T over 22-28 min was not different between trials. There was a trend for duty

cycle to be lower throughout volitional hyperpnoea, although a significant ($P < 0.05$) interaction effect was only observed at 26 min.

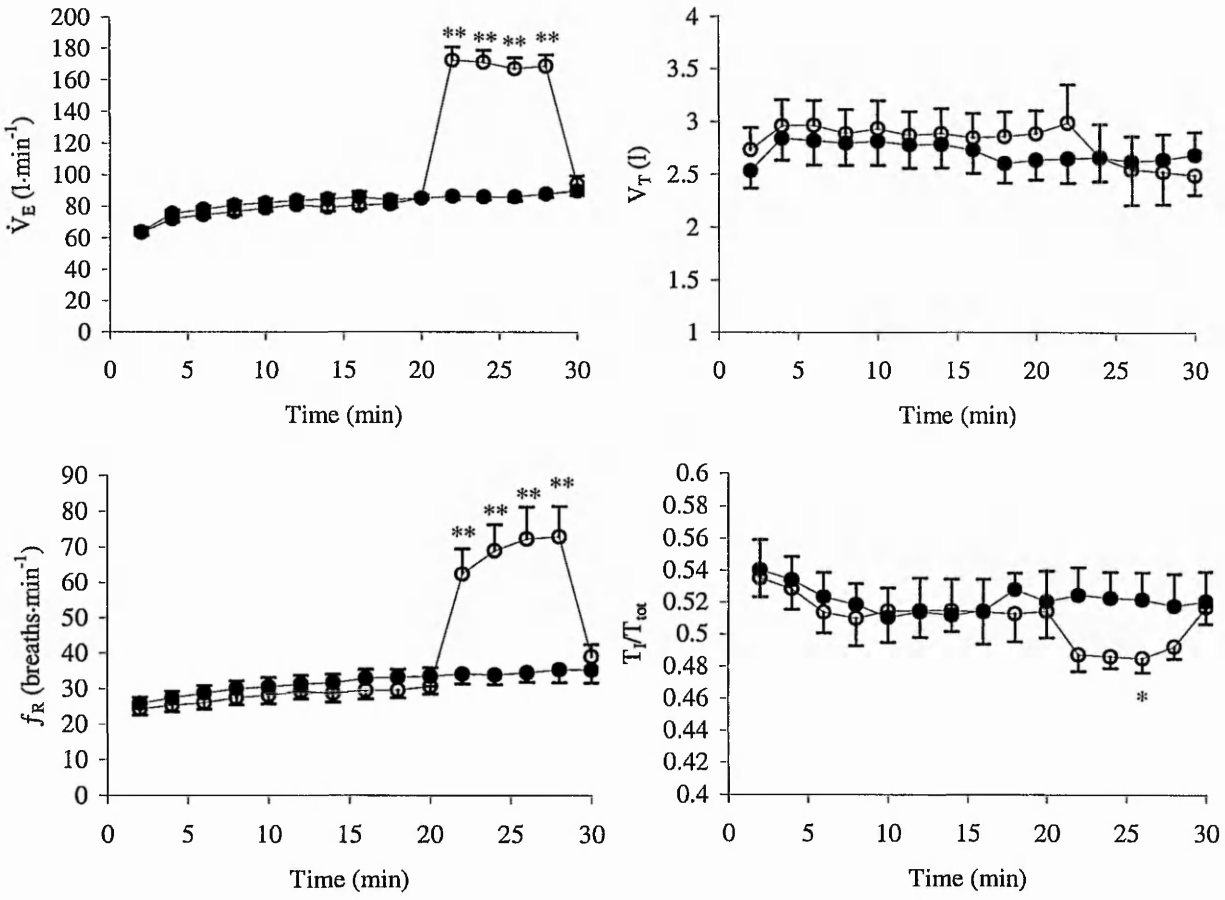

Figure 6.1 Ventilatory response to exercise at MLSS power in control (•) and experimental (o) trials (mean ± SEM). $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, respiratory frequency; T_I/T_{tot}, inspiratory time/total breath time (duty cycle). *Significant interaction effect ($P < 0.05$). **Significant interaction effect ($P < 0.01$).

6.3.2 BREATHING PATTERN: EXPERIMENTAL TRIAL VS. $\dot{V}_E$ MAX

The V_T at each measurement time during volitional hyperpnoea was not different from the 3.29 ± 0.27 l corresponding to $\dot{V}_E$ max. However, following 4 min of volitional hyperpnoea f_R was significantly ($P < 0.05$) greater than that corresponding to $\dot{V}_E$ max (69.0 ± 7.3 vs. 53.6 ± 5.2 breaths·min^{-1}) and differences existed up to 28 min. The duty cycle, inspiratory flow rate, and expiratory flow rate during volitional hyperpnoea was not

different from the 0.49 ± 0.01, 6.01 ± 0.30 $l \cdot s^{-1}$, and 5.68 ± 0.38 $l \cdot s^{-1}$, respectively, corresponding to $\dot{V}_E$ max.

6.3.3 BLOOD PARAMETERS

During control and experimental trials rectal temperature increased progressively, and similarly, from 37.4 ± 0.1 and 37.6 ± 0.1 °C at 2 min to 38.3 ± 0.1 and 38.5 ± 0.1 °C at 30 min, respectively. Note that although it is not possible to attain a true PaO_2 from arterialised venous blood sampled from a superficial dorsal hand vein (as was the method used in this study), we recorded, from 10-30 min of exercise, a mean PO_2 of arterialised venous blood of 78.2 ± 2.9 and 80.3 ± 3.9 mmHg during control and experimental trials, respectively (no significant difference between trials). These values suggest that our arterialisation was successful. Changes in SaO_2 during exercise were also not different between trials. From 10-30 min of exercise SaO_2 remained relatively constant and averaged 93.6 ± 0.6 and 92.8 ± 1.1% during control and experimental trials, respectively. Changes in all remaining blood parameters during the steady state period were not different between or within trials (Figure 6.2). All blood parameters also showed no change over time in the control trial from 20-30 min of exercise.

During the control trial $[lac^-]_B$ increased transiently during the first 10 min, after which it remained at a steady state concentration of 3.7 ± 0.1 $mmol \cdot l^{-1}$. During the first 20 min of the experimental trial changes in $[lac^-]_B$ were not different from those observed during the control trial. However, during volitional hyperpnoea, and during the last 2 min of exercise, $[lac^-]_B$ increased continually at a rate of 0.20 ± 0.05 $mmol \cdot l^{-1} \cdot min^{-1}$. Following 6 min of volitional hyperpnoea $[lac^-]_B$ had significantly ($P < 0.01$) increased above steady state, and at the end of the experimental trial $[lac^-]_B$ was 4.7 ± 0.3 $mmol \cdot l^{-1}$, which represented a 24.9 ± 5.5% increase from steady state. Significant ($P < 0.05$) differences in

$[lac^-]_B$ were observed between trials from 26-30 min, inclusive.

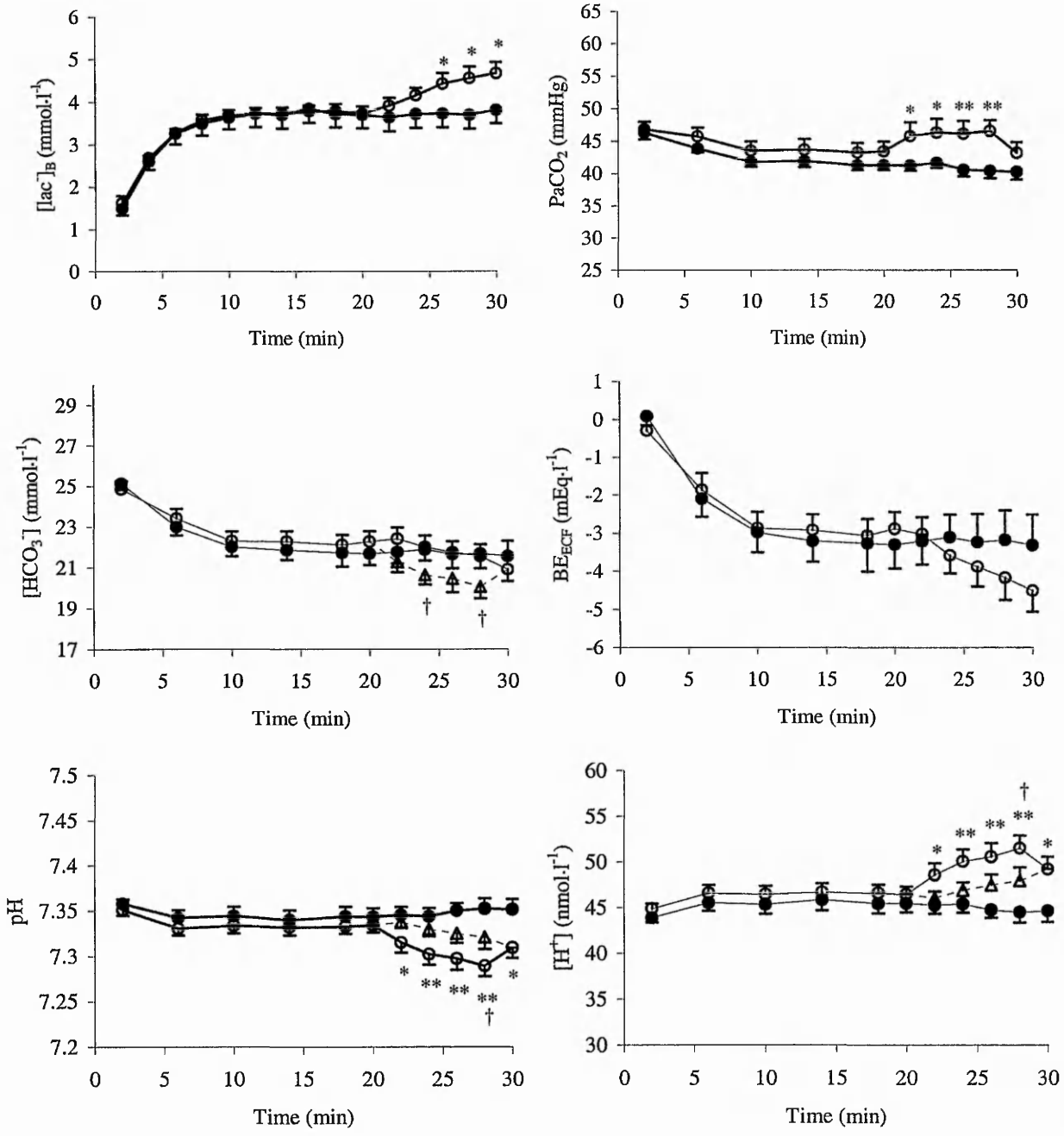

Figure 6.2 Changes in blood lactate concentration ($[lac^-]_B$) and blood acid-base balance during exercise at MLSS power in control (•) and experimental (o) trials (mean ± SEM). $PaCO_2$, partial pressure of carbon dioxide in arterialised venous blood; $[HCO_3^-]$, actual plasma bicarbonate concentration; BE_{ECF}, base excess of the extracellular fluid; $[H^+]$, hydrogen ion concentration. Open triangles (Δ) denote standard $[HCO_3^-]$ and changes in pH (and thus $[H^+]$) according to the standard $[HCO_3^-]$ and control $PaCO_2$. *Significant interaction effect (• vs. o) ($P < 0.05$). **Significant interaction effect (• vs. o) ($P < 0.01$). †Significant interaction effect (• vs. Δ) ($P < 0.05$).

A degree of hypercapnia was evident during volitional hyperpnoea, in that $PaCO_2$ increased significantly ($P < 0.05$) from 43.4 ± 1.5 mmHg at 20 min to 46.3 ± 2.12 mmHg at 24 min. Thereafter, $PaCO_2$ remained relatively constant up to 28 min. $PaCO_2$ at 30 min (43.2 ± 1.6 mmHg) during the experimental trial was not different from that observed at 20 min. Significant differences in $PaCO_2$ were observed between trials from 22-28 min, inclusive ($P < 0.05$ and 0.01 for 22-24 and 26-28 min, respectively).

During the control trial actual [HCO_3^-] remained at a steady state of 21.7 ± 0.6 $mmol \cdot l^{-1}$ over 20-30 min of exercise. Conversely, a continual decline was observed during and following volitional hyperpnoea, such that actual [HCO_3^-] recorded at 30 min was significantly ($P < 0.01$) lower than that observed at 20 min (20.9 ± 0.6 vs. 22.3 ± 0.5 $mmol \cdot l^{-1}$). However, although there was a significant ($P < 0.05$) interaction effect (trial × time) for actual [HCO_3^-] over 20-30 min of exercise, paired t-tests did not reveal significant differences between trials because the data were disordinal (i.e. crossing over). There was, however, a greater decline in standard [HCO_3^-] compared to actual [HCO_3^-] during volitional hyperpnoea, suggestive of a combined respiratory and metabolic acidosis. During the experimental trial standard [HCO_3^-] from 22-30 min, inclusive, was significantly ($P < 0.01$) lower than that recorded at 20 min. Standard [HCO_3^-] was also significantly ($P < 0.05$) lower than actual [HCO_3^-] recorded in both control and experimental trials at 24 and 28 min.

The ANOVA analysis for BE_{ECF} revealed a significant ($P < 0.05$) interaction effect (trial × time) over 20-30 min of exercise; however, because the data were disordinal, differences between trials at each sample time were not significant. This response is, however, to be expected given that changes in BE_{ECF} usually mirror changes in actual

$[HCO_3^-]$ (see previous paragraph). From 20-30 min BE_{ECF} during the control trial was maintained at a steady state of –3.23 ± 0.69 $mEq \cdot l^{-1}$. In contrast, a continual decline in BE_{ECF} was observed during volitional hyperpnoea. Specifically, BE_{ECF} decreased significantly ($P < 0.01$) from –2.89 ± 0.43 $mEq \cdot l^{-1}$ at 20 min to –3.89 ± 0.51 $mEq \cdot l^{-1}$ at 26 min, and further reductions were evident for the remainder of exercise.

The actual $[H^+]$ increased continually during volitional hyperpnoea from 46.4 ± 0.8 $nmol \cdot l^{-1}$ (pH = 7.33 ± 0.01) at 20 min to 51.5 ± 1.4 $nmol \cdot l^{-1}$ (pH = 7.29 ± 0.01) at 28 min ($P < 0.01$). The actual $[H^+]$ declined during the final 2 min of exercise as subjects returned to spontaneous breathing, although values were still significantly ($P < 0.01$) elevated above that recorded at 20 min. Also shown on the $[H^+]$ and pH figures are changes in these variables when calculated using the standard $[HCO_3^-]$ and control $PaCO_2$. The $[H^+]$ calculated from the standard $[HCO_3^-]$ and control $PaCO_2$ (i.e. the metabolically derived $[H^+]$) increased significantly ($P < 0.01$) during the experimental trial from 46.3 ± 0.1 $nmol \cdot l^{-1}$ (pH = 7.33 ± 0.01) at 20 min to 49.2 ± 1.3 $nmol \cdot l^{-1}$ (pH = 7.31 ± 0.01) at 30 min. The less pronounced increase in metabolically derived $[H^+]$ compared to actual $[H^+]$ during volitional hyperpnoea is suggestive of the presence of a mixed metabolic and respiratory acidosis. Actual $[H^+]$ was significantly greater than the metabolically derived $[H^+]$ at 24 ($P < 0.01$) and 26-28 min ($P < 0.05$), inclusive, and it was also greater than that recorded during the control trial at 22 ($P < 0.05$), and 24-30 min ($P < 0.01$), inclusive. The metabolically derived $[H^+]$ at 28 min was significantly ($P < 0.05$) greater than that recorded during the control trial.

6.3.4 PULMONARY GAS EXCHANGE

Changes in $\dot{V}O_2$ and $ETCO_2$ are shown in Figure 6.3. Changes in $ETCO_2$ throughout exercise were not different between trials. Changes in $\dot{V}O_2$ over the steady state period were also not different between trials. During the control trial, $\dot{V}O_2$ increased up to 12 min, after which it remained at a steady state of 3.46 ± 0.20 $l \cdot min^{-1}$ for the remainder of exercise. However, during volitional hyperpnoea there was a notable increase in $\dot{V}O_2$ above steady state. Significant differences in $\dot{V}O_2$ were observed between trials at all times from 22-28 min, inclusive. Once elevated at 22 min, $\dot{V}O_2$ remained unchanged for the remainder of volitional hyperpnoea. The mean $\dot{V}O_2$ during volitional hyperpnoea was 3.83 ± 0.20 $l \cdot min^{-1}$, thus the oxygen cost of the additional hyperpnoea was calculated at 4.3 ± 0.8 ml O_2 per $l \cdot min^{-1}$ of $\dot{V}_E$.

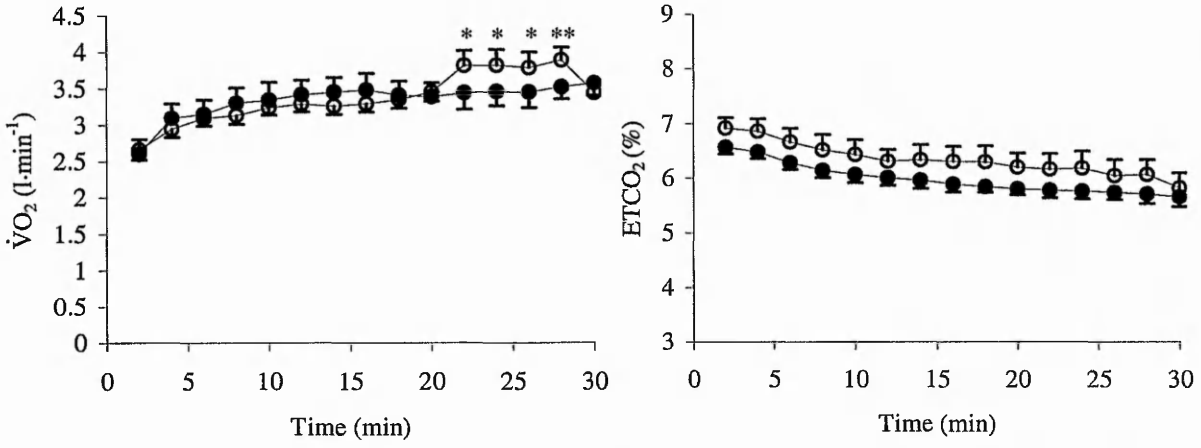

Figure 6.3 Changes in pulmonary oxygen uptake ($\dot{V}O_2$) and end-tidal CO_2 ($ETCO_2$) during exercise at MLSS power in control (•) and experimental (o) trials (mean ± SEM). *Significant interaction effect ($P < 0.05$). **Significant interaction effect ($P < 0.01$).

6.3.5 CARDIOVASCULAR RESPONSES

Cardiovascular responses to control and experimental trials are shown in Figure 6.4. With the exception of HR, which, perhaps owing to an anticipatory increase, was

significantly ($P < 0.05$) higher in the experimental trial at 20 min (162.1 ± 2.1 vs. 157 ± 2.8 beats·min^{-1}), the cardiovascular response to the initial 20 min of exercise was not different between control and experimental trials. Conversely, the mean HR during volitional hyperpnoea was significantly ($P < 0.05$) greater than that recorded over the same time period during the control trial (166.8 ± 2.7 vs. 158.5 ± 2.7 beats·min^{-1}). Additionally, compared to that recorded during the control trial, HR was significantly higher during volitional hyperpnoea at all times from 24-28 min ($P < 0.01$), and at 30 min ($P < 0.05$).

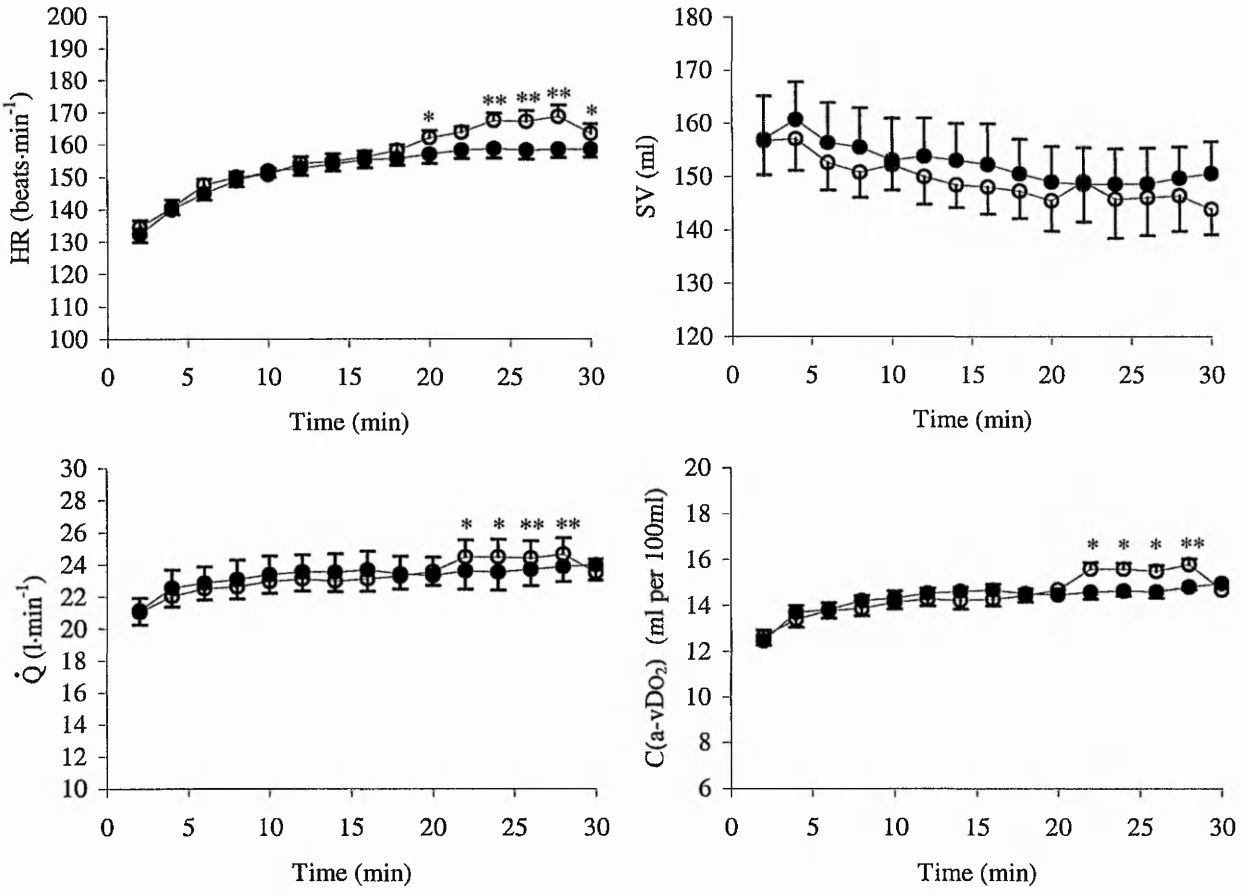

Figure 6.4 Changes in heart rate (HR), stroke volume (SV), cardiac output ($\dot{Q}$), and arteriovenous oxygen content difference [$C(a\text{-}vDo_2)$] during exercise at MLSS power in control (•) and experimental (o) trials (mean ± SEM). *Significant interaction effect ($P < 0.05$). **Significant interaction effect ($P < 0.01$).

The increased HR during volitional hyperpnoea primarily accounted for the concurrent increase in estimated $\dot{Q}$ since changes over time in SV were not different

between trials. The mean $\dot{Q}$ during volitional hyperpnoea was significantly ($P < 0.01$) greater than that observed over the same time period during the control trial (24.5 ± 1.1 $l \cdot min^{-1}$ vs. 23.7 ± 1.1 $l \cdot min^{-1}$). Significant differences between trials were also observed for $\dot{Q}$ at all times from 22-28 min, inclusive.

Expectedly, the increase in $\dot{V}O_2$ and $\dot{Q}$ during volitional hyperpnoea was paralleled by an increased O_2 extraction from the arterial blood. The mean $C(a\text{-}vDO_2)$ from 22-28 min was significantly ($P < 0.05$) higher in the experimental trial compared to the control trial (15.6 ± 0.3 vs. 14.6 ± 0.2 ml per 100 ml). Significant differences in $C(a\text{-}vDO_2)$ were also noted between trials at all times from 22-28 min, inclusive.

6.3.6 CORRELATIONS BETWEEN VARIABLES

The relative change in $[lac^-]_B$ during volitional hyperpnoea was not correlated with either the relative or absolute increase in $\dot{V}_E$, nor was it correlated with any index of pulmonary or respiratory muscle function, or endurance training status ($\dot{V}O_2$ peak and $\dot{W}$ max). There was also no correlation between relative changes in $[lac^-]_B$ and changes in parameters of acid-base balance. However, the relative increase in $[lac^-]_B$ was significantly ($P < 0.01$) correlated (positively) with both the relative change in duty cycle (r = 0.95) and the fraction of MVV_{12} utilised (r = 0.94) (Figure 6.5).

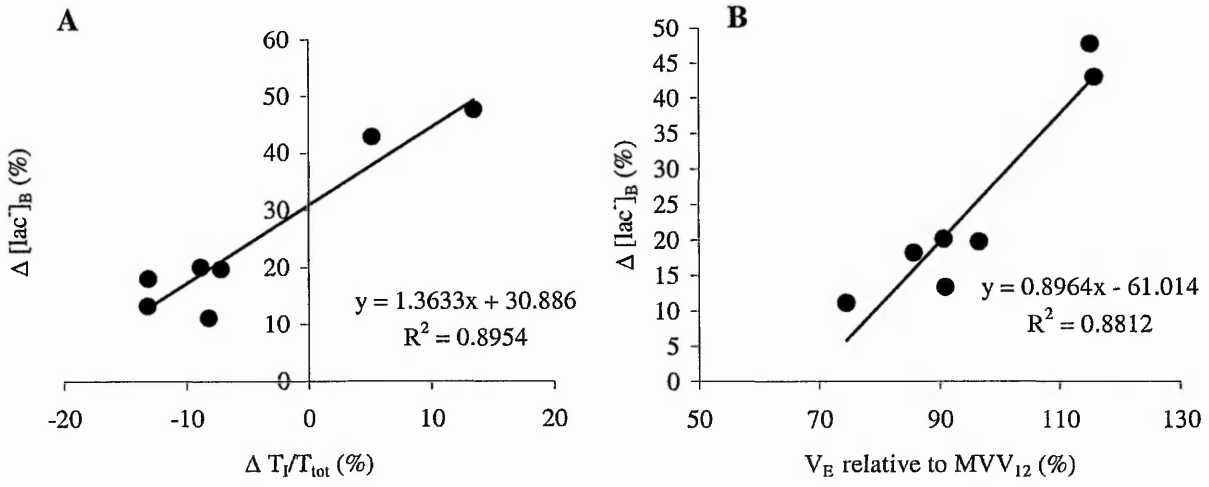

Figure 6.5 Relative change in blood lactate concentration ($[lac^-]_B$) versus relative change in duty cycle (T_I/T_{tot}) (A), and V_E as a percentage of maximum voluntary ventilation in 12 s (MVV_{12}) (B). Note: correlation coefficients significant ($P < 0.01$).

6.4 Discussion

This study examined the effects of maximal voluntary hyperpnoea on the $[lac^-]_B$ response to cycle ergometry exercise at MLSS. In agreement with the hypothesis, $[lac^-]_B$ increased (+24.9%) significantly during exercise at MLSS when $\dot{V}_E$ was elevated to a level comparable with that observed in maximal exercise. Therefore, these findings suggest that the respiratory muscles may affect $[lac^-]_B$ during heavy endurance exercise.

The findings of this study appear contrary to previous studies that have shown minimal or no change in $[lac^-]_B$ when maximal exercise $\dot{V}_E$ is reproduced voluntarily under resting conditions (Babcock et al. 1995; Martin et al. 1984). A key factor in this discrepancy is that the paradigm adopted in this study was able to unmask the influence of additional respiratory muscle work on $[lac^-]_B$ by superimposing it upon exercise at MLSS. Under these conditions there is very limited capacity to counter further lactate appearance because the system is operating at a point where lactate appearance and removal are in equilibrium. However, these data are harder to resolve with the observation that $[lac^-]_B$ does not change when the work of breathing is increased (via an inspiratory resistance), or

decreased (via proportional assist ventilation), during maximal exercise (Harms et al. 1997). There are two possible explanations for this. Firstly, that the recruitment patterns of the respiratory muscles under conditions of external loading and voluntary hyperpnoea differ (McCool et al. 1992) and result in different lactate responses; secondly, that compartmental lactate turnover associated with different exercise intensities may be critical to the resulting response of $[lac^-]_B$ to additional respiratory muscle work. For example, during exercise of maximal intensity, as was used in the study of Harms et al. (1997), any influence the respiratory muscles have on $[lac^-]_B$ may be overshadowed by the action of the much larger locomotor muscle mass.

Since only systemic $[lac^-]_B$ was measured in the present study, it is not possible to identify whether the increased $[lac^-]_B$ during volitional hyperpnoea resulted from a greater rate of lactate appearance, a decline in the rate of lactate clearance, or a combination of these processes. Further, it can only be speculate in which muscle groups these changes may have occurred. However, it does seem likely that either the active locomotor muscles of the legs, the respiratory muscles, or both, were responsible for the changes in $[lac^-]_B$. A potential mechanism for a change in lactate kinetics in the locomotor muscles may reside in the respiratory muscle metaboreflex that probably occurs during heavy endurance exercise when the respiratory muscles become fatigued (Harms et al. 1997), as is likely (although not measured) to have happened in the present study. The subsequent decrease in leg blood flow, which presumably results in greater perfusion of respiratory musculature, could attenuate lactate uptake by locomotor muscles through reducing the extra-to-intramuscular lactate concentration gradient. However, the fact that inspiratory muscle loading during maximal exercise caused no change in the arterial and femoral venous $[lac^-]_B$, even in the presence of a reflex reduction in the locomotor muscle blood flow of 7.1% (Harms et al. 1997), suggests that locomotor muscle may not be the source of the

increased systemic lactate that was observed during voluntary hyperpnoea at MLSS. However, since the study design of this investigation was quite different from that of the present study, the possibility remains that limb lactate kinetics were indeed influenced by volitional hyperpnoea. Moreover, we should not discount the possibility that a local vasoconstriction in other tissues capable of removing lactate (e.g. liver) perhaps contributed to decreased lactate clearance in the present study.

Thus, it remains possible that at least part of the increase in $[lac^-]_B$ during volitional hyperpnoea resulted from lactate production and release from, or reduced lactate clearance by, active respiratory muscles. Though it has been suggested that the diaphragm, because of its small mass (about 0.5% of body mass), is unlikely to influence $[lac^-]_B$ (Wetter and Dempsey 2000), it should be recognised that maximal exercise hyperpnoea requires substantial activity by many other muscles of the trunk, such as the intercostals, scalenes, sternomastoids, abdominal muscles, and muscles of the chest and back, all of which may contribute to lactate turnover. It is noteworthy that the relative change in $[lac^-]_B$ during volitional hyperpnoea was positively correlated with the ratio of $\dot{V}_E$ to MVV_{12}. Martin et al. (1984) reported a similar relationship in subjects performing volitional hyperpnoea at rest, although the authors did not elaborate on this observation. An explanation for this finding could be that subjects utilising the greatest fraction of MVV_{12} may also be utilising a greater proportion of their maximal respiratory muscle power output, which may thus result in greater metabolic perturbations (i.e. lactate production). Furthermore, the relative change in duty cycle during volitional hyperpnoea was also correlated with changes in $[lac^-]_B$. Increasing duty cycle would have increased the fraction of each breath for which the perfusion vessels of the inspiratory muscles were constricted during contraction, with a consequent restriction of inspiratory muscle blood flow (Bellemare et al. 1983). This might have influenced lactate exchange between inspiratory muscles and blood, depending upon

the role of the inspiratory muscles as net consumers or producers of lactate under the conditions of this experiment.

Because of the delay in acquiring $PaCO_2$ measurements from the blood-gas analyser, an attempt was made to maintain isocapnia during volitional hyperpnoea through keeping $ETCO_2$ (measured breath-by-breath) constant. However, despite the precision with which $ETCO_2$ was maintained, an approximate 6.4% increase, from steady state, in $PaCO_2$ was observed during volitional hyperpnoea. This underestimate of $PaCO_2$ from $ETCO_2$ could have been partly related to the increased f_R and thus shortened expiratory duration, which may have provided insufficient time for $ETCO_2$ to increase and subsequently reflect alveolar PCO_2. More importantly, however, it is possible that this increase in $PaCO_2$ affected the $[lac^-]_B$ in the present study. The greater decline in standard $[HCO_3^-]$ compared to actual $[HCO_3^-]$ during volitional hyperpnoea confirms the existence of a mixed respiratory and metabolic acidosis. The changes in pH as calculated using the actual and standard $[HCO_3^-]$ along with the control $PaCO_2$ lends further support to this conclusion. However, during exercise, an increase in $PaCO_2$, or respiratory acidosis, has been consistently shown to result in a reduction in $[lac^-]_B$ (Ehrsam et al. 1982; Graham et al. 1986; Graham and Wilson 1983; Graham et al. 1982). For example, Graham et al. (1982) showed a 16.3% increase in $PaCO_2$ (achieved through hypercapnic breathing) during exercise at 65% $\dot{V}O_2$max resulted in a 0.9 $mmol \cdot l^{-1}$ reduction in $[lac^-]_B$. Thus, it is highly unlikely that the presence of a respiratory acidosis explains the increase in $[lac^-]_B$ observed in the present study; if anything, it is more likely to have led to a slight underestimate of the increase in $[lac^-]_B$.

The findings of the present study indicate that respiratory muscle work is capable of influencing the $[lac^-]_B$ associated with heavy endurance exercise. This observation is entirely consistent with observations made in several studies of RMT, in which $[lac^-]_B$

during whole-body endurance exercise is attenuated post-RMT (reviewed in McConnell and Romer 2004a; McConnell and Sharpe 2005). Recently, McConnell and Sharpe (2005) observed an approximate 1 mmol·l^{-1} reduction in the MLSS $[lac^-]_B$ following a 6 week pressure-threshold inspiratory muscle training regimen, although the MLSS cycling power remained unchanged. The implications of these findings are that the respiratory muscles may affect the $[lac^-]_B$ of even steady state submaximal exercise in which $\dot{V}_E$ may approximate only 50% of maximal exercise $\dot{V}_E$.

The oxygen cost of volitional hyperpnoea in the present study (4.3 ± 0.84 ml O_2 per l·min^{-1} of $\dot{V}_E$) exceeds the 2.85 ml O_2 per l·min^{-1} of $\dot{V}_E$ reported by Aaron et al. (1992), who had subjects mimic, at rest, the $\dot{V}_E$ (range = 117-147 l·min^{-1}) and associated breathing pattern demonstrated during maximal exercise. The greater oxygen cost of hyperpnoea in the present study could be due, in part, to volitional hyperpnoea being less efficient than spontaneous hyperpnoea (Coast et al. 1993; Klas and Dempsey 1989), which would result in an increased work of breathing and respiratory muscle $\dot{V}O_2$. Note, however, that the relative cost of breathing increases with $\dot{V}_E$ (Aaron et al. 1992), thus the greater oxygen cost of breathing in this study would be expected given the level of $\dot{V}_E$ achieved during volitional hyperpnoea (168.3 ± 7.0 l·min^{-1}). However, given the above consideration, a limitation to the present study, which may have influenced the results, is that the breathing and respiratory muscle recruitment patterns adopted during volitional hyperpnoea were not consistently matched with those adopted spontaneously during exercise (i.e. $\dot{V}_E$max), thus the work of breathing during volitional hyperpnoea may have been greater than that experienced in exercise (Coast et al. 1993). However, the lack of correlation between changes in f_R during volitional hyperpnoea (the only parameter of breathing pattern that was different from that associated with $\dot{V}_E$max) and changes in $[lac^-]_B$ suggests that the

progressive tachypnoea can not entirely explain the increase in $[lac^-]_B$. It should also be noted that the oxygen cost of breathing remained constant throughout the 8 minutes of volitional hyperpnoea, thus further suggesting that the progressive change in breathing pattern did not greatly affect the work of breathing. Thus although this model does not exactly simulate the dynamics of breathing inherent to the spontaneously regulated control system, the findings, like those of Harms et al. (1997; 1998b) who used mechanical loading and unloading of the inspiratory muscles to quantify the effects of inspiratory muscle work on $\dot{Q}$ distribution during exercise, speak towards what might potentially occur during spontaneous exercise hyperpnoea. Specifically, the results of the present study and those of Harms et al. (1997; 1998b) suggest that during heavy whole-body exercise the respiratory muscles may impart a major influence on $[lac^-]_B$ and $\dot{Q}$ distribution, respectively. However, despite these considerations, we still cannot discount the possibility that smaller increases in $[lac^-]_B$ may have been observed in the present study had all elements of breathing pattern been controlled during volitional hyperpnoea.

A large part of the exaggerated work of breathing during voluntary hyperpnoea results from the generation of excessive expiratory pressures beyond the maximal effective expiratory pressures (Klas and Dempsey 1989). During exercise, excessive gastric pressures due to abdominal or diaphragmatic contraction causes a reduction in venous return from the legs, subsequently attenuating increases in SV and thus $\dot{Q}$ (Miller et al. 2005; Stark-Leyva et al. 2004). Therefore, if limb blood flow were compromised in the present study due to an attenuated $\dot{Q}$ secondary to excessive expiratory pressure development during volitional hyperpnoea, then lactate clearance by leg musculature may have declined, thus further contributing to the increase in $[lac^-]_B$.

Consistent with the well-established relationship between $\dot{V}O_2$ and $\dot{Q}$, the increase in $\dot{V}O_2$ during volitional hyperpnoea was accompanied by an approximate 1.0 $l \cdot min^{-1}$ increase in $\dot{Q}$. Although thoracic pressure swings exert a significant effect on $\dot{Q}$, this primarily occurs through changes in SV (Boutellier and Farhi 1986; Harms et al. 1998b). Therefore, since the increase in $\dot{Q}$ during volitional hyperpnoea resulted from an increase in HR, and was also accompanied by an increase in $\dot{V}O_2$, the increase in $\dot{Q}$ was likely to reflect the increased metabolic demands of the respiratory muscles. However, considering that $\dot{V}_E$ increased approximately two-fold during volitional hyperpnoea, a 1.0 $l \cdot min^{-1}$ increase in $\dot{Q}$ seems lower than might be expected. Anholm et al. (1987) observed a 4.3 (SD 1.0) $l \cdot min^{-1}$ increase in $\dot{Q}$ during sustained maximal ventilation ($\dot{V}_E$ ranged from 127-193 $l \cdot min^{-1}$) and suggested an upper limit of respiratory muscle blood flow of 3-5 $l \cdot min^{-1}$. The possibility therefore exists that there was limited vasodilator reserve in the respiratory muscles during volitional hyperpnoea in the present study. Partial support for this contention is provided by the observations of Manohar (1986) who failed to observe, during adenosine infusion, further dilation in the diaphragm and intercostal muscles of maximally exercising ponies, although a significant vasodilator reserve was observed for the myocardium. Therefore, although inter-species differences preclude direct comparisons, it is possible that the upper limit of respiratory muscle blood flow is attained during maximal exercise.

In summary, the present study has shown a significant effect of maximal volitional hyperpnoea on $[lac^-]_B$ during cycling exercise at MLSS. These findings suggest that the respiratory muscles are capable of influencing $[lac^-]_B$ during heavy endurance exercise and are consistent with previous observations that RMT reduces $[lac^-]_B$ at equivalent intensities of exercise. Further studies are required to establish whether: (I) volitional hyperpnoea

causes similar increases in $[lac^-]_B$ when the adopted breathing and respiratory muscle recruitment patterns are precisely matched with those demonstrated during maximal exercise; (II) exercise hyperpnoea affects limb lactate kinetics; and (III) specific RMT attenuates the lactate accumulation that is observed when maximal hyperpnoea is performed during exercise at MLSS.

CHAPTER 7

EFFECTS OF MAXIMAL EXERCISE HYPERPNOEA ON BLOOD LACTATE CONCENTRATION AT REST

7.1 INTRODUCTION

In the preceding chapter superimposing voluntary isocapnic hyperpnoea at a level commensurate with maximal exercise $\dot{V}_E$ ($\dot{V}_E$max) on leg cycling exercise at maximal lactate steady state resulted in 1.0 mmol·l^{-1} increase (+25%) in $[lac^-]_B$. These findings were therefore indicative of a prominent role of the respiratory muscles in lactate turnover during maximal exercise. However, because only systemic $[lac^-]_B$ was determined in the previous chapter it was not possible to identify whether increases in $[lac^-]_B$ during volitional hyperpnoea resulted from altered lactate kinetics in respiratory muscles, active locomotor muscles, or both. Potentially, the increased metabolic demands of the respiratory muscles during volitional hyperpnoea resulted in a "steal" of locomotor muscle perfusion (Harms et al. 1997), which may have reduced the delivery and therefore uptake of lactate by the locomotor muscles. However, it is also possible that the respiratory muscles become net lactate producers during heavy endurance exercise, thus the increases in $[lac^-]_B$ observed in the preceding chapter may have partly resulted from increased lactate efflux from active respiratory muscles.

Evaluating the $[lac^-]_B$ response to volitional hyperpnoea under resting conditions could resolve some of the ambiguity surrounding the mechanisms by which $[lac^-]_B$ increases when volitional hyperpnoea is superimposed on heavy exercise. For example, it is tenable to presume that an increase in $[lac^-]_B$ whilst mimicking $\dot{V}_E$max at rest primarily results from increased lactate production and efflux from respiratory muscles; accordingly, this could be taken as evidence that increased $[lac^-]_B$ when $\dot{V}_E$max is superimposed on heavy exercise is also, in part, the result of increased lactate efflux from respiratory muscles. However, in the previous chapter it was also reasoned that the influence of additional respiratory muscle work on $[lac^-]_B$ at rest might be masked by a considerable reserve for lactate clearance under these conditions. Thus it is also intriguing to test the

hypothesis that under resting conditions volitional hyperpnoea results in smaller increases in $[lac^-]_B$ compared to when volitional hyperpnoea is superimposed on exercise.

Therefore, the aim of the present study was to examine changes in $[lac^-]_B$ when $\dot{V}_E$ max and the associated breathing pattern is mimicked voluntarily under resting conditions. It was hypothesised that mimicking the ventilatory response to maximal exercise under resting conditions would result in an increase in $[lac^-]_B$, but that the magnitude of increase would be smaller than that observed when volitional hyperpnoea was superimposed on exercise.

7.2 METHODS

7.2.1 PARTICIPANTS

Following local ethics approval and written informed consent, 12 non-smoking, recreationally active subjects (1 female) were recruited. The age, height, and body mass of the subjects were 25.1 (4.7) years, 173.9 (9.0) cm, and 76.3 (7.7) kg, respectively. Subjects were instructed not to partake in strenuous exercise the day before an exercise test. On test days, subjects were asked to refrain from taking any caffeine containing substances, and were instructed not to eat or ingest any substance of nutritional value during the 2 h prior to testing. Tests for each subject were performed at a similar time of day so to minimise diurnal fluctuation effects.

7.2.2 EXPERIMENTAL DESIGN

Initially, pulmonary and respiratory muscle function tests were performed. Subsequently, subjects performed a maximal incremental exercise test on an electromagnetically-braked cycle ergometer (see section 2.1) to establish $\dot{V}O_2$ peak and $\dot{V}_E$ max. Then on a subsequent day changes in $[lac^-]_B$ were assessed whilst subjects

mimicked, under isocapnic conditions and while seated on the cycle ergometer, $\dot{V}_E$ max and the associated breathing pattern for a duration of 10 min.

7.2.3 MEASUREMENTS

Pulmonary function, MIP, and MEP were assessed in accordance with the procedures outlined in section 2.5. Arterialised venous blood was drawn from a superficial dorsal hand vein via an indwelling cannula for the determination of $[lac^-]_B$ and blood-gas variables, as described in section 2.6. Note, however, that because body core temperature was not expected to change from rest (37° C) in this study blood-gas parameters were not temperature corrected. Actual plasma bicarbonate concentration ($[HCO_3^-]$) and base excess of the extracellular fluid (BE_{ECF}) were calculated as described in section 6.2.3.

Respiratory variables were measured during exercise and voluntary hyperpnoea using an on-line breath-by-breath respiratory system (see section 2.2). Note, however, that a two-way non-rebreathing valve (model 2730, Hans Rudolph, Kansas City, Missouri) was connected distally to the turbine flowmeter.

7.2.4 MAXIMAL EXERCISE TEST

Subjects performed an incremental cycling test to the limit of exercise tolerance. Cycling power was increased, starting from 0 W, every 15 s by a suitable increment so to elicit exercise intolerance within approximately 10 min. Subjects were instructed to exercise for as long as possible or exercise was terminated when cycling cadence could not be maintained above 60 rpm. The power output at which exercise intolerance ensued defined $\dot{W}$ max, and $\dot{V}O_2$ peak and $\dot{V}_E$ max were taken as the highest value averaged over any 30 s period.

7.2.5 VOLITIONAL HYPERPNOEA AT REST

Subjects performed the volitional hyperpnoea trial whilst seated on the cycle ergometer in a position identical to that adopted during the maximal exercise test. This was done so that any use of the arms during exercise hyperpnoea was mimicked and so that body position differences would have limited effect on the results. Following 3 min of rest, subjects were instructed to gradually increase and attempt to maintain $\dot{V}_E$ as close as possible to $\dot{V}_E$ max for 10 min (subjects visually inspected the computer-generated, breath-by-breath plot of $\dot{V}_E$). A metronome was set to double the f_R associated with $\dot{V}_E$ max, thus establishing similar inspiratory and expiratory durations. Note that technical difficulties prevented $\dot{V}O_2$ being measured at rest, thus a resting $\dot{V}O_2$ of 3.5 $ml \cdot kg^{-1} \cdot min^{-1}$ was assumed for each subject (ACSM 2000). Isocapnia was maintained according to the procedures outlined in section 6.2.6. Blood samples were taken every 2 min from 0-10 min, inclusive.

7.2.6 STATISTICAL ANALYSES

Values derived during maximal exercise and volitional hyperpnoea were compared using paired t-tests. A one-way ANOVA established changes over time during volitional hyperpnoea. A significant F ratio was followed by Tukey's HSD post-hoc analysis. Pearson's product moment correlation coefficient (r) was calculated to determine the degree of association between selected variables. The critical significance level was set at $P < 0.05$. Results are presented as means (1 SD) unless otherwise indicated. Statistical analyses were performed using the 12.0 release version of SPSS for Windows (SPSS Inc., Chicago, IL, USA).

7.3 RESULTS

Resting pulmonary and respiratory muscle function are presented in Table 7.1. All values were within normal limits.

Table 7.1 Resting pulmonary and respiratory muscle function. Mean (SD).

Variable	Measurement	% Predicted value
FVC (l)	5.29 (0.64)	109 (8)
FEV_1 (l)	4.31 (0.62)	106 (9)
FEV_1/FVC (%)	81.7 (8.0)	101 (10)
PEF ($l \cdot s^{-1}$)	9.7 (1.5)	97.9 (12.2)
PIF ($l \cdot s^{-1}$)	8.7 (1.6)	98.8 (14.8)
MVV_{12} ($l \cdot min^{-1}$)	172.2 (23.0)	117.4 (15.5)
MIP (-cmH_2O)	146 (31)	126 (30)
MEP (cmH_2O)	179 (50)	114 (32)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF and PIF, peak expiratory and inspiratory flow, respectively; MVV_{12}, maximal voluntary ventilation in 12 s; MIP and MEP, maximal inspiratory and expiratory mouth pressure, respectively. Normative values for pulmonary and respiratory muscle function were taken from Cotes (1993) and Wilson et al. (1984), respectively.

7.3.1 VENTILATORY RESPONSES TO MAXIMAL EXERCISE AND VOLITIONAL HYPERPNOEA

$\dot{W}$ max and $\dot{V}O_2$ peak were 338 (63) W (range 240-430 W) and 3.67 (0.62) $l \cdot min^{-1}$ (range 2.69-4.8 $l \cdot min^{-1}$), respectively. $\dot{V}_E$ max and the associated f_R, V_T, and duty cycle are shown in Table 7.2. There was considerable inter-subject variation in $\dot{V}_E$ max, which ranged from 100.5-215.0 $l \cdot min^{-1}$. On average, $\dot{V}_E$ max represented 81% (12) of MVV_{12}. The mean ventilatory response to volitional hyperpnoea is also given in Table 7.2, whereas changes over time are shown in Figure 7.1. Most subjects were unable to maintain $\dot{V}_E$ max

throughout volitional hyperpnoea, thus due to a significantly ($P < 0.05$) lower V_T the mean $\dot{V}_E$ during volitional hyperpnoea was significantly ($P < 0.01$) lower than $\dot{V}_E$ max.

Table 7.2 Ventilatory response to maximal exercise and volitional hyperpnoea. Mean (SD).

Variable	Maximal exercise	Volitional hyperpnoea
$\dot{V}_E$ ($l \cdot min^{-1}$)	154.0 (33.0)	139.4 (27.4)**
f_R ($breaths \cdot min^{-1}$)	53.5 (13.4)	55.0 (9.7)
V_T (l)	2.97 (0.61)	2.59 (0.52)*
T_i/T_{tot}	0.52 (0.03)	0.51 (0.05)

$\dot{V}_E$, minute ventilation; f_R, respiratory frequency; V_T, tidal volume; T_i/T_{tot}, inspiratory time/total breath time (duty cycle). *Significant difference from maximal exercise ($P < 0.05$). **Significant difference from maximal exercise ($P < 0.01$).

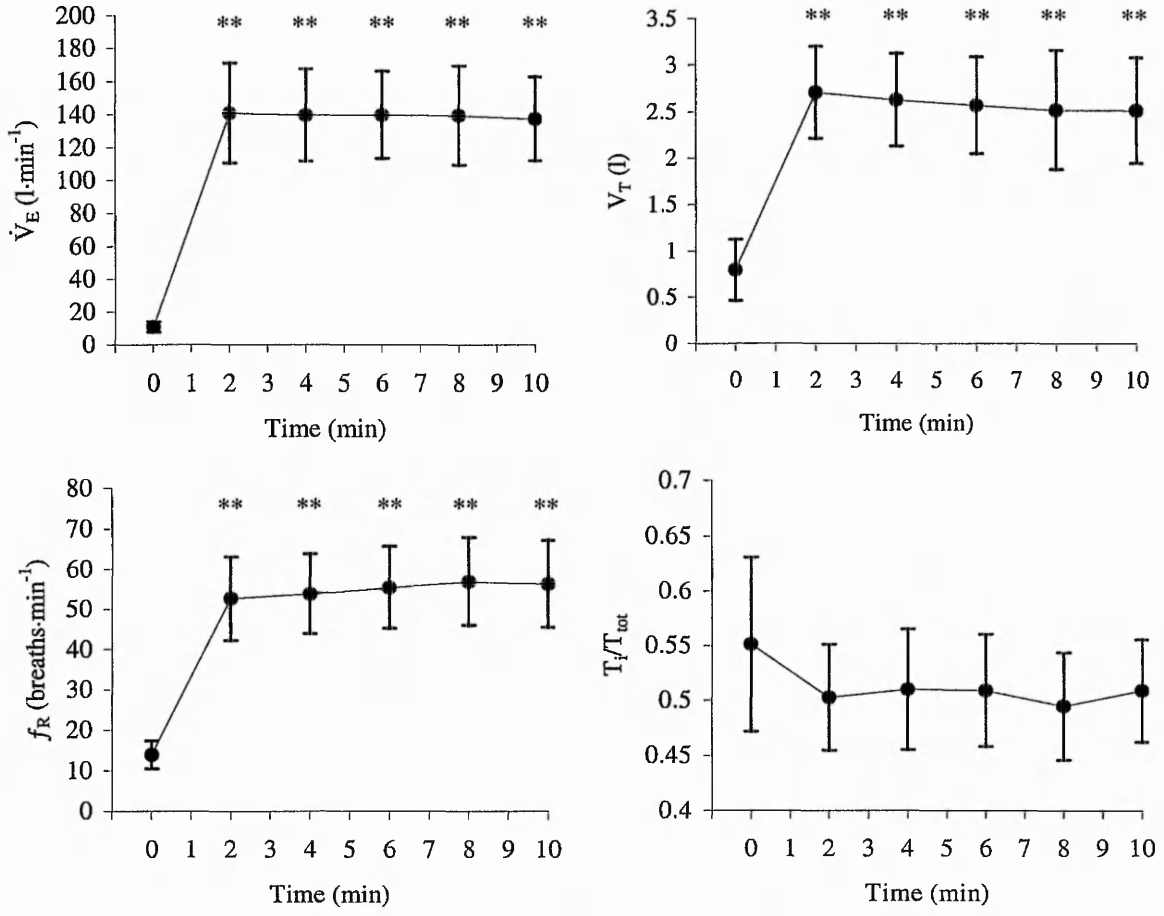

Figure 7.1 Ventilatory response to 10 min of volitional hyperpnoea. $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_R, respiratory frequency; T_i/T_{tot}, inspiratory time/total breath time (duty cycle). Values are mean (SD). **Significant difference from rest ($P < 0.01$).

7.3.2 PULMONARY GAS EXCHANGE

Changes in $\dot{V}O_2$ and $ETCO_2$ are shown in Figure 7.2. $ETCO_2$ was not different from rest throughout volitional hyperpnoea. Conversely, $\dot{V}O_2$ was significantly ($P < 0.01$) increased above rest throughout volitional hyperpnoea and by 10 min $\dot{V}O_2$ was 1.09 (0.36) $l \cdot min^{-1}$, which represented a 0.82 (0.35) $l \cdot min^{-1}$ increase above baseline. Despite the constancy of $\dot{V}_E$ during volitional hyperpnoea (Figure 7.1), the oxygen cost of volitional hyperpnoea increased significantly ($P < 0.01$) from 4.54 (0.95) ml O_2 per $l \cdot min^{-1}$ of $\dot{V}_E$ following 2 min to 6.68 (2.93) ml O_2 per $l \cdot min^{-1}$ of $\dot{V}_E$ following 10 min.

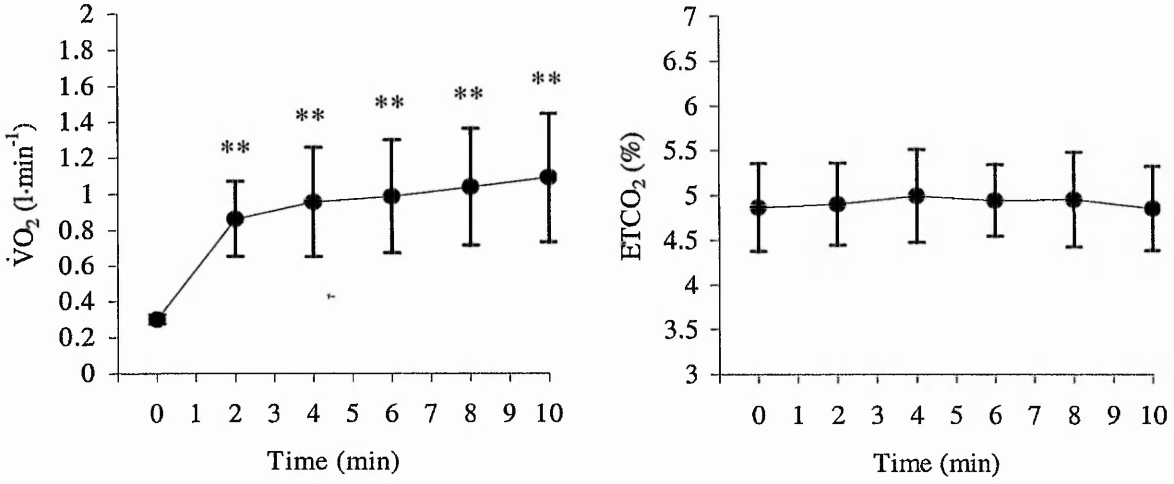

Figure 7.2 Changes in pulmonary oxygen consumption ($\dot{V}O_2$) and end-tidal CO_2 ($ETCO_2$) during volitional hyperpnoea. Values are mean (SD). **Significant difference from rest ($P < 0.01$).

7.3.3 BLOOD PARAMETERS

Individual changes in $[lac^-]_B$ following 10 min of volitional hyperpnoea are shown in Figure 7.3 and group mean changes in all blood parameters throughout volitional hyperpnoea are shown in Figure 7.4. There was a gradual increase in $[lac^-]_B$ during volitional hyperpnoea and a significant ($P < 0.01$) 0.59 (0.54) $mmol \cdot l^{-1}$ increase from rest was recorded after 6 min. Following 10 min of volitional hyperpnoea $[lac^-]_B$ had increased by 0.77 (0.65) $mmol \cdot l^{-1}$ ($P < 0.01$). However, there was considerable inter-subject variation

in the $[lac^-]_B$ response to volitional hyperpnoea (Figure 7.3), in that the absolute increase ranged from 0-2.0 $mmol \cdot l^{-1}$.

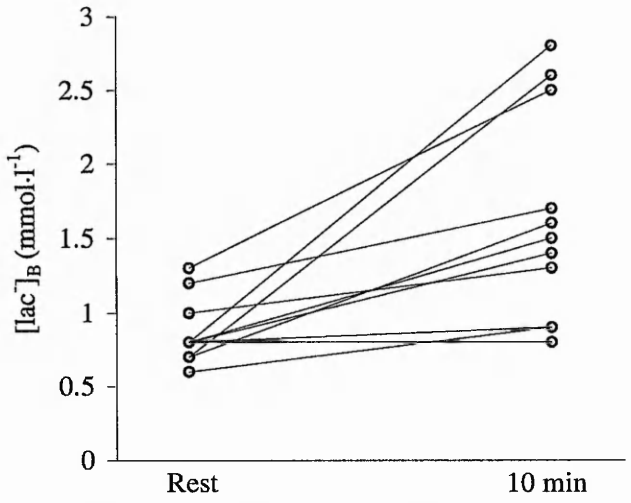

Figure 7.3 Individual blood lactate concentrations ($[lac^-]_B$) at rest and following 10 min of volitional hyperpnoea.

There were no changes over time in $PaCO_2$, pH, or $[H^+]$. There was a significant ($P < 0.05$) 0.80 (1.1) $mmol \cdot l^{-1}$ increase in $[HCO_3^-]$ during the first 2 min of volitional hyperpnoea, after which values were not different from rest. The increased $[HCO_3^-]$ during the first 2 min of volitional hyperpnoea was, expectedly, mirrored by similar changes in BE_{ECF}, which increased significantly ($P < 0.01$) by 0.92 (0.81) $mEq \cdot l^{-1}$ after 2 min. After 4 min of volitional hyperpnoea BE_{ECF} had declined slightly but was still 0.65 (0.73) $mEq \cdot l^{-1}$ above rest ($P < 0.05$); thereafter, values were not different from rest.

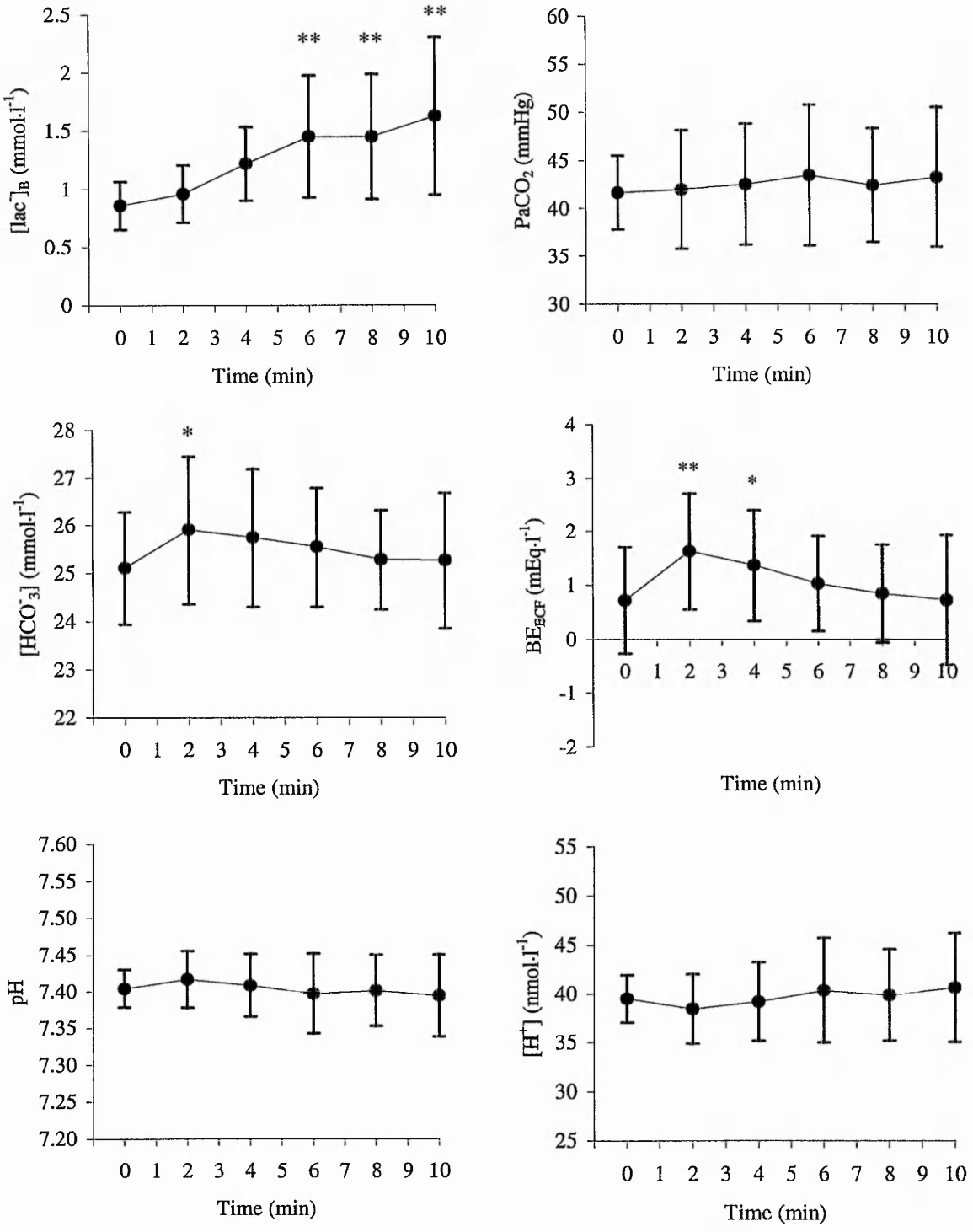

Figure 7.4 Changes in blood lactate concentration ($[lac^-]_B$) and blood acid-base balance during volitional hyperpnoea. Values are mean (SD). $PaCO_2$, partial pressure of carbon dioxide in arterialised venous blood; $[HCO_3^-]$, plasma bicarbonate concentration; BE_{ECF}, base excess of the extracellular fluid; $[H^+]$, hydrogen ion concentration. *Significant difference from rest ($P < 0.05$). **Significant difference from rest ($P < 0.01$).

7.3.4 CORRELATIONS BETWEEN VARIABLES

Absolute changes in $[lac^-]_B$ were not correlated with the $\dot{V}_E$ achieved during volitional hyperpnoea, whether expressed as an absolute value or relative to MVV_{12}. Changes were also not correlated with any index of pulmonary function or endurance training status ($\dot{V}O_2$peak and $\dot{W}$max). However, absolute changes in $[lac^-]_B$ during volitional hyperpnoea were significantly correlated, positively, with MIP ($r = 0.59$, $P < 0.05$), the mean f_R ($r = 0.67$, $P < 0.05$) and $\dot{V}O_2$ ($r = 0.78$, $P < 0.01$) achieved during volitional hyperpnoea, the mean oxygen cost of the volitional hyperpnoea ($r = 0.64$, $P < 0.05$), and also the absolute increase of $\dot{V}O_2$ over 3-10 min of hyperpnoea ($r = 0.69$, $P < 0.05$).

7.4 DISCUSSION

The primary finding of this study was that $[lac^-]_B$ increased significantly by, on average, 0.77 $mmol \cdot l^{-1}$ when $\dot{V}_E$max and the associated breathing pattern were mimicked voluntarily under resting conditions. Because these changes were not accompanied by changes in $PaCO_2$, the increased $[lac^-]_B$ can be attributed, primarily, to lactate production and efflux from active respiratory muscles. Therefore, these results provide evidence to suggest that at least some of the increase in $[lac^-]_B$ when volitional hyperpnoea is superimposed on heavy exercise (chapter 6) results from lactate production and efflux from respiratory muscles.

The 0.77 $mmol \cdot l^{-1}$ increase in $[lac^-]_B$ during 10 min of volitional hyperpnoea in the present study exceeds the 0.5 $mmol \cdot l^{-1}$ increase reported by Martin et al. (1984), who also had subjects mimic under resting conditions $\dot{V}_E$max and the associated breathing pattern. This difference is not likely to be explained by different levels of $\dot{V}_E$, which were almost

identical in the two studies (139 $l \cdot min^{-1}$ in the present study vs. 138 $l \cdot min^{-1}$ in Martin et al's study). However, a key factor in this discrepancy appears to be the duration of volitional hyperpnoea used (10 min in the present study vs. 5 min in Martin et al.'s study). In support, the increase in $[lac^-]_B$ during the first 5 min of volitional hyperpnoea in the present study was identical [0.5 (0.5) $mmol \cdot l^{-1}$] to that reported by Martin et al. (1984). In another study, Freedman et al. (1983) observed a 1.1 $mmol \cdot l^{-1}$ increase in $[lac^-]_B$ in subjects performing 10 min of maximal voluntary hyperpnoea. However, these authors did not match the breathing pattern with that associated with the spontaneous exercise hyperpnoea, and subjects performed voluntary hyperpnoea whilst semi-recumbent. These factors limit the external validity of the findings and the extent to which comparisons can be made with the present study.

In the preceding chapter it was argued that because of a limited reserve to counter further lactate appearance, the influence of $\dot{V}_E$max on $[lac^-]_B$ would be more discernable when superimposed on heavy whole-body exercise compared to when $\dot{V}_E$max was reproduced under resting conditions. Although the use of different subjects precludes direct comparisons being made between this study and that of the preceding chapter, several observations lend credence to this paradigm. Firstly, note that the duration of volitional hyperpnoea in the present study (10 min) was 2 min greater than that used in the preceding chapter. When the data from the present study were analysed over the first 8 min of volitional hyperpnoea, the increase in $[lac^-]_B$ was 0.59 (0.51) $mmol \cdot l^{-1}$, somewhat less than the 0.81 (0.57) $mmol \cdot l^{-1}$ increase observed following 8 min of volitional hyperpnoea superimposed on exercise. Moreover, when voluntary hyperpnoea was superimposed on exercise an increase in $[lac^-]_B$ of more than 0.3 $mmol \cdot l^{-1}$ was observed following 8 min in all but one subject, in whom an increase of exactly 0.3 $mmol \cdot l^{-1}$ was observed. Conversely, in the present study 4 subjects demonstrated an increased $[lac^-]_B$ during voluntary

hyperpnoea of 0.3 $mmol \cdot l^{-1}$ or less (1 subject showing no increase at all). Another important difference between the two studies is that whereas in the present study isocapnia was maintained throughout volitional hyperpnoea, in the preceding chapter voluntary hyperpnoea was performed in the presence of a respiratory acidosis, which may have resulted in an underestimate of the increase in $[lac^-]_B$ (Ehrsam et al. 1982; Graham et al. 1986; Graham and Wilson 1983; Graham et al. 1982). Thus it would seem that the influence of additional respiratory muscle work on $[lac^-]_B$ is indeed more profound when superimposed on exercise in which there is limited reserve to counter further lactate appearance

A comparison of changes in acid-base balance during volitional hyperpnoea at rest and whilst simultaneously performing heavy exercise lends further credence to the argument that greater metabolic perturbations transpire under the latter condition. Specifically, when volitional hyperpnoea was superimposed on exercise at maximal lactate steady state an inexorable metabolic acidosis (i.e. increased $[H^+]$) was manifest, thus suggesting an imbalance between the rate of proton production and the rate of proton buffering and removal. Conversely, despite an increase in $[lac^-]_B$ during volitional hyperpnoea in the present study $[H^+]$ remained unchanged from rest, which suggests that the rate of proton production was matched by the rate of proton removal and buffering. That $[H^+]$ remained unchanged in the present study despite an increase in $[lac^-]_B$ is perhaps explained by the greater number of membrane transport mechanisms for H^+ compared to lactate (Juel 1998). Importantly, however, it is attractive to speculate that if most of the proton accumulation was removed from the systemic circulation in the present study during volitional hyperpnoea, then some of this removal may have been coupled with lactate removal if lactate-proton cotransport via the monocarboxylate transporters was the mechanism through which protons were transported into skeletal muscle (Juel 2001).

Taken together, the collective findings of the present and preceding chapter suggest that caution is warranted when inferences concerning responses to whole-body exercise are made from loaded breathing protocols performed under resting conditions.

Note, however, that it is also possible that the different $[lac^-]_B$ responses observed in the present study and the preceding chapter may have been partly related to differences in breathing pattern. Specifically, in the preceding chapter subjects adopted a self-selected breathing pattern during volitional hyperpnoea, whereas in the present study breathing pattern was matched to $\dot{V}_E$ max. Consequently, f_R during volitional hyperpnoea in the preceding chapter [69.1 (SEM 7.9) breaths·min^{-1}] was higher than that observed in the present study [55.0 (SD 9.7) breaths·min^{-1}]. This may have also contributed to the greater increase in $[lac^-]_B$ when volitional hyperpnoea was superimposed on exercise since in the present study a significant correlation was found between f_R during volitional hyperpnoea and increases in $[lac^-]_B$.

In the present study the increase in $[lac^-]_B$ during volitional hyperpnoea ranged from 0-2.0 mmol·l^{-1}. This observation is consistent with previous reports of large inter-subject variation in the $[lac^-]_B$ response to voluntary hyperpnoea under resting conditions (Freedman et al. 1983; Martin et al. 1984). However, since only systemic $[lac^-]_B$ was measured in these studies (including the present study), it is not possible to identify whether the inter-subject variation results from different rates of lactate production and release from respiratory muscles, different rates of lactate clearance, or a combination of these processes. A potential mechanism explaining inter-subject differences in lactate clearance by inactive skeletal muscle may reside in the respiratory muscle metaboreflex that is activated under resting conditions when the respiratory muscles reach a threshold of force output sufficient to cause fatigue (reviewed in Dempsey et al. 2002). The subsequent reduction in limb blood flow might compromise lactate uptake by reducing the extra-to-

intramuscular lactate concentration gradient. Although respiratory muscle fatigue was not assessed in this study, several subjects reported a transient abdominal pain in the right lumbar region, which was perhaps caused by diaphragmatic ischaemia (Morton and Callister 2000). Note also that in a previous study just 2 min of maximal isocapnic hyperpnoea was sufficient to cause a 22% drop in twitch Pdi during phrenic nerve stimulation (Luo et al. 2001). Respiratory muscle fatigue would also provide a logical explanation for the inability of most subjects to maintain $\dot{V}_E$ at $\dot{V}_E$max. Therefore, part of the inter-subject variation in the $[lac^-]_B$ response to volitional hyperpnoea may result from inter-subject differences in rates of lactate clearance at peripheral sites due to changes in cardiac output distribution, which in turn might be partly dependent upon the severity of respiratory muscle fatigue.

However, given the considerable lactate clearance capacity that exists under resting conditions it seems inconceivable that the inter-subject variation in the $[lac^-]_B$ response to volitional hyperpnoea does not result from, primarily, different rates of lactate production and release from active respiratory muscles. However, we can only speculate upon why these differences exist. Increases in $[lac^-]_B$ during volitional hyperpnoea were positively correlated with MIP, and there was also a trend for increases in $[lac^-]_B$ to be correlated with both the mean inspiratory flow rate during hyperpnoea ($r = 0.51$, $P = 0.089$) and baseline PIF ($r = 0.57$, $P = 0.052$). Because MIP is proportional to maximal inspiratory muscle force generation, and inspiratory flow rates are proportional to muscle shortening velocity, these indices may give some indication of the morphology and/or fibre type recruitment of the inspiratory muscles, since force and velocity of muscle shortening are partly dependent upon muscle fibre type composition. Compared to type I fibres type II fibres possess greater capacity to generate force, shorten more quickly (Åstrand et al. 2003), and achieve higher glycolytic, and thus lactate production, rates (Brooks 1986). Therefore, although

speculative it is possible that subjects demonstrating the highest MIP's and inspiratory flow rates possessed and/or recruited a greater proportion of type II fibres during volitional hyperpnoea, subsequently resulting in greater rates of lactate production.

In addition to their distinct contractile properties, metabolic differences can also characterise different muscle fibre types. Type II fibres are less efficient energetically, having a greater $\dot{V}O_2$ for any given rate of ATP resynthesis compared to type I fibres (Gaesser and Poole 1996). In the present study, a significant relationship was noted between increases in $[lac^-]_B$ and the increase in $\dot{V}O_2$ over 3-10 min, which can be taken as a close estimate of the $\dot{V}O_2$ slow component amplitude (Gaesser and Poole 1996; Roston et al. 1987), i.e. the continued rise in $\dot{V}O_2$ that follows the initial exponential $\dot{V}O_2$ response to muscular work performed above critical power (Gaesser and Poole 1996). This finding is consistent with previous studies in which increases in $[lac^-]_B$ during heavy whole-body exercise were correlated ($r = 0.64–0.86$) with the amplitude of the $\dot{V}O_2$ slow component (Barstow et al. 1996; Roston et al. 1987). Although lactate per se is not thought to cause the $\dot{V}O_2$ slow component (Poole et al. 1996), the association between increases in $[lac^-]_B$ and the slow component amplitude is suggestive of increased recruitment of type II muscle fibres. In support, Barstow et al. (1996) and Pringle et al. (2003) compared subjects with a range of type I and type II muscle fibres of vastus lateralis, and found that subjects demonstrating the greatest slow component during whole-body exercise also possessed a greater proportion of type II fibres. Therefore, it is possible that the positive relationship between increases in $[lac^-]_B$ during volitional hyperpnoea and the increase in $\dot{V}O_2$ over 3-10 min (i.e. the slow component amplitude) reflected, in part, inter-subject differences in respiratory muscle fibre types and/or motor unit recruitment patterns.

Although it has been argued that the respiratory muscles were the primary source of the increased $[lac^-]_B$ during volitional hyperpnoea in the present study, it is also possible that tonic activation of non-respiratory muscles also influenced systemic lactate kinetics. For example, to aid stabilisation of the thorax during volitional hyperpnoea subjects may have intuitively increased their grip on the handlebars, thus increasing forearm muscle activity and subsequently lactate production (Catcheside and Scroop 1993).

Consistent with the assertion that the $\dot{V}O_2$ slow component represents an increasing metabolic inefficiency as exercise proceeds at a constant-load, there was a significant increase in the oxygen cost of breathing (at constant $\dot{V}_E$) as volitional hyperpnoea progressed in the present study. This finding supports the notion of Carra et al. (2003), who argue that the respiratory muscles behave like locomotor muscles when working under load, i.e. there is a progressive decrease in muscular efficiency. Metabolic inefficiency is likely to occur whenever a physiological system is operating above its critical power (Gaesser and Poole 1996), as the respiratory muscles probably were in the present study. The decline in breathing efficiency in the present study may have resulted from increased recruitment of less efficient type II muscle fibres (Barstow et al. 1996; Pringle et al. 2003), perhaps because type I fibres became progressively fatigued. However, the metabolic cost of hyperpnoea may have also increased due to increased recruitment of less efficient accessory rib cage muscles (DiMarco et al. 2004; Hart et al. 2002), increased end-expiratory lung volume, expiratory pressure development beyond the maximum effective pressure, and chest wall distortion (Aaron et al. 1992; Collett and Engel 1986; Klas and Dempsey 1989). Moreover, because only $\dot{V}_E$max and the associated breathing pattern were mimicked in the present study, such alterations in breathing mechanics may have been accentuated, subsequently resulting in an increased work of breathing. Support for this notion is provided by the observation that the mean oxygen cost

of breathing in the present study was approximately twofold greater than that reported by Aaron et al. (1992), who had subjects mimic the respiratory muscle recruitment patterns and the flow-volume and pressure-volume characteristics of the spontaneous maximal exercise hyperpnoea. The possibility therefore exists that smaller increases in $[lac^-]_B$ may have been observed in the present study had all elements of breathing and respiratory muscle recruitment pattern been controlled.

The observation of a significant influence of respiratory muscle work on systemic $[lac^-]_B$ is consistent with several studies of respiratory muscle training (RMT), in which $[lac^-]_B$ during whole-body exercise is attenuated post-RMT (reviewed in McConnell and Romer 2004a; McConnell and Sharpe 2005). However, whereas in the present study increases in $[lac^-]_B$ can be largely attributed to lactate efflux from active respiratory muscles, it is not possible to identify whether reduced $[lac^-]_B$ following RMT results from altered lactate kinetics in respiratory muscles, active locomotor muscles and/or other metabolically active tissues, or a combination of these. Volitional hyperpnoea under resting conditions may thus offer a useful model with which to explore the, hitherto ambiguous, mechanisms by which RMT reduces $[lac^-]_B$. Assessing the $[lac^-]_B$ response to volitional hyperpnoea pre- and post-RMT would perhaps shed light on whether the respiratory muscles make a direct contribution (reduced lactate efflux or increased lactate clearance), or an indirect contribution (associated with changes in cardiac output distribution), to the lowered $[lac^-]_B$ during exercise post-RMT.

In conclusion, mimicking $\dot{V}_E$max and the associated breathing pattern under resting conditions resulted in an increased $[lac^-]_B$ in most, but not all, subjects. These findings suggest that some of the increase in $[lac^-]_B$ when volitional hyperpnoea is superimposed on heavy exercise results from lactate production and efflux from respiratory muscles. However, the results of the present study, together with those of the preceding

chapter, suggest that greater metabolic perturbations result when volitional hyperpnoea is superimposed on heavy exercise compared to when it is performed under resting conditions. There appears to be large inter-subject variation in the $[lac^-]_B$ response to volitional hyperpnoea, which may be related to inter-subject differences in respiratory muscle morphology and/or recruitment patterns of respiratory muscle fibre types.

CHAPTER 8

GENERAL DISCUSSION

8.1 PULMONARY LIMITATIONS TO ENDURANCE EXERCISE

8.1.1 HISTORICAL PERSPECTIVE

Historically, endurance exercise performance was not considered to be limited by the pulmonary system in healthy individuals, let alone athletes. That $\dot{V}_E$ during even maximal exercise was always below the MVV was taken as evidence that the capacity of the respiratory muscles to ventilate the lungs was never exhausted during spontaneous exercise hyperpnoea (Åstrand et al. 2003). It was also unclear whether the combination of the magnitude of respiratory muscle pressure development, respiratory muscle shortening velocity, and frequency of contraction, were sufficient to cause respiratory muscle fatigue during even maximal physiological exercise (Dempsey 1986). Based on the $[lac^-]_B$ response of healthy humans and animals performing loaded breathing at rest the respiratory muscles were considered to be highly resistant to anaerobic metabolism and likely to consume rather than produce lactate (Eldridge 1966; Rochester and Briscoe 1979). An early study of breathing mechanics during exercise in healthy untrained subjects demonstrated that the lung architecture was adequate to accommodate the airflow rates achieved during even maximal exercise (Olafsson and Hyat 1969). Moreover, that even healthy untrained subjects maintained SaO_2 near resting levels during maximal exercise was taken as evidence that the lungs' diffusion capacity was more than adequate (Dempsey 1986). Accordingly, the healthy pulmonary system was commonly held as being somewhat "overbuilt" with respect to the demands placed upon it during even maximal exercise, and the cardiovascular and skeletal muscle systems were outlined as the main physiological determinants of endurance exercise performance. However, our knowledge of respiratory exercise physiology has advanced considerably over the last 20 years and new perspectives have emerged.

8.1.2 EXERCISE-INDUCED ARTERIAL HYPOXAEMIA

Trained endurance athletes are an exception to the view that the structural capacities of the lung are capable of meeting the demands for pulmonary oxygen transport. Exercise-induced arterial hypoxaemia (EIAH) is observed in approximately 50% of young, adult, endurance trained male athletes performing moderate-through-maximal exercise (Dempsey and Wagner 1999; Prefaut et al. 2000). EIAH may occur because trained endurance athletes have undergone adaptation in the cardiovascular and skeletal muscle systems but not in the structural elements responsible for pulmonary gas exchange (Dempsey 1986). In females EIAH occurs at a lower percentage of $\dot{V}O_2$ peak compared to height and age matched males (the prevalence is therefore higher in females), which might be related, in part, to their anatomically smaller lungs (including airways) compared to males (Harms et al. 1998a; Sheel et al. 2004).

The severity of EIAH correlates most closely and inversely with the $P(A\text{-}a)O_2$, which indicates an abnormality in gas exchange across the alveolar-capillary matrix (Dempsey and Wagner 1999). However, many athletes exhibiting EIAH also demonstrate a relative hypoventilation, which lowers PAO_2 and further widens the $P(A\text{-}a)O_2$ (Dempsey et al. 1984; Harms and Stager 1995). Inadequate hyperventilation during exercise may be related to a low chemoresponsiveness (Harms and Stager 1995), and/or a mechanical constraint on hyperpnoea due to expiratory flow limitation, although the latter would only occur during heavy exercise (Dempsey and Wagner 1999).

EIAH may compromise maximal oxygen transport, as evidenced by the increase in $\dot{V}O_2$ peak when SaO_2 is restored to resting values using small increases in inspired oxygen concentration (Harms et al. 2000). The severity of quadriceps muscle fatigue, as assessed using magnetic stimulation of the femoral nerve, and ratings of limb discomfort and dyspnoea, are also attenuated when EIAH is prevented during high-intensity exercise by

using increases in inspired oxygen concentration (Romer et al. 2006). Furthermore, these changes are likely to contribute to enhanced and impaired exercise endurance when EIAH is prevented and exacerbated, respectively (Koskolou and McKenzie 1994; Nielsen et al. 1999; Nielsen et al. 2002; Romer et al. 2006). These findings thus provide evidence that during high-intensity exercise the lung may limit oxygen transport and subsequently exercise performance.

8.1.3 Mechanical constraints on exercise hyperpnoea

Although trained endurance athletes achieve higher levels of $\dot{V}_E$ compared to sedentary or recreationally active individuals, the maximal flow-volume loop is insensitive to training (Johnson et al. 1992). Consequently, during heavy exercise expiratory flow limitation develops. This effectively constrains exercise hyperpnoea and results in an increase in end-expiratory lung volume, which subsequently increases the elastic load on the inspiratory muscles (Johnson et al. 1992; McClaran et al. 1999). Importantly, because expiratory flow limitation, along with EIAH, are phenomena primarily observed in highly-trained endurance athletes, this suggests that the incapacity of the lung to adapt structurally to whole-body endurance training may result in the primary limitation to oxygen transport and performance shifting from the skeletal muscle and cardiovascular systems to the pulmonary system (Dempsey 1986).

8.1.4 Exercise-induced respiratory muscle fatigue

Chapters 4 and 5 show that time-trial and constant power cycling exercise can cause significant reductions in MIP. These findings support a body of evidence showing the inspiratory muscles of even highly trained endurance athletes to be susceptible to fatigue during heavy exercise (see section 1.4.5). The findings of chapters 4 and 5 lend

credence to the notion that baseline MIP partly determines the extent with which MIP declines following heavy exercise (McConnell et al. 1997), since from a baseline MIP of around 140-150% of the predicted value exercise-induced decrements in MIP were typically less than 10%. In comparison, trained cyclists demonstrating baseline MIP's close to predicted values experienced an 18% reduction in MIP following a 20 km time-trial (Romer et al. 2002a). Inter-subject differences in baseline MIP are probably not explained by differences in endurance training status, height, nor body mass (Coast et al. 1990; McConnell et al. 1997; McCool et al. 1997; Robinson and Kjeldgaard 1982). Thus alternatively, differences in baseline MIP might be explained, in part, by normal variation (genetic predisposition) in the muscularity of the inspiratory muscle pump (McCool et al. 1997).

Johnson et al. (1993) showed that the severity of exercise-induced diaphragm fatigue increases with the exercise intensity. However, in chapter 5 exercise-induced decrements in MIP were greater following the lower intensity, more prolonged (10-30 min) constant power exercise bouts compared to the shorter (<10 min), higher intensity exercise bouts. Note, however, that MIP probably reflects, primarily, the strength of the inspiratory rib cage muscles (McCool et al. 1992). Therefore, lower exercise intensities may spare diaphragm fatigue, but at the expense of greater inspiratory rib cage muscle fatigue. This pattern of fatigue is consistent with the pattern of inspiratory muscle recruitment during heavy exercise. Specifically, as heavy endurance exercise progresses the inspiratory rib cage muscles make a progressively greater, and the diaphragm a progressively smaller, contribution to the total respiratory muscle pressure output (Johnson et al. 1993); this may thus increase the likelihood of inspiratory rib cage muscle fatigue during prolonged exercise bouts.

It is not known whether the inspiratory muscle fatigue observed in chapters 4 and 5 had any serious implications for exercise performance. There are probably two possible ways human performance may be limited by inspiratory muscle fatigue: (I) through compromising leg blood flow subsequent to respiratory muscle metaboreflex activation (Harms et al. 1997), and (II) by an increase in dyspnoea. If fatiguing respiratory muscles "steal" blood flow from locomotor muscles during exercise then this could accentuate locomotor muscle fatigue (Romer et al. 2004) and the perceptual response to exercise, subsequently reducing exercise tolerance (Harms et al. 2000). However, respiratory muscle metaboreflex activation may only occur during high-intensity or maximal exercise (Harms et al. 1997; Wetter et al. 1999), thus whether this phenomenon has any bearing on, for example, prolonged (>30 min) time-trial exercise performance is uncertain. On the other hand, inspiratory muscle fatigue may develop during even submaximal exercise and contribute to increased dyspnoea. This is crucial since exercise intensity during time-trial exercise, and exercise tolerance during constant-load exercise, is largely governed by the overall central perception of exercise exertion, to which dyspnoea is likely to make a significant contribution. Inspiratory muscle fatigue would exacerbate dyspnoea because to achieve the same mechanical output a greater motor command would be required, which may result in greater corollary discharge from respiratory neurones to the sensory cortex and thus an increased awareness of respiratory discomfort (El-Manshawi et al. 1986).

8.2 Respiratory muscle energetics during exercise

8.2.1 Physiological cost of exercise hyperpnoea

Aaron et al. (1992) estimated the oxygen cost of maximal exercise $\dot{V}_E$ at approximately 3.0 ml O_2 per $l \cdot min^{-1}$ of $\dot{V}_E$, thus in some highly trained endurance athletes the oxygen cost of maximal exercise hyperpnoea can equate to 15% of $\dot{V}O_2$ peak. These

demands are met through a substantial increase in respiratory muscle perfusion that may equate to 16% of the available cardiac output (Harms et al. 1998b). The oxygen cost of hyperpnoea in chapters 6 and 7 of this thesis ($\geq$4.30 ml O_2 per l·min^{-1} of $\dot{V}_E$) was greater than that reported by Aaron et al. (1992), probably because volitional hyperpnoea is less efficient than spontaneous hyperpnoea (reviewed in section 1.3).

During volitional hyperpnoea under resting conditions (chapter 7) a progressive increase in $\dot{V}O_2$ was recorded over 3-10 min, the magnitude of which can be taken to represent the $\dot{V}O_2$ slow component amplitude (Gaesser and Poole 1996; Roston et al. 1987). The respiratory muscles, like locomotor muscles, thus demonstrate a progressive decrease in muscular efficiency when working above a certain threshold, or critical power. Therefore, during exercise in which the respiratory muscles are working above their critical power, they are likely to contribute to the $\dot{V}O_2$ slow component, not only because of an increasing $\dot{V}_E$ and thus respiratory muscle $\dot{V}O_2$ (Gaesser and Poole 1996), but because the respiratory muscles, like the locomotor muscles, are also experiencing a decrease in muscular efficiency.

8.2.2 New perspectives on lactate kinetics of the respiratory muscles

Empirical studies suggest that the respiratory muscles do not produce lactate during exercise (Fregosi and Dempsey 1986; Manohar et al. 1988; Manohar and Hassan 1991; Manohar and Hassan 1990). However, these studies were performed on quadruped animals, thus their findings have limited relevancy to the exercising human. Moreover, the belief that the respiratory muscles do not produce lactate is at odds with the acceptance of respiratory muscle metaboreflex activation during high-intensity exercise in humans (Dempsey et al. 2002; Harms et al. 1997), which suggests metabolite accumulation within the respiratory muscles.

Contrary to that reported in animal studies, chapters 6 and 7 suggest that the respiratory muscles of humans make a significant contribution to the $[lac^-]_B$ of heavy exercise, largely through increased lactate production and release. The effects of additional respiratory muscle work on $[lac^-]_B$ are also more apparent when superimposed on heavy exercise in which there is limited reserve to counter further lactate appearance. Thus accordingly, it seems unlikely that accurate inferences concerning whole-body exercise responses can be made from loaded breathing protocols performed at rest.

8.2.3 PUTATIVE EXPLANATIONS FOR INTER-SUBJECT VARIATION IN THE BLOOD LACTATE RESPONSE TO VOLITIONAL HYPERPNOEA

Large inter-subject variation in the $[lac^-]_B$ response to volitional hyperpnoea has been consistently demonstrated (chapters 6 and 7; Freedman et al. 1983; Martin et al. 1984). A potential explanation for this variability is that increases in $[lac^-]_B$ during volitional hyperpnoea partly depend upon the proportion of maximal respiratory muscle power output utilised (chapter 6; Martin et al. 1984). However, in chapter 7 a significant correlation was found between changes in $[lac^-]_B$ during volitional hyperpnoea at rest and the magnitude of increase in $\dot{V}O_2$ over 3-10 min, i.e. the $\dot{V}O_2$ slow component amplitude. The $\dot{V}O_2$ slow component amplitude during heavy whole-body exercise is also correlated with increases in $[lac^-]_B$ (Barstow et al. 1996; Roston et al. 1987), and in addition, the proportion of type II fibres in vastus lateralis (Barstow et al. 1996; Pringle et al. 2002). Thus it is possible that inter-subject variation in the $[lac^-]_B$ response to volitional hyperpnoea is also causatively linked to differences in respiratory muscle morphology and/or muscle fibre recruitment patterns.

8.2.4 IMPLICATIONS FOR PERFORMANCE

The findings of the present research point towards a significant, hitherto unacknowledged, contribution of the respiratory muscles to inexorable increases in $[lac^-]_B$ and $\dot{V}O_2$, and thus presumably exercise intolerance, during heavy endurance exercise. However, because lactate per se is not likely to limit muscle contractility (Robergs et al. 2004), but instead provide a means of "shuttling" a carbohydrate fuel source between different tissues (Brooks 1986), an increase in $[lac^-]_B$ by itself is not likely to reduce exercise tolerance. On the other hand, lactate accumulation is a good indirect marker for cellular metabolic conditions associated with the evolution of metabolic acidosis (Robergs et al. 2004). Although at physiological temperatures acidosis has little direct effect on muscle function, it may augment the perceptual response to exercise by stimulating group III/IV nerve afferents in muscle (Westerblad et al. 2002). It is also significant that the hydrogen ion may be an important mediator of metaboreflexes from contracting skeletal muscle (Dempsey et al. 2002; Rodman et al. 2003). Therefore, if the increased $[lac^-]_B$ observed in chapters 6 and 7 reflected, in part, lactate production by respiratory muscles and a concomitant exacerbation of acidosis within the interstitium of the respiratory muscles, then during spontaneous heavy exercise this could have implications for respiratory muscle fatigue (Babcock et al. 1995), limb blood flow (Harms et al. 1997) and subsequently exercise tolerance (Harms et al. 2000). Furthermore, because lactate provides a good indirect marker of metabolic conditions, the production of lactate by the respiratory muscles could also suggest increased production of other fatigue-inducing metabolites (e.g. inorganic phosphate), which may not only affect respiratory muscle function, but also the development of locomotor muscle fatigue (Romer et al. 2004; Westerblad et al. 2002).

8.3 INSPIRATORY MUSCLE TRAINING

8.3.1 HISTORICAL PERSPECTIVE

In 1976 Leith and Bradley showed that the respiratory muscles of healthy humans were responsive to both strength and endurance training stimuli. However, these authors doubted that respiratory muscle training could impact on exercise performance. Specifically, Leith and Bradley (1976) wrote: "We see three kinds of applications to be explored, in which ventilatory muscle fatigue may limit human performance and in which prior ventilatory muscle training might then improve performance or at least minimise decrements. The first is in sports, if it turned out that exercise capacity in fit individuals were ever limited by ventilatory muscle endurance. This is not generally thought to be the case". The refutation of the possibility that respiratory muscle training (RMT) might enhance athletic performance was consistent with the belief that the healthy pulmonary system was somewhat "overbuilt".

Following the seminal work of Leith and Bradley (1976) much attention was focused on whether RMT could indeed improve endurance exercise performance, although contradictory findings were consistently reported. However, the controversy that has surrounded the ergogenic potential of RMT can be largely explained by methodological factors (McConnell and Romer 2004a). For example, the present research (chapters 4 and 5) highlights how the choice of performance evaluation test can influence the efficacy of RMT. Thus what was once considered an unnecessary adjunct to regular whole-body endurance training is now accepted, under certain circumstances, as a powerful ergogenic aid to athletic performance (McConnell and Romer 2004a).

8.3.2 Role of baseline inspiratory muscle strength in determining the extent of adaptation with training

In chapters 4 and 5 the 12.6-15.3% improvement in MIP following pressure-threshold inspiratory muscle training (IMT) concurred with the 8% improvement reported by Sonetti et al. (2001), but was considerably less than the 28-45% increase reported in numerous other pressure-threshold IMT studies (Huang et al. 2003; Romer and McConnell 2003; Romer et al. 2002a; Volianitis et al. 2001). A key factor in these inter-study discrepancies appears to be baseline MIP; whereas previous studies report baseline MIP values close to the predicted value, baseline MIP in the present research and in Sonetti et al.'s (2001) study approximated 140% of the predicted value. IMT-mediated increases in MIP may thus be dependent upon baseline MIP values, which is consistent with the notion that the baseline status of a physiological system, or genetic endowment, plays a crucial role in determining the extent of adaptation with training (Kraemer 2000).

8.3.3 Effects of inspiratory muscle training upon endurance exercise performance

The findings of chapter 4 suggest that inspiratory muscle function may limit time-trial performance in competitive cyclists. The 3.0% improvement in 25 km cycling time-trial performance following 6 weeks of pressure-threshold IMT is comparable to previously documented improvements in short-term (6 min) rowing and 20 and 40 km cycling time-trial performances following IMT (Romer et al. 2002a; Volianitis et al. 2001). Moreover, the present research suggests that significant improvements in cycling time-trial performance can be achieved following IMT in the absence of a substantial improvement in MIP. However, IMT-mediated improvements in 25 km cycling time-trial performance were not conveyed to constant power cycling endurance (chapter 5), thus suggesting that

the choice of exercise performance evaluation test (time-trial vs. T_{lim} test) imparts a significant influence on the efficacy of IMT. The dependency of IMT's ergogenic effect on the choice of performance evaluation test may be related to the different physiological factors that determine exercise tolerance under different conditions of physiological exercise (i.e. constant power exercise vs. exercise performed at a self-selected intensity). Accordingly, previous studies repudiating the efficacy of RMT on the basis that constant-load exercise endurance was not improved (Fairbarn et al. 1991; Morgan et al. 1987; Williams et al. 2002) may have observed a performance enhancement had a time-trial based performance measure been utilised.

8.3.4 Putative mechanisms of performance enhancement following inspiratory muscle training

Although under the right circumstances IMT can have a positive influence on exercise endurance, the physiological mechanisms that underlie this ergogenic effect remain elusive. An IMT-mediated attenuation/delay of inspiratory muscle fatigue (Romer et al. 2002a; Volianitis et al. 2001) could have several benefits, including a concomitant reduction in dyspnoea, and less reflex activity from type III/IV receptors of inspiratory muscles that ultimately increase sympathetic vasoconstrictor activity in the limbs, thereby resulting in a decrease in locomotor muscle perfusion (McConnell and Romer 2004a). However, the findings of chapter 4 suggest that exercise endurance may be improved following IMT in the absence of any substantial reduction in the extent with which global inspiratory muscle strength declines following exercise. This result thus calls into question the physiological significance of an exercise-induced reduction in MIP. It also raises the possibility that impairment in some other aspect of inspiratory muscle function (e.g.

muscle shortening velocity), which is subsequently alleviated with IMT, has greater significance for exercise performance.

The findings of chapter 5, together with those of McConnell and Sharpe (2005), suggest that an increase in maximal sustainable power output (critical power or maximal lactate steady state) is an unlikely mechanism by which IMT improves exercise endurance. Walsh (2000) suggests that the main physiological determinant of critical power is the ability to utilise oxygen. It is tenable to speculate therefore that IMT has little influence on the maximal sustainable rate of oxygen transport and utilisation.

The findings of chapter 4 add to a growing body of literature showing a significant influence of IMT on lactate turnover during exercise (McConnell and Sharpe 2005; Romer et al. 2002c; Volianitis et al. 2001). How IMT attenuates $[lac^-]_B$ during exercise is, however, unclear at present, but may be related to altered lactate kinetics in respiratory muscles, active locomotor muscles, other metabolically active tissues, or a combination of these. As previously discussed, lactate is unlikely to impair muscle function (Robergs et al. 2004), thus a lower $[lac^-]_B$ per se is not likely to contribute to enhanced exercise endurance. However, a concomitant reduction of cellular acidosis may be the factor closely related to lactate turnover that is affecting performance, although the influence of acidosis is probably perceptual rather than via a direct effect on muscle contractility (Westerblad et al. 2002). Therefore, if IMT resulted in favourable changes in acid-base balance during exercise, then this might attenuate sensory input into the central nervous system and thus increase exercise tolerance.

In light of the above, and in the absence of any (measurable) influence of IMT on oxygen transport and utilisation during exercise, a reduced perceptual response to exercise may be the most important mechanism by which IMT improves exercise endurance. In chapter 4 improvements in 25 km cycling time-trial performance following IMT were

accompanied by a reduction in the intensity with which both limb and respiratory efforts were perceived. Previous studies have also reported post-IMT reductions in the perceptual response to constant-load and incremental cycling exercise (Gething et al. 2004b; Romer et al. 2002b), and also incremental rowing exercise (Volianitis et al. 2001). However, constant-load or incremental tests bear little relevance to an athlete's competitive experience, thus the novel finding of an IMT-mediated reduction in the perceptual response to time-trial exercise has far greater external validity. Consistent with a previous study (Romer et al. 2002b), the findings of chapter 4 show that improvements in 5 km interval times following IMT were greater during the latter stages of the 25 km time-trial test. An attenuation/delay of dyspnoea and/or limb discomfort may thus allow cyclists to maintain a higher, or at least the same, external power output for a given duration.

How IMT attenuates the perception of limb and respiratory effort during exercise is unclear at present, not least because the sensation of physical exertion is itself a complex phenomenon that probably involves an integration of feedback and feedforward mechanisms (St Clair Gibson et al. 2003). As highlighted previously, favourable changes in acid-base balance might reduce sensory input into the central nervous system, thus contributing to reduced limb and respiratory discomforts. An IMT-mediated increase in MIP would reduce the proportion of maximum force utilised for a given inspiratory load, thus the outgoing motor command would be perceived as being less intense (Huang et al. 2003; Kellerman et al. 2000). IMT may also modify breathing pattern during exercise, thus reducing the extent of "functional weakening" of the inspiratory muscles and therefore the severity of dyspnoea (McConnell and Romer et al. 2004b).

8.4 SUMMARY OF MAJOR FINDINGS

8.4.1 EMPIRICAL

Pressure-threshold IMT performed over 6 weeks in competitive cyclists resulted in a significant 15.3% increase in MIP and a significant 3.0% improvement in 25 km cycling time-trial performance. IMT-mediated changes in performance significantly exceeded changes in performance observed in a sham hypoxic-training placebo group. Following IMT the $[lac^-]_B$ during time-trial exercise was reduced by approximately 1.0 $mmol \cdot l^{-1}$. There were also reductions in the intensity with which both leg and respiratory muscle efforts were perceived, although the extent of global inspiratory muscle fatigue, as diagnosed using MIP, was not reduced.

Improvements in cycling time-trial performance following IMT were not transferred to constant power cycling endurance ranging in duration from approximately 3-30 min. Therefore, it seems that the choice of performance evaluation test is fundamental when evaluating the ergogenic effects of IMT. Changes in cycling time-trial performance were not explained by an alteration in the power-T_{lim} relationship, as neither critical power nor anaerobic work capacity was influenced by IMT. Therefore, an increase in maximal sustainable cycling power is an unlikely mechanism by which IMT improves exercise endurance.

Superimposing 8 min of voluntary, isocapnic hyperpnoea at a level commensurate with maximal exercise $\dot{V}_E$ on leg cycling exercise at maximal lactate steady state disrupted the equilibrium between lactate appearance and clearance and resulted in a significant increase in $[lac^-]_B$ of approximately 1.0 $mmol \cdot l^{-1}$ (+25%). Therefore, these findings are the first to suggest that the respiratory muscles may make a significant contribution to systemic lactate kinetics during maximal exercise.

Voluntarily mimicking the maximal exercise $\dot{V}_E$ and associated breathing pattern (f_R, V_T, and duty cycle) under resting conditions for 10 min resulted in a significant increase in $[lac^-]_B$ of approximately 0.8 mmol·l^{-1}, although the inter-subject variation was large, ranging from 0-2.0 mmol·l^{-1}. These findings suggest that a large part of the increase in $[lac^-]_B$ when volitional hyperpnoea is superimposed on exercise results from lactate production and efflux from respiratory muscles. Increases in $[lac^-]_B$ during volitional hyperpnoea at rest were significantly correlated with $\dot{V}O_2$ over 3-10 of hyperpnoea (r = 0.69), which suggests that inter-subject differences in the $[lac^-]_B$ response to volitional hyperpnoea are related, in part, to differences in respiratory muscle morphology, in particular the proportion of type II respiratory muscle fibres. When compared over the same duration (8 min) increased $[lac^-]_B$ when volitional hyperpnoea was superimposed on exercise was approximately 0.2 mmol·l^{-1} greater compared to when volitional hyperpnoea was performed at rest. Moreover, the extent of metabolic acidosis was greater when volitional hyperpnoea was superimposed on exercise compared to when volitional hyperpnoea was performed at rest. These observations suggest that greater metabolic perturbations result when additional respiratory muscle work is superimposed on heavy exercise.

8.4.2 Methodological

The lactate minimum test underestimated maximal lactate steady state power by, on average, 12 W. This discrepancy is perhaps explained by the observation that temporal changes in $[lac^-]_B$ during the incremental phase of the lactate minimum test did not reflect changes observed during constant power exercise at equivalent intensities. This latter finding therefore suggests a flaw in the theoretical basis of the lactate minimum test. The dissimilar $[lac^-]_B$ responses to constant power exercise and the incremental phase of the

lactate minimum test might be related, in part, to the considerable influence that the preceding lactate elevation phase imparts on the metabolic response to the subsequent incremental phase; specifically, performing the lactate elevation phase using arm-cranking exercise precluded a U-shaped $[lac^-]_B$ profile, and thus lactate minimum power determination, during a subsequent leg cycling incremental phase.

The lactate minimum test was shown to be dependent on other aspects of the test protocol; specifically, starting the incremental phase at a higher power output, and including a 1 min test interruption between each stage of the incremental phase, both modified temporal changes in $[lac^-]_B$ during the incremental phase and resulted in an approximate 9 W increase in lactate minimum power.

8.5 Limitations and directions for future research

Because subjects in chapters 4 and 5 demonstrated a relatively modest IMT-mediated increase in MIP subsequent to high baseline MIP values, it was reasoned that the baseline strength of the inspiratory pump might be a crucial factor determining the extent with which MIP increases with IMT. However, since the subjects recruited in the present research did not demonstrate a range of baseline MIP values, a conclusive relationship between baseline MIP and the magnitude of IMT-mediated increase in MIP could not be resolved. Therefore, future study should examine IMT's effect on MIP in subjects demonstrating a range of baseline MIP values.

Due to technical and ethical constraints lactate measurements throughout this thesis were restricted to arterialised venous or venous $[lac^-]_B$ measurements. Because $[lac^-]_B$ represents a balance between processes of lactate appearance and clearance, it is difficult to interpret changes that result from empirical interventions such as respiratory muscle training (RMT) and volitional hyperpnoea superimposed on heavy exercise. Reduced

$[lac^-]_B$ following RMT and increased $[lac^-]_B$ during volitional hyperpnoea may both result, in part, from altered lactate kinetics in working locomotor muscles, perhaps due to sympathetically-mediated changes in limb blood flow. Therefore, future studies should examine the influence of both these interventions on leg blood flow, and arteriovenous lactate concentration difference across the leg together with measures of tracer measured lactate uptake by the leg to account for simultaneous lactate uptake and release. Alternatively, study of the $[lac^-]_B$ response to volitional hyperpnoea under resting conditions pre- and post-RMT offers another paradigm with which to explore the mechanisms by which RMT lowers $[lac^-]_B$. It is tenable to presume that increased $[lac^-]_B$ under these conditions primarily results from lactate production and efflux from active respiratory muscles, thus any reduction in the $[lac^-]_B$ response to volitional hyperpnoea post-RMT can be primarily attributed to less lactate production and release from, and/or increased lactate clearance by, respiratory muscles. The external validity of this study would be enhanced if all elements of breathing pattern during volitional hyperpnoea were matched with those associated with the spontaneous exercise hyperpnoea (see also final paragraph below).

A putative mechanism by which RMT improves exercise tolerance is via an attenuation of exercise-induced respiratory muscle fatigue. In the present research global exercise-induced inspiratory muscle fatigue was diagnosed using MIP measurements pre- and post-exercise. Although MIP has many practical advantages, it does lack specificity and objectivity. Conversely, phrenic nerve stimulation with simultaneous measures of Pdi provides an objective method of assessing diaphragm function, although technical constraints precluded such measurements being made in the present research. Given the potential implications of exercise-induced diaphragm fatigue (see below) measures of Pdi during phrenic nerve stimulation pre and post-RMT are warranted to objectively evaluate

the influence of RMT on diaphragm fatigue. Moreover, the effect of RMT via voluntary isocapnic hyperpnoea on any measure of exercise-induced respiratory muscle fatigue has yet to be examined.

The efficacy of IMT is probably related to some improvement in inspiratory muscle function. However, it is not yet known what specific facet(s) of inspiratory muscle function enhancement (i.e. inspiratory muscle strength, inspiratory muscle shortening velocity, or rate of inspiratory pressure development) is/are responsible for the ergogenic effects of IMT. This might be resolved, in part, by examining the effects of different IMT regimens on exercise performance, e.g. high-inspiratory flow, low-pressure IMT vs. low-inspiratory flow, high-pressure IMT, which focus on opposite axes of the pressure-flow (force-velocity) relationship.

A respiratory muscle metaboreflex is considered to be responsible for the "steal" of locomotor muscle perfusion during fatiguing ventilatory work. If RMT reduced/delayed respiratory muscle metaboreflex activation during exercise then the increased leg blood flow might contribute to enhanced exercise tolerance, and as discussed above, the lowering of $[lac^-]_B$. Therefore, the effect of RMT on respiratory muscle metaboreflex activation offers an interesting avenue for future research. This paradigm could be addressed using loaded breathing under resting conditions with simultaneous measures of muscle sympathetic nerve activity using intraneural electrodes in the peroneal nerve (St Croix et al. 2000). Alternatively, sympathetically-mediated changes in leg blood flow can be inferred from noradrenaline spillover across the limb (Harms et al. 1997), which thus offers a means with which to examine IMT's effect on respiratory muscle metaboreflex activation during whole-body exercise.

As demonstrated in chapters 4 and 5 the choice of performance evaluation test (T_{lim} test vs. time-trial) is crucial when evaluating the ergogenic influence of RMT. Thus future

studies should duly consider their experimental aims (i.e. effects of RMT on physiological responses to exercise vs. effects of RMT on exercise performance) and select an appropriate test accordingly.

In chapters 6 and 7 the effects of volitional hyperpnoea on $[lac^-]_B$ were addressed. Although several parameters of breathing pattern during volitional hyperpnoea were not different from that associated with spontaneous exercise hyperpnoea, it is possible that respiratory muscle recruitment patterns, end-expiratory lung volume, and expiratory muscle pressure generation, differed between the two conditions. The result may have been an increased work of breathing during volitional compared to spontaneous hyperpnoea, which may have influenced the $[lac^-]_B$ data. Therefore, future study should examine the $[lac^-]_B$ response to volitional hyperpnoea with breathing pattern, end-expiratory lung volume, and tidal flow-volume and pressure-volume loops matched to those achieved during spontaneous exercise.

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List of Publications

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.

List of Conference Presentations

Johnson, M., Mellor, P., Forbes-Bell, O. and Sharpe, G. (2005). Comparison of lactate kinetics during the lactate minimum test and constant power cycling. *Proceedings of the 10th Annual Congress of the European College of Sports Science, Belgrade, Serbia and Montenegro.*

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