ALTERED CHEMORECEPTOR RESPONSE AND IMPROVED CYCLING PERFORMANCE FOLLOWING RESPIRATORY MUSCLE TRAINING

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FUFILLMENT OF THE REQUIRMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOMEDICAL SCIENCES (PHYSIOLOGY)

May 2003

By
Michael E. McMahon

Dissertation Committee:

Richard M. Smith, Chairperson
G. Causey Whittow
David A. Lally
John R. Claybaugh
Jan Prins

ACKNOWLEDGEMENTS

I would like to thank my family, friends, sponsors, and coworkers who contributed to the success of this study. Especially,

Dr. Richard Smith whose discussions, commitment, and ability to motivate me with positive criticism brought out the best in me and helped me accomplish more than I thought possible.

Dr. David Lally whose extensive knowledge has impressed and inspired me since my first exercise physiology class at the University of Hawaii.

Dr. Jan Prins who has lent his expertise to assist me to improve, whether it be my swim stroke, training clinics, or intellect, since I first moved to Hawaii.

Dr. Causey Whittow and Dr. John Claybaugh and the entire dissertation committee whose flexibility and willingness to help me meet deadlines in a short-notice allowed me to finish in two months what I had trouble completing during the last two years.

Many thanks to Urs Boutellier, who provided the opportunity to conduct this research at his facility and so much more, and Christina Spengler. Special thanks to K. Kulik and the subjects of this study for their time and effort. Funding was provided by the Hermann Klaus-Foundation and the Eidgenoessische Sportkommission. Nutritional supplementation was provided by Wander AG.

ABSTRACT

Cross-sectional studies have shown that well trained endurance athletes frequently have a lower peripheral and central chemoreceptor response (pR_c and cR_c) and a lower minute ventilation ($\dot{V}_E$) during exercise compared to untrained individuals. Some recent prospective studies support these observations. We speculated that the reductions in chemoreceptor response and $\dot{V}_E$ may be the specific result of the high rates of ventilation occurring during endurance training. To test this idea, subjects performed voluntary eucapnic hyperpnea to simulate exercise hyperpnea while avoiding the metabolic consequences of physical exercise. We therefore examined the effects of respiratory muscle training (RMT: 20x30min sessions of voluntary eucapnic hyperpnea) on the pR_c, cR_c, cycling performance, and $\dot{V}_E$. Twenty endurance trained cyclists were randomized into RMT or control-groups. To indicate cR_c both the hypercapnic ventilatory response at rest ($HCVR_R$) and during light exercise ($HCVR_{EX}$) were measured in a background of 50% O_2. The pR_c was assessed by measuring the ventilatory response to a modified Dejours O_2 test (4-6 trials of 10-12 breaths of 100% O_2) during light exercise. Endurance performance and $\dot{V}_E$ were measured during a fixed-rate cycling endurance test, performed at 85% of the maximal workload until exhaustion.

The RMT-group's cycling endurance improved significantly compared to controls (+3.26±4.98min versus -1.46±3.67min, p=0.027) but $\dot{V}_E$ was unchanged

at all times analyzed. The pR_c was significantly reduced in the RMT-group but unchanged in controls (-5.8±6.0% versus +0.1±4.6%, p=0.032). The cR_c, both at rest and during exercise, was not significantly altered following RMT in either group. However, the X-intercept of $HCVR_{EX}$ exhibited a significant shift to the left (-5.83±10.68mmHg, +0.38±2.48mmHg, p=0.047, RMT-group and controls respectively). The importance of this leftward shift and the reduced pR_c, though statistically significant, is unclear because there were no significant changes in $\dot{V}_E$ during any test nor were there correlations between $\dot{V}_E$ or performance or the altered chemoreceptor responses. We conclude that exercise hyperpnea, as simulated by RMT in this study, is accompanied by a reduction in pR_c and a leftward shift in the $HCVR_{EX}$, and improves cycling endurance; however, the altered chemoreceptor responses had little impact on $\dot{V}_E$ suggesting that their role in the control of ventilation during exercise is minor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I INTRODUCTION

Simulating Exercise Hyperpnea

Separating the respiratory muscles' role in exercise from the effects of whole-body exercise is difficult. The far larger mass of the locomotor muscles certainly overshadows any potential effects of the smaller respiratory muscles on the body as a whole. Furthermore, the muscular and cardiovascular systems' potential non-specific effects on the respiratory system confound the examination of the specific effects of exercise hyperpnea on the respiratory system itself. Not surprisingly, it would be extremely difficult to expose the body to the stresses of exercise without concomitant increases in ventilation. However, it is possible to simulate or even exaggerate the minute rate of pulmonary ventilation ($\dot{V}_E$) achieved during exercise while minimizing or removing the concurrent metabolic and cardiovascular stresses of endurance exercise. This can be done by a mode of respiratory muscle exercise called voluntary eucapnic hyperpnea. The stimulus elicited by voluntary eucapnic hyperpnea is highly specific to components of the respiratory system and the load can be high relative to the respiratory muscles functional capacity.

When voluntary eucapnic hyperpnea is performed repeatedly in a training regime designed for endurance conditioning, it is known as respiratory muscle training (RMT). The level of $\dot{V}_E$ occurring with RMT can be similar to, or higher

than, the limits of $\dot{V}_E$ reached during intensive exercise and can be sustained longer and repeated more often. RMT simulates exercise $\dot{V}_E$ without the exercise. This eliminates many of the typical physiological responses accompanying whole-body exercise, thus preventing, or at least minimizing, the adaptations that occur during whole-body training. In this way, RMT enables the isolation of the effects high levels of $\dot{V}_E$ on the respiratory system and on whole-body exercise performance.

This uncoupling of exercise hyperpnea from the metabolic changes of exercise is of particular relevance to this study because of two findings regarding the crossover responses to whole-body endurance training on the respiratory system: 1) whole-body endurance training seems to result in a lower exercise $\dot{V}_E$ and this lowered exercise $\dot{V}_E$ is sometimes linked to improved performance in athletes (see training effects on exercise ventilation); and 2) whole-body endurance training seems to affect respiratory chemosensitivity (see training effects on chemosensitivity and exercise and chemoreceptor response). It is not possible to determine whether the stimulus for these conditioning responses to the respiratory system is the result of the nonspecific and indirect effects of whole-body endurance exercise or the nonspecific but direct effects of the exercise hyperpnea.

We wondered whether the reduced $\dot{V}_E$ and reduced chemosensitivity associated with whole-body endurance training might be the specific result of the high levels of $\dot{V}_E$ that occur during whole-body endurance exercise. This study

examined the effects of exercise hyperpnea per se on the peripheral chemoreceptors (PC), on the central chemoreceptors (CC) responsiveness and on cycling performance, by uncoupling it from the other affects of whole-body exercise.

The Respiratory System as an Exercise Limiting Factor

Overview

If a specific training program for the respiratory muscles is to result in improved endurance exercise performance, a component of the respiratory system must play a limiting or "governing" role to some extent during endurance exercise. Although it now seems cliché, traditionally (and as this study began) the respiratory system was not considered to be a limiting factor during exercise in healthy young subjects (as opposed to the elderly or diseased) exercising under normal standardized conditions (as opposed to environmental stress e.g., hypoxia or heat; Bye, Farkas, & Roussos, 1983). This view was based, at least in part, on the ability of the healthy respiratory system to maintain homeostasis while working within its maximum capacity, which it largely seems to do even considering mechanical factors of ventilation, gas exchange, acid-base regulation, lung solute balance, and cardiopulmonary interactions.

During maximal exercise, the energy requirements of exercising muscle result in high partial pressures of mixed venous carbon dioxide (P_{CO_2}), which requires high levels of $\dot{V}_E$ up to 30 times of that at rest to maintain homeostasis.

The respiratory system meets this substantial requirement adequately in normal sedentary and moderately fit subjects under standard conditions. Despite the large increases in $\dot{V}_E$ from rest to exercise, this level of $\dot{V}_E$ is only about 50-60% (healthy untrained subjects) of the maximal capacity (15-s maximum voluntary ventilation). In part, this has led to the notion that $\dot{V}_E$ and, by inference, the respiratory muscles are not considered to limit exercise performance. However, the "text book" view that the excess capacity to meet demand rules-out any possible governing effect on the respiratory system to limit exercise, has been overly simplified (see previous work below).

Previous Work

In some extremely well trained athletes, the lung's limits of expiratory airflow can be reached; this impinges on the maximal effort expiratory flow-volume curve over a large portion of the expired volume (Grimby, Saltin, & Wilhelmsen, 1977; Hesser, Linnarsson, & Fagraeus, 1981). Furthermore, in the early eighties, Dempsey et al. (Dempsey, Hanson, & Henderson, 1984), demonstrated that the healthy respiratory system's ability to maintain homeostasis was indeed challenged during near-maximal and maximal exercise leading to arterial hypoxemia. This occurred in some, but not all, highly trained athletes (Dempsey et al., 1984). Nevertheless, these very specific examples of expiratory-flow limitation and arterial hypoxemia in the healthy respiratory system under normal conditions did little to change the widely held view that the

respiratory system's enormous capacity to meet demand somehow exempted it from being an exercise limiting factor. The lack of other "objective" evidence to the contrary was often used to bolster this view.

Ironically, and despite the extraordinary capability of the respiratory system, it was shown over 50 years ago that lung capacity (lung volumes and breathing capacity) could be further increased by non-specific training (Carey, Schaefer, & Alvis, 1956; Gilson & Hugh-Jones, 1949). More than a quarter of a century ago, Leith and Bradely showed that respiratory muscle strength and endurance could "be specifically increased by appropriate ventilatory muscle training programs" (Leith & Bradley, 1976). They concluded that ventilatory muscle training might improve sporting performance (Leith & Bradley, 1976). Additionally, it was shown that the 15-s maximum voluntary ventilation (MVV) is likely not to be the best measure of ventilatory capacity when evaluating exercise. Freedman showed that the 4-min MVV was approximately 70% of the 15-s MVV and as low as 40% in some subjects (Freedman, 1970). Therefore, resting MVV cannot be considered a reliable measure of the ventilatory capacity available during exercise.

In the late 1980's and early 1990's, subjective evidence hinting at the potential limitation of performance by the respiratory system began to mount. As early as 1985, Aaron, Seow, Johnson, and Dempsey, (1992b) demonstrated that reducing the physical and metabolic costs of breathing by unloading the respiratory muscles with a low density helium/oxygen gas mixture improved

endurance exercise performance. In addition, several other investigations examined the effect of various training programs specific to the respiratory muscles on exercise performance. Many, but not all, showed endurance exercise performance improved following the respiratory muscle training programs (Boutellier, Büchel, Kundert, & Spengler, 1992; Boutellier & Piwko, 1992; Markov, Spengler, Knopfli-Lenzin, Stuessi, & Boutellier, 2001; Spengler, Roos, Laube, & Boutellier, 1999).

An interest in respiratory muscle fatigue drew the efforts of several other investigators. The results of some of these studies showed respiratory muscle function impairment could last several days following long duration exercise (Boussana et al., 2003; Hill, Jacoby, & Farber, 1991; Ker & Schultz, 1996; McConnell, Caine, & Sharpe, 1997). These reductions in respiratory muscle function were taken to be indirect evidence of respiratory muscle fatigue. Others have shown that exhaustive respiratory muscle work prior to exercise resulted in reduced endurance exercise performance (Mador & Acevedo, 1991; Martin, Heintzelman, & Chen, 1982). The finding that specific respiratory muscle training programs improved endurance cycling performance suggests that exercise performance prior to the training program was limited by the respiratory system. However, this concept did little to alter the contemporary view - that the respiratory system does not play a limiting role in exercise. Certainly, this was partially due to the subjective nature of the tests and measures used in these studies which provided only indirect evidence.

In 1993, Johnson et al. (Johnson, Babcock, Suman, & Dempsey, 1993), and Mador et al. (Mador, Maglang, Rodis, & Kufel, 1993), demonstrated direct and objective evidence of respiratory muscle fatigue (diaphragm) following high intensity exercise (80-95% of the maximum oxygen consumption: $\dot{V}_{O_2MAX}$) of short duration (10-30 min). This was accomplished by showing a reduction in diaphragm contractility using bilateral supramaximal electrical phrenic nerve stimulation. If the diaphragmatic fatigue measured in these studies was enough to impair endurance exercise performance then the respiratory system (i.e., respiratory muscles) must be considered an exercise limiting factor.

A series of studies by the Dempsey group (Harms & Dempsey, 1999; Harms et al., 1998; Harms, Wetter, St Croix, Pegelow, & Dempsey, 2000; Harms et al., 1997) provided evidence that the "work of breathing normally incurred during maximal exercise causes vasoconstriction in locomotor muscles and compromises locomotor muscle perfusion and oxygen consumption." (Harms et al., 1997) This landmark study shows that the respiratory system, or at least the cost of meeting the respiratory system's metabolic demands, "governs" the blood flow to the locomotor muscles and suggested this competition could limit endurance exercise performance.

Summary

With direct and objective evidence showing that the respiratory system can be an exercise limiting factor, the idea of a specific training program for the

respiratory muscles leading to improvements in endurance exercise performance is more tenable. This leaves one to ponder, how could such respiratory training result in performance improvements? Potential mechanisms include delaying the onset of respiratory muscle fatigue, reducing the perception of respiratory exertion and breathlessness, reducing competition for blood flow between the respiratory muscles and locomotor muscles, or improving respiratory muscle and ventilatory efficiency (by mechanical, metabolic, or neural means).

Of the mechanisms to account for increases in exercise performance an improvement in ventilatory efficiency is of particular interest and relevance to this study. By increasing their maximum capacity, the respiratory muscles would then work at a smaller percentage of this maximum capacity during a fixed level of exercise. The improvements in respiratory muscle or ventilatory efficiency (e.g., a simple reduction in $\dot{V}_E$ at a given exercise intensity) could spare resources that could then be made available to the locomotor muscles resulting in improved endurance exercise performance. These changes may affect or be mediated by respiratory control mechanisms, such as the chemoreceptors, which will be the focus of this study.

The Respiratory Muscles

Overview

To better understand the effects of specific respiratory muscle training and the mechanisms by which it may improve endurance exercise performance,

it is necessary to review the anatomy, physiology, and neural regulation of the respiratory muscles. Of particular interest and importance is respiratory muscle function and control during exercise.

The respiratory muscles are the muscles which are involved in the process of moving air in and out of the lungs. "By definition, any skeletal muscle that changes the dimensions of the chest wall is a respiratory muscle." (Rowell & Sheperd, 1996). More accurately, any skeletal muscle that changes or *maintains* the dimensions of the chest wall is a respiratory muscle. The respiratory muscles may be active (or not) to varying degrees during inspiration or expiration or both depending on the ventilatory requirement. Some of the respiratory muscles are also involved in postural support and even have a locomotor function during exercise. In fact, their ventilatory role may sometimes be secondary.

Biochemically, morphologically, and physiologically the respiratory muscles are similar to skeletal muscle (NHLBI, 1990). In fact, the respiratory muscles are skeletal muscles; however, some important differences exist.

Respiratory muscle control is under both volitional (cortex) and automatic control (upper medulla and brainstem), whereas peripheral skeletal muscle is under voluntary control (cortex). Respiratory muscle contractions are rhythmic, this may (or not) be true of skeletal muscles depending on their required action at the time of contraction. Some (i.e., diaphragm, intercostals) are constantly active and their actions are life sustaining. In this respect, the function of these

respiratory muscles more closely resembles cardiac muscle than skeletal muscle. On the whole, respiratory muscles have a greater oxidative capacity, are more fatigue resistant, have a higher capillary density, and increased maximal blood flow compared to peripheral skeletal muscle (Epstein, 1994). These physiological differences between skeletal muscle and respiratory muscles are a consequence of the functional properties of the respiratory muscles and are important in terms of their ability to adapt to increased loads.

Mechanically, respiratory muscles differ from skeletal muscle in that they contract to overcome resistive (airways) and elastic loads (chest wall and lungs; Fenn, 1963). Skeletal muscle contracts against inertial forces. Inertial forces are usually negligible for the respiratory muscles (Rowell & Sheperd, 1996). The resting position of the limb muscles is determined actively or by gravity. Functional residual capacity, the resting position for the respiratory muscles, is determined passively by the mechanical relationship between the lungs and the chest wall (Berne & Levy, 1993). The maximum force is generated at the resting length, and as with limb muscle, the maximum tension developed by respiratory muscles depends on the muscle length (length-tension relationship) and the velocity of shortening (force-velocity relationship; Green & Moxham, 1985). The difference with respiratory muscles is that pressure is used to estimate the tension or force generated by the respiratory muscles and muscle length and shortening velocity are determined by lung volume and respiratory flow, respectively (Rowell & Sheperd, 1996).

Primary and Accessory Muscles of Ventilation

Overview

The respiratory muscles may be grouped into two categories, the primary and accessory muscles. The primary muscles are the inspiratory muscles that routinely contract during normal quiet breathing. The accessory muscles are inactive during resting ventilation but play a ventilatory role under certain circumstances such as exercise. The accessory muscles may have an inspiratory or expiratory function or both and may even perform postural and locomotor functions.

Primary Muscles of Ventilation

The diaphragm is the principle muscle of inspiration. It is actually two muscles, the costal and crural diaphragm (De Troyer, Sampson, Sigrist, & Macklem, 1981). Anatomically, it consists of three distinct parts, the costal, sternal and crural regions, all of which are connected to the central tendon (Leak, 1979). Functionally, the costal and sternal regions are closely associated whereas the crural region may be recruited differently. The costal diaphragm (larger) originates at the upper inner margins of the six lower ribs and the adjoining costal cartilage (costal region) and from the posterior aspect of the xyphoid process (sternal region). Both regions insert at the central tendon. The

crural (thicker) diaphragm runs from the anterolateral aspect of the upper three lumbar vertebrae to the central tendon (Loring & DeTroyer, 1985).

Together the diaphragm muscles form a domed-shaped sheet that separates the thoracic and abdominal cavities. During inspiration the diaphragm contracts and shortens which pulls the central dome downward in a piston-like manner causing pleural pressure to fall which increases lung volume. This motion also compresses the abdominal contents which increases intra-abdominal pressure. By using the abdomen as a fulcrum the pressure can be transmitted outward to the lower rib cage (through the zone of apposition) causing it to expand and further decrease pleural pressure (Green & Moxham, 1985). Contraction of the costal fibers, because the descent of the diaphragm is opposed by the abdominal contents and muscles, lifts the lower rib cage. Due to the shape and articulations of the ribs the upward movement of the rib cage is accompanied by a forward motion (pump-handle) and an outward (bucket-handle) motion of the ribs (Green & Moxham, 1985).

Needle electromyography has shown the parasternal intercostal, the external intercostal, and the scalene muscles are primary muscles of respiration as they are active during every normal resting inspiration (De Troyer & Estenne, 1984; De Troyer, S., & Zin, 1983; De Troyer & Sampson, 1982). The scalene muscles run between the transverse process of the last five cervical vertebrae and the upper surface of the first two pairs of ribs. Their contraction lifts the sternum and the first two pair of ribs. This produces an expansion of the upper

rib cage in the sagittal plane (anteroposterior direction) often referred to as a pump-handle movement of the ribs (Epstein, 1994).

The parasternal muscles run downward and laterally from the periphery of the sternum and interchondral cartilage to the upper surface of the ribs. Contraction results in lateral expansion of the upper ribcage (bucket-handle movement) which decreases intrathoracic pressure, thereby assisting inspiration. Also, the parasternal and scalene muscles jointly aid inspiration by preventing the diaphragm from having an expiratory effect on the upper rib cage (Loring & DeTroyer, 1985).

The external intercostal muscles slope downward (caudally) and forward (ventrally) in the intercostal spaces running between upper and lower surfaces of adjacent ribs. Their contraction elevates the rib cage, assisting inspiration. However, their action is not without controversy. Studies in dogs indicate their action may be lung volume dependent. At low lung volumes, they do indeed serve inspiration but they may have an expiratory function at high lung volumes (De Troyer et al., 1983). However, recent modeling based on humans, suggests their isolated action is solely inspiratory, expanding the rib cage in the pump-handle motion (Loring, 1992).

Accessory Muscles of Ventilation

Almost any muscle of the trunk can be recruited as an accessory muscle to assist inspiration during heavy loads. The Sternocleidomastiod muscle

connects the mastoid process of the temporal bone and the occipital bone with the medial aspect of the clavicle and the top of the sternum (manubrium). It assists inspiration during high levels of ventilation and high lung volumes (Moxham, Wiles, Newham, & Edwards, 1980) causing a pump-handle expansion of the rib cage (Loring, 1992).

The internal intercostal muscles lie in the intercostal spaces beneath the external intercostal muscles. The fiber orientation runs in an inferior (caudal) and posterior (dorsal) direction. This orientation and needle electromyography indicate that they are active during expiration (De Troyer et al., 1983). As with the external intercostals, controversy has surrounded their action. Most recent evidence indicates they have an unambiguous expiratory action (pump-handle) (Loring, 1992).

The levator costae muscles extend from the tip of the transverse process of the thoracic vertebrae to the angle of the rib below. Their action is inspiratory and duplicates that of the external intercostal muscles (Loring, 1992).

The pectoralis major extends laterally and caudally from the manubrium and medial portion of the clavicle to the humerus. If the arms are fixed, as during cycling, its contraction can assist expiration and if the humerus and shoulder are "fixed" by other muscles (e.g., the trapezius) it can assist inspiration (Epstein, 1994).

The triangularis sterni muscle connects between the lower half of the body of the sternum to the costal cartilage of the third through seventh ribs.

Contraction occurs simultaneously with the abdominal muscles during expiration and pulls the ribs caudally (Epstein, 1994).

The four abdominal muscles (internal and external obligue and rectus and transversus abdominus) contract individually and not as a unit (De Troyer, Estenne, Ninane, Van Gansbeke, & Gorini, 1990). The internal oblique and tranversus abdominus make up an inner muscular layer which runs around the circumference of the abdomen. The external oblique and rectus abdominus form the outer abdominal layer and connect the lower rib cage to the pelvis. The result of their contraction pulls the rib cage inwards and caudally and elevates the diaphragm by pushing the abdominal area upwards. The abdominal muscle group can also play an inspiratory role under several circumstances, for instance during exercise. They do so by maintaining contraction during early inspiration, this lengthens the fibers of the diaphragm which improves the diaphragm's contractility (Epstein, 1994).

Other accessory muscles of ventilation may include the trapezius, the latissimus dorsi, the serratus (anterior and posterior), and theneck and the upper airway muscles. The trapezius, latissimus dorsi, and serratus muscles have an accessory role during inspiration, although the serratus activation can extend into expiration. The platysma, mylohyoid, and sternohyoid (neck muscles) are phasically active during inspiration in tetraplegics so they could be active in other extreme situations. The muscles of the upper airway are required for maintaining the patency of the airways during inspiration. These muscles are

active during inspiration and potentially during active expiration. The laryngeal adductor muscles' coordinated activation with the primary muscles of ventilation can prevent upper airway collapse, reduce airway resistance, and decrease the work of breathing (Epstein, 1994). The regular activity pattern and importance of the muscles of the upper airway for inspiration makes one ask why they aren't *considered to be primary respiratory muscles.*

Summary

Many muscles are involved with ventilation. Some of these muscle can have both an inspiratory and expiratory function and others also play a supporting role as postural or locomotor muscles. There are fewer muscles with an expiratory function than muscles with an inspiratory function. In fact, the internal intercostals are the sole expiratory muscle with a primary expiratory function. The abdominal muscle group has a primary and substantial postural role in conjunction with its expiratory activity and can even have an inspiratory function. Given the number and varied arrangement (i.e., origins and insertions) of respiratory muscles the enormous complexity of their interaction and control during breathing, especially the hyperpnea of exercise, is difficult to imagine.

Biochemistry of the Respiratory muscles

Overview

Skeletal muscles have a large plasticity based on their pattern of usage. The fiber type, capillary supply, and enzymatic activity of locomotor muscles and respiratory muscles are a direct result of this pattern of usage. Due to the differences in their recruitment pattern and workload, limb and respiratory muscles exhibit notable difference in these muscle fiber characteristics.

Fiber Type

Like other skeletal muscle, respiratory muscles in humans exhibit heterogeneous motor unit types both within and between muscles. The difference between motor unit types is based on the characteristics of their associated fibers. Muscle fibers are classified into three predominate types, type I, type IIa, and type IIb. This is done by incubation at different pH values and histochemical staining for myosin-ATPase.

Type I fibers are generally activated first, are highly resistant to fatigue and generate comparatively lower force than the other fiber types. They have the highest oxidative capacity, the slowest maximal shortening velocity,(and a low glycolytic enzyme capacity), and are innervated by slow-twitch motor neurons. Type IIa fibers have an intermediate oxidative capacity and a high maximal shortening velocity. They are innervated by fast-twitch motor neurons. These

fibers are moderately to highly fatigue-resistant, and have an intermediate glycolytic capacity. Type IIb fibers have the lowest oxidative capacity, the highest maximal shortening velocity and the highest glycolytic capacity of the fiber types. They are innervated by fast-twitch motor neurons. These fibers are the last to be activated, produce the greatest force, and are highly susceptible to fatigue. "The physiological significance of the various muscle fiber types is the differences between fibers in oxidative capacity and maximal shortening velocity" (Rowell & Sheperd, 1996).

Fiber type distribution determines the range of possible contractile responses for a muscle. In general, muscles with a preponderance of slow twitch fibers are well suited for sustained, low-intensity efforts. Muscles with a high proportion of fast twitch fibers, particularly type IIb fibers, are capable of fast, high-powered, but non-sustainable contractions (Epstein, 1994). The relative proportions of fiber types varies between muscle groups, but can be linked to the muscle's activity pattern. Postural muscles, for example, which are subjected to long periods of tonic stimulation, contain a higher proportion of fatigue resistant fibers (more type I than Type IIa fibers) when compared to phasically active limb muscles. Fiber type composition also appears linked to activity pattern when comparing muscle biopsies among untrained, strength trained, and endurance trained individuals

The activity pattern of the muscles involved with respiration differs from that of other skeletal muscles. Unlike peripheral skeletal muscles, many

respiratory muscles are stimulated repetitively and contract rhythmically. Some respiratory muscles also have a postural function. When performing a postural role these muscles can be tonically stimulated for long periods of time. The inspiratory muscles are constantly active and are subject to rhythmic stimulation. Some respiratory muscles, or some muscles performing a respiratory role, are active only under instances of increased ventilatory demand, such as exercise. In this situation, the respiratory muscles are stimulated phasically like peripheral skeletal muscle. The differences in activation pattern between respiratory skeletal muscle and limb skeletal muscle are likely responsible for the increased fatigue resistance, increased maximal blood flow, greater oxidative capacity, and higher capillary density found in respiratory muscle compared to the limb skeletal muscles (Roussos & Macklem, 1985).

In peripheral skeletal muscle, fiber type differentiation is completed within the first two years. This is true for the diaphragm and intercostal muscles as well (Keens et al., 1977). It is therefore likely that the percentage of slow (type I) to fast (type II) twitch fibers remains the same in respiratory muscles. However, like other skeletal muscle, the plasticity of type II fibers is greater than type I, and therefore the potential for alterations of type IIb to type IIa fibers exists.

The adult diaphragm contains a high percentage of fatigue resistant fibers (50% type I and 25% type IIa) (Mizuno, 1991). In general, limb muscle from an untrained individual will contain between 40%-50% slow twitch (type I) fibers (Poortmans, 1993) and a nearly equal mix of fast twitch fibers (type IIa and type

IIb). Of course, the specific proportions of fiber types will depend on the activity pattern of the muscle sampled and the genetic disposition of the individual from which the muscle sample was taken. For untrained humans, the fiber type distribution of the diaphragm resembles that of peripheral skeletal muscle (Poortmans, 1993).

The other respiratory muscles also exhibit a high but variable proportion of type I fibers. The relative occurrence for the intercostals range from 57%-66%, the scalenes about 59%, the abdominal muscles from 30%-60%, and the latissimus dorsi from 44%-48% (Epstein, 1994; Hards, Reid, Pardy, & Pare, 1990). The important fact is that the respiratory muscles contain large numbers of fibers with a potentially high oxidative capacity.

The intercostal muscles of humans with normal ventilatory function exhibit a similar but slightly higher occurrence of slow twitch fibers (57%-69%). The expiratory and inspiratory intercostal muscles have at least 10% more slow twitch fibers than most other skeletal muscle and the diaphragm (Mizuno, 1991). The expiratory intercostals (lateral internal intercostals) have 33% more type IIa fibers (about 35% of the total fiber number) than the inspiratory intercostal muscles (internal parasternal and the external lateral intercostal muscles) (Mizuno, 1991). The expiratory intercostals have far fewer type IIb fibers (1%) than the inspiratory intercostals muscles (19%). Thus, "the fiber type distribution of the inspiratory intercostal muscles is similar to that of limb skeletal muscle in non-athletes, whereas the almost complete lack of fast twitch b fibers in the

expiratory intercostal muscles makes them resemble, and even exceed, fiber type distribution of muscles from extremely well-trained athletes in endurance events." (Mizuno, 1991) The fiber type charateristics of the expiratory (internal) intercostals are those of a muscle in between slow and fast twitch and which is especially well suited for prolonged activation.

The fiber type distribution of the scalene muscles is similar to that for the inspiratory intercostal muscles (type I 59%, type IIa 22%, and type IIb 17%). The proportion of fatigue resistant fibers exceeds that found in most skeletal muscles including the diaphragm (Mizuno, 1991).

The sternocleidomastoid muscles have smaller proportion of slow twitch fibers (35%) than other respiratory muscles. "Explanation of this finding, as well as information on the proportion of subgroups of fast twitch fibers is lacking." (Mizuno, 1991) Nevertheless, it is likely their fiber type is accounted for by the activation pattern of the muscle which is dictated by their function.

The expiratory extrathoracic muscles, consisting of the four abdominal muscles - including the rectus and transverse as well as the external and internal oblique - have 54% slow twitch fibers in normal humans and in patients undergoing abdominal surgery . In these patients fast twitch IIa (20%) and fast twitch IIb (23%) fibers were evenly distributed (Mizuno, 1991).

Fiber Size of the Respiratory Muscles

The size of the muscle fiber affects the diffusion capacity of the muscle. The distance from the capillaries to the muscle cells' interior increases as muscle fibers become larger. This increases the time required for the exchange of gases, nutrients, and metabolites. Of course, larger muscle fibers are able to generate greater force. Generally, smaller muscles fibers have smaller diffusion distances, and faster diffusion times. If diffusion capacity is increased, muscle fibers are better suited for endurance exercise. However, the diffusion capacity, while influenced by fiber size, is ultimately dependent on capillarization.

Skeletal muscle adapts to the pattern of activity and recruitment. Endurance exercise results in relatively less hypertrophy than strength training. The role of these factors is evident in the fiber size of the respiratory muscles.

The average cross-sectional area of muscle fibers of the costal diaphragm (2,200 μm^2) is smaller than that of limb skeletal muscle (>4,000 μm^2). The type I fibers (2,400 μm^2) are slightly larger than type IIa (2,200 μm^2) and type IIb (1,800 μm^2) (Mizuno, 1991).

The fiber size of the intercostal muscles varies. The average cross-sectional area of muscle fibers (type I and IIa) of the expiratory internal intercostals is 4,300 μm^2. This is large compared to the muscle fibers (type I, IIa, and IIb) of inspiratory intercostals (parasternal internal and lateral external intercostal) which have an average fiber size of 2,900 μm^2. The type IIa (5,400 μm^2) fibers of the expiratory (internal) intercostals have a larger fiber size than

the type I fibers (3,700 μm^2). The inspiratory (external) intercostals have a similar fiber size among all three fiber types. The hypertrophy of the expiratory intercostals suggests they have they ability to sustain relatively higher loads than the inspiratory intercostals.

The scalenes have smaller fibers than other inspiratory muscles. This is the result of comparatively smaller fast twitch fibers (type IIa 1,500 μm^2 and type IIb 1,200 μm^2). The average cross-sectional area of the scalene type I fibers is 2,300 μm^2. The scalenes resemble the diaphragm in the fact that the slow twitch fibers are larger than the fast twitch fibers (Mizuno, 1991).

The fiber size of the other respiratory muscles has not been determined or has been measured by other techniques (e.g., least mean diameter).

Capillarzation of the Respiratory Muscles

The capillarity of a muscle is most effectively seen by a vascular corrosion cast. However, the disadvantage of this procedure is that it is not ethically possible in living humans. The next best method for estimating the capillarization in muscle is the capillary-to-muscle fiber ratio. This measure is unaffected by fiber size and the sharing of capillaries with adjacent muscle fibers (Rowell & Sheperd, 1996). Only estimates of capillarity using the capillary/muscle fiber ratio method are considered in the following observations.

The average capillary/muscle fiber ratio in untrained humans is approximately 1.5 capillaries per fiber. Physiologically, a muscle with high

capillarization is beneficial because it improves the diffusion capacity of the microvascular unit. This occurs because the diffusion distance between capillaries and muscle fibers is decreased and the red blood cell transit time is increased. Functionally, the changes facilitate oxygen extraction and the exchange of nutrients and metabolites between the blood and muscle, which can enhance aerobic work capacity (Brooks, Fahey, & White, 1996).

The average capillary/muscle fiber ratio for the costal diaphragm is 1.9 (range 1.5-2.4) (Mizuno et al., 1990). Somewhat surprisingly, this is somewhat similar to that of limb skeletal muscle found in untrained humans.

Of the intercostal muscles, the expiratory have a greater capillary/muscle fiber ratio (2.3) than the inspiratory (1.6). Again these values are similar to (but slightly higher than) limb skeletal muscle in normal untrained individuals. This difference between the inspiratory and expiratory intercostals suggests the latter have better perfusion. This is somewhat surprising given their larger fiber size (see earlier, fiber size of respiratory muscles). Data on the capillarity of the extrathoracic respiratory muscles has not been published.

From the above data it is clear that the number of capillaries surrounding each muscle fiber is similar around most skeletal muscle, respiratory or limb, but with that of the respiratory muscles being slightly greater. Perhaps a better estimate of the diffusion distance between the capillaries and muscle fibers is their cross-sectional area/capillary number ratio (Mizuno et al., 1990). The lower the ratio the shorter the diffusion distance between the muscle fibers and the

nutrient and oxygen rich capillaries, thus facilitating exchange. The cross-sectional area to capillary number ratio is comparatively lower in respiratory muscles than in some limb muscle (vastus lateralis) (Mizuno et al., 1990).

Enzymatic Profile of the Respiratory Muscles

Quantitative biochemical analysis of enzymatic activity offers another indication of the pattern and type of recruitment the muscle experiences. The oxidative capacity of muscle fibers can be assessed by their mitochondrial (krebs cycle) enzymatic profile. The three oxidative enzymes commonly assessed in skeletal muscle are citrate synthase (CS), Succinate dehydrogenase (SDH), and 3-hydroxyacyl-coenzyme A dehydrogenase (HAD).

Unfortunately, due to their invasive nature, few studies are available on the enzymatic activities of healthy human respiratory muscle. This makes it difficult to compare the activity of oxidative and glycolytic enzymes between respiratory and limb skeletal muscle. However, numerous studies on rats have demonstrated that the activity of oxidative enzymes is 35% - 65% higher in the diaphragm compared to limb locomotor muscle (Grinton et al., 1992; Powers et al., 1994).

Endurance of Individual Respiratory Muscles

The individual endurance of the diaphragm, inspiratory muscles working as a group, and the expiratory muscles has been assessed. To do this endurance was measured by the duration submaximal target pressures could be

sustained against an external resistance. From these results, it appears the endurance of the inspiratory muscles is greater than the diaphragm, which is great than the expiratory muscles. The inspiratory muscles can sustain 50-80% of their maximal inspiratory pressure, whereas the diaphragm can sustain about 40%, and the expiratory muscles only about 16% (Roussos, 1995). This suggests that the expiratory muscles are more susceptible to fatigue than the inspiratory muscles and seems paradoxical when considering their morphological and biochemical characteristics (see fiber type, fiber size, and capillarization of the respiratory muscles). Perhaps it is related to the activation pattern of the respiratory muscles. The inspiratory muscles may be able to diminish the relative load by sharing it among many individual muscle groups or by being recruited to varying degrees which would permit periods of recovery or both. The fewer expiratory muscles would not be able to achieve this to the same extent.

Summary of the Respiratory Muscles

It is interesting to note that some characteristics of the expiratory intercostals differ from the other respiratory muscles. The expiratory intercostals exhibit hypertrophy of the muscle fibers, a lack of type IIb fibers, and increased capillarization. These properties suggest a recruitment pattern indicative of relatively large force development during repeated contractions. The explanation for the apparent intense use of the expiratory intercostals compared to the other respiratory muscles may be that their relatively small mass may at times be

solely responsible for meeting the requirements of expiration or only partially assisted by the abdominal muscle group which also stabilizes the trunk and aids inspiration. The inspiratory load is shared by the diaphragm, parasternal and external intercostal, scalene, and sternocleidomastoid muscles (Mizuno, 1991) and may not adapt to such an extent under normal conditions.

In summary, the respiratory muscles have a high proportion of fatigue resistant fibers (type I and type IIa), are highly vascularized, and have a high oxidative enzyme capacity. These characteristics give the respiratory muscles qualities of energy economy and resistance to fatigue, which enable them to contract rhythmically in the ceaseless process of moving air in and out of the lungs. It should be clear from the preceding information that the respiratory muscles are skeletal muscles which have developed to be specifically well suited for their life-sustaining role and as we will see have potential for even further adaptability (see respiratory muscle adaptations in response to training).

The Work of Breathing

Overview

The respiratory muscles must perform metabolic work (consuming oxygen to convert substrates to energy) to produce mechanical work (changes in pressure and volume). There are five types of mechanical forces that respiratory muscles must overcome while breathing. They are:

1.) elastic forces, caused by changes in volume to the lung and chest. During exercise, elastic work is done during inspiration for lung and chest wall expansion. During expiration during exercise elastic work is done to decrease the expiratory time and to decrease the end-expiratory lung volume (EELV) below the functional residual capacity;

2.) flow - resistive forces, which are dependent on the caliber of the airways which can be affected by lung volume and the flow rate and pattern (laminar or turbulent);

3.) inertial forces, which are dependent on the mass of the tissues (and gas). The work done to overcome the inertial forces is probably negligible or would be recovered (i.e., the work done during the phase of the breathing cycle requiring acceleration would be recovered during the phase when deceleration occurs);

4.) gravitational forces, which are considered part of the inertial forces;

5.) distorting forces of the chest wall, which come into play when breathing through external resistance or at high levels of ventilation such as during exercise (Roussos, 1995).

In practice, the total mechanical work done can only be estimated and these estimates have obvious shortcomings. Two different approaches are used to assess the work of breathing: 1) to measure the mechanical work done by the respiratory muscles (using pressure and volume changes) and 2) to estimate the total energy cost of breathing. The ratio of mechanical work to energy cost gives

the mechanical efficiency of the respiratory muscles. During exercise, the rate of the work of breathing (power) and $\dot{V}_E$ have a curvilinear relationship with an upward concavity and ever increasing slope (Roussos & Macklem, 1985), such that at extremely high $\dot{V}_E$, the power required becomes substantial.

Methods for Estimating the Work of Breathing

The metabolic cost of breathing includes the work done to overcome the mechanical forces mentioned above, energy used for isometric contractions and the energy lost as heat. Measuring the oxygen cost of breathing gives an approximation of the metabolic work (aerobic) or energy cost that the respiratory muscles expend for a given level of ventilation. This can be done directly by measuring the respiratory muscle blood flow (not yet possible in humans) or indirectly by measuring the change in oxygen consumption ($\dot{V}_{O_2}$) for the whole body while altering ventilation. Using this indirect method, Aaron, Johnson, Seow, and Dempsey (1992a) demonstrated the oxygen cost of breathing at rest is about 2% of the total $\dot{V}_{O_2}$. This proportion increases to 3-5% of the total $\dot{V}_{O_2}$ during moderate exercise and about 8-10% of the total $\dot{V}_{O_2}$ (13-16% in some subjects) during maximal exercise.

These values in humans are supported by values obtained by measuring the blood flow to the respiratory muscles in dogs and ponies. Microshpere measurement of blood flow showed that blood flow to inspiratory and expiratory muscles (diaphragm, chest wall, and abdominal respiratory muscles) increased

to as much as 16% of total cardiac output during peak exercise in ponies (Manohar, 1986). Additionally, it was shown that the peak values for diaphragm blood flow (per 100g muscle) were most often greater than those for the primary locomotor muscles (gluteus medius and biceps femoris). This supports the studies by Harms et al., (Harms et al., 1997) that demonstrated changes in leg blood flow which were consequent to alterations in respiratory muscle work, suggesting a competitive relationship between respiratory and locomotor muscles for a limited cardiac output, which the respiratory muscles win.

The efficiency of breathing is simply the ratio of the mechanical work of breathing to the metabolic cost of the respiratory muscles in performing the work. The difficulty is in the accurate estimation of the mechanical and metabolic work. The efficiency of large muscle groups performing aerobic work is generally in the range of 20-30%. However, the overall efficiency of the respiratory muscles probably approximates a value closer to 10% (Roussos, 1995). Of course, a single value for respiratory muscle efficiency does not exist, because it is susceptible to numerous changing factors.

Oxygen cost of Breathing

Even during maximal exercise, the oxygen cost of breathing is not considered substantial enough to negatively affect performance. Nevertheless, the metabolic expenditure of the respiratory muscles cannot be discounted. The rate of oxygen consumption of 1.5-2.0 ml O_2/l O_2 doesn't include locomotor and

postural functions of the respiratory muscles. Thus, the oxygen costs of the respiratory muscles to generate the $\dot{V}_E$ during exercise would be increased further by the amount of $\dot{V}_{O_2}$ the respiratory muscles require for locomotor and postural functions.

Summary

The comparatively low efficiency of the respiratory muscles (~10%) suggests the potential for improvement in respiratory muscle efficiency is considerable when compared to the efficiency of other muscle groups (~25%). Large improvements in efficiency of the respiratory muscles may only result in small changes to an already small percentage of total $\dot{V}_{O_2}$, but the effect of such a small change on exercise performance can not be dismissed in situations that test the limits of human physiology, where even the smallest improvements can make large difference (e.g., winning an Olympic medal or not).

Breathing Pattern

Overview

Breathing pattern is used to refer both to the pattern of specific respiratory muscle recruitment and their level of activation and to the mechanical pattern of breathing (tidal volume: V_T, EELV, end-inspiratory lung volume: EILV, breathing frequency: f_b, inspiratory and expiratory flow rates, and respiratory timing) created by the respiratory muscles. The complexity and dynamics of respiratory

muscle fiber orientation, along with the shape of the rib cage and its articulations make it difficult to assess or model the ventilatory actions of the individual respiratory muscles, especially in humans. An increasingly large number of muscles with a respiratory function are recruited during increased ventilatory load making assessment even more difficult in exercise. A dynamic combination of respiratory muscles can achieve a given level of ventilation by a numerous variety of combinations of volumes, frequency, and flow rates. The optimum combination is that which meets the ventilatory requirements with the lowest energy cost. The specifics of the movement, recruitment, and control of their coordinated response to the substantial requirements of exercise is a mystery not yet completely understood.

Resting Breathing Pattern

During rest, a respiratory cycle begins with extrinsic laryngeal muscle contraction to maintain the patency of the upper airway (Epstein, 1994). Next, the diaphragm and the external intercostal muscles contract which serves to expand the thoracic cavity. This creates a negative intrapulmonary pressure and air moves into the respiratory passages and lungs. During quiet breathing, expiration is passive and is caused by the recoil of the chest wall, lungs and diaphragm. This occurs as the inspiratory muscles relax and may be assisted by the internal intercostal muscles. When ventilatory needs increase, such as during exercise, the accessory inspiratory and expiratory muscles are recruited.

Exercising Breathing Pattern

The breathing pattern during exercise is quite different than at rest. Ventilation during exercise becomes a complex symphony which must combine and coordinate the talents of multiple orchestras (cardiovascular, nervous, skeletal and pulmonary systems) to meet the increased demand for ventilation, gas exchange, and blood flow, while minimizing the energy costs.

Both V_T and f_b increase during exercise. Although V_T can be up to 8 times greater during maximal exercise than at rest and f_b up to 4 times greater; V_T normally stabilizes at about 50 - 60% of vital capacity while f_b continues to increase with increasing exercise intensity. In fact, V_T may decrease at very high levels of exercise in some subjects, requiring even greater increases in f_b to meet the increase in $\dot{V}_E$ (Rowell & Sheperd, 1996). To meet the increase in f_b, both inspiratory time and expiratory time decrease; however, expiratory time shortens the most.

The increases in V_T initially occur by expiratory muscle recruitment which decreases the EELV. This is efficient because large increases in V_T can occur while breathing on the linear part of the respiratory flow-volume curve (Rowell & Sheperd, 1996). Also, far greater increase in inspiratory muscle work would be required to achieve the same increase in V_T (Rowell & Sheperd, 1996). Nevertheless, the EELV eventually plateaus and further increases in V_T are accomplished by greater inspiratory muscle recruitment which increases the EILV. The determination of EELV is limited by respiratory mechanics and can

even rise in some subjects with expiratory flow limitation (Johnson, Saupe, & Dempsey, 1992) or elite endurance athletes (Fee, Smith, & English, 1997). Increasing EILV may enhance passive chest wall and lung recoil, thereby sparing some expiratory muscle work and may also mitigate expiratory flow limitation.

Relatively little data exists about the recruitment of the specific respiratory muscle groups during exercise hyperpnea. The most recent research indicates that during heavy prolonged exercise the ventilatory contribution of the diaphragm levels off while the contribution of other inspiratory muscles (e.g., external intercostals) continues to increase (Johnson et al., 1992). It also seems inspiratory and expiratory muscles both increase during light to moderate exercise, but as $\dot{V}_E$ increases further expiratory muscle activation increases proportionally more than inspiratory muscle activation (Krishnan, Zintel, McParland, & Gallagher, 1991). This seems unusual given that expiration is flow limited (see work of breathing).

Breathing pattern and $\dot{V}_E$ are not only dependent on the intensity, but duration and mode of exercise as well. Prolonged high intensity endurance exercise exhibits lower V_T and a higher f_b than the shorter steps of incremental exercise of the same intensity, presumably due to the longer duration of endurance exercise. The mechanisms for the tachypnea are unknown, but possibilities include respiratory muscle fatigue and altered respiratory mechanics (Syabbalo, Krishnan, Zintel, & Gallagher, 1994) or perhaps changes in chemoreceptor sensitivity.

The form of exercise can affect the f_b, V_T, and $\dot{V}_E$ and thus the recruitment pattern of respiratory muscles that were involved in producing the changes. For instance, arm cranking results in higher f_b and lower V_T than cycling at equivalent levels of $\dot{V}_E$. Compared to cycling, treadmill running causes a greater gastric pressure and a larger decrease in EELV, which is indicative of greater abdominal muscle activation while running (Henke, Sharratt, Pegelow, & Dempsey, 1988).

Summary

There are a large variety of respiratory muscles and numerous choices of breathing pattern capable of achieving a given $\dot{V}_E$. The choices are influenced by the intensity, duration, and mode of exercise. Presumably, the optimum choice would be the combination that meets the ventilatory requirements with the lowest energy costs. In any case, understanding the interaction of the various respiratory muscles and the numerous breathing patterns involved in maintaining high levels of $\dot{V}_E$ over a long duration, such as during the hyperpnea of exercise, is a daunting task.

Respiratory Muscle Training and Exercise

Overview

The respiratory muscles can be conditioned by nonspecific or specific stimuli. Whole body exercise can cause a nonspecific effect on the muscles involved in ventilation in addition to the training effect on the locomotor muscles.

Specific stimuli for the respiratory muscles can be grouped into two basic categories: (1) flow dependent stimulus (high frequency - low load contractions); and (2) resistance (force) dependent stimulus (high load - low frequency contractions). Of course, the overall conditioning response to these stimuli are dependent on a number of factors (e.g., the nature of the training regime).

A large variety of training exercises exist within the two specific stimuli for conditioning the respiratory muscles. However, researchers examining the respiratory muscles have used primarily two forms of respiratory muscle exercise: (i) voluntary eucapnic hyperpnea, primarily a flow dependent stimulus; and (ii) inspiratory resistive loading, primarily a resistance-dependent stimulus. These respiratory muscle exercises exhibit some crossover characteristics by having stimuli with both flow and resistive (force) components. These crossover *characteristics can be amplified by the training technique and regime.*

Interestingly, training of the expiratory muscles has been largely overlooked. This is because expiration is limited by airway mechanics rather than the pressure generated by the expiratory muscles. The maximal expiratory flow at a given volume is reached well before the pressure generated by the expiratory muscles is maximal. Further increases in pleural pressure are not effective in increasing expiratory flow over a large portion of the flow-volume relationship because of this maximal expiratory flow limitation (Hyatt & Flath, 1966). Despite the fact that the expiratory pressure at which flow limitation is reached is only a small fraction of the pressure the expiratory muscles are

capable of achieving, generally during exercise, they do not generate excessive and inefficient pressures beyond what is required (Henke et al., 1988). While the argument of a greater capacity than necessary to meet demand is not without logic, it is only based on theory. As already discussed, such logic has been inappropriately applied toward the respiratory system as an exercise limiting factor.

The principle of training specificity is an extrapolation of the fundamental principle of specificity (i.e., a specific stimulus for adaptation initiates specific structural and functional changes in specific components of skeletal muscle) partially based on the observations that stress from a particular activity has minimal or no affect other activities (Brooks et al., 1996). A training regime which mimics the requirements of a desired exercise (or task) will elicit specific adaptations that can enhance the performance of that specific exercise. Given that the respiratory muscles are skeletal muscles, it is assumed this principle applies to them; thus, to maximize the benefits (conditioning response) the training of the respiratory muscles should simulate ventilatory conditions that are encountered during the particular exercise. Given that the respiratory muscles primarily work against low loads for the duration of their life span (even during exercise) it seems reasonable that an endurance testing and training regime would be more informative and beneficial than a strength testing and training regime.

Based on the *principle of specificity of training* (Brooks et al., 1996) and that ventilation during exercise is primarily flow-dependent (high-flow and low-resistance respiratory work) it follows that a flow-dependent stimulus would best simulate conditions encountered by the respiratory muscles during exercise. Additionally, the training regime itself should also be performed to simulate techniques which improve endurance; individual sessions should be aerobic in nature, of sufficient duration (20- 30minutes), and repeated several times per week for a time course of several weeks. Therefore, the subjects participating in the present study performed a flow dependent version of respiratory muscle training best referred to as voluntary eucapnic hyperpnea in an endurance-oriented fashion. Although flow-dependent, voluntary eucapnic hyperpnea may have crossover characteristics because high flow rates will increase the resistive load to the respiratory muscles. Because the subjects involved with this research performed voluntary eucapnic hyperpnea, the remainder of this document will focus on voluntary eucapnic hyperpnea endurance training (for simplicity, respiratory muscle training: RMT) and its consequences. The training is not "hyperventilation" because near normal CO_2 (eucapnic) levels are maintained during the RMT sessions by a special training device (see Methods).

Respiratory Muscle Adaptations in Response to Training

Overview

Despite the extraordinary capability of the respiratory system, it was shown over 50 years ago that this capacity could be further increased (Carey et al., 1956; Gilson & Hugh-Jones, 1949). More than a quarter century ago, Leith and Bradely showed that respiratory muscle strength and endurance could "be specifically increased by appropriate ventilatory muscle training programs" (Leith & Bradley, 1976). Furthermore, these adaptive changes in the functional capacity of the respiratory muscles are similar to the adaptations which occur with limb skeletal muscles which have undergone physical exercise training (O'Kroy & Coast, 1993). Like other skeletal muscles, the respiratory muscles respond to high frequency - low load contractions with an endurance conditioning response and to a high load - low frequency stimulus with a strength conditioning response. It is now known that there is a crossover effect between some forms of conditioning stimulus (O'Kroy & Coast, 1993) and this results in a dual-conditioning response for the muscles of ventilation.

As with the locomotor muscles it seems likely that the respiratory muscles would undergo structural and biochemical changes appropriate to specific training stimuli. In humans, measurement of these changes is prohibitive. Such changes could be examined in animals but animals have yet to participate in a specific RMT study. However, studies on animals which examined the effects of

whole-body training on the respiratory muscles (primarily the diaphragm) do exist. Unfortunately, the non-specific nature of the ventilatory load which occurs during whole-body training is likely to have a relatively smaller affect on conditioning the respiratory muscles compared to the specific effect of a RMT program. Despite the obvious shortcomings, animal research must be relied upon to provide indirect insight into the structural and biochemical response of human respiratory muscles to training. Nevertheless, measures of respiratory capacity are possible in humans following specific training of the respiratory muscles.

The literature suggests that the respiratory muscles do exhibit similar adaptations to locomotor muscles after physical training. In humans and animals, the conditioning response of the respiratory muscles arising from *normal whole-body endurance training shows changes in many biochemical and* physical factors, such as, increased oxidative enzymes, improved respiratory muscle endurance and other measures of respiratory capacity, reductions in $\dot{V}_E$ during exercise, and perhaps changes in peripheral and central chemosensitivity.

Training Effects on Enzymes

Most studies have used whole-body endurance training to examine the costal diaphragm of rodents. These studies report increases in mitochondrial enzyme activity (citrate synthase, succinate hydrogenase), antioxidant enzyme activity (superoxide dismutase, glutathione peroxidase), and resting glycogen

levels (Powers & Dempsey, 1996). Glycolytic enzymatic changes also occur; increases in hexokinase and decreases in lactate dehydrogenase have been observed (Reid et al., 1994). Given a strong enough conditioning stimulus, changes in oxidative and antioxidant enzymatic activity have been shown to occur in accessory respiratory muscles too. The crural diaphragm, the parasternal and external intercostal muscles (inspiratory) (Metzger & Fitts, 1986; Moore & Gollnick, 1982; Powers et al., 1990) and the abdominal muscles (expiratory) (Grinton et al., 1992) have all shown significant changes in their enzymatic capacity (Powers, Grinton, Lawer, Criswell, & Dodd, 1992).

Training Effects on Fiber Type

Training-induced shifts in diaphragmatic fiber type have not been found (ATPase histochemistry). However, changes in the myosin heavy chain isoforms (composition) have been demonstrated in the costal diaphragm (Green, Plyley, Smith, & Kile, 1989). The investigators observed a reduction in the percentage of type IIb fibers and an increase in type I and type IIa myosin heavy chains. It is presumed that these adaptations result in functional improvements in the endurance properties of the diaphragm (Fitts, 1994).

The above results on rodents are not unexpected, given the enzymatic plasticity of vertebrate skeletal muscles, and it is tempting to extrapolate them to human respiratory muscles.

Training Effects on Respiratory Functional Capacity

In humans, whole-body endurance training has resulted in improved functional capacity of the respiratory muscles as evidenced by increases in the maximal sustainable ventilatory capacity (MSVC), maximal voluntary ventilation (MVV) and breathing (voluntary hyperpnea) endurance (Clanton, Dixon, Drake, & Gadek, 1987; O'Kroy & Coast, 1993; Robinson & Kjeldgaard, 1982). Robinson and Kjeldgaard (1982), found significant increases in MVV and MSVC after 10 weeks of endurance training. After another 10 weeks (20 weeks total) the subjects improved more. The overall improvements were 13.6% for MVV and 15.8 %MSVC. More recently, Markov et al. (2001), did not report a significant increase in MVV or breathing endurance following whole-body endurance training. However, the breathing endurance improvements were large (but not *significant*), *from* 6.5 *min* (*range* 1.5-15.8 min) *min* pre-training to 9.4 min (range 1.4-40 min) post-training. The surprising lack of statistical significance is likely the result of two factors: 1) the high variability of the breathing endurance test (BET) used; and 2) post-training BET were terminated after 40 min (Markov et al., 2001).

Comparisons between athletes and non-athletes have supported the idea that whole-body training improves respiratory capacity (Coast, Clifford, Henrich, Stray-Gundersen, & Johnson, 1990; Martin & Stager, 1981). For instance, the MVV was shown to be nearly 4 times greater in athletes compared to non-athletes. Furthermore, comparisons between highly trained endurance athletes

and untrained subjects provide clues that endurance training protects the inspiratory muscles from the acute loss of strength following maximal exercise. (Coast et al., 1990).

Training Effects on Exercise Ventilation

As with functional capacity, cross-sectional comparisons between endurance trained athletes and non-athletes have supported the idea that endurance training lowers exercise $\dot{V}_E$ (Martin, Sparks, Zwillich, & Weil, 1979). Other such studies have concluded that physical training can result in larger V_T and decreased f_b during exercise (Mahler, Shuhart, Brew, & Stukel, 1991). The deeper slower pattern of breathing has been indirectly linked to fatigue resistance (see below RMT, Effect on $\dot{V}_E$).

Longitudinal studies have examined changes in exercise $\dot{V}_E$ following endurance training. (Gaesser & Poole, 1986; McParland, Krishnan, Lobo, & Gallagher, 1992; Taylor & Jones, 1979; Yerg, Seals, Hagberg, & Holloszy, 1985). At any given external work rate of $\dot{V}_{O_2}$, the trained person shows a significant reduction in the ventilatory response to exercise. The training effect can be quite large, as much as 20-30% the decrement in total $\dot{V}_E$ or a 4-6 mmHg increase in arterial blood partial pressure of CO_2 (P_aCO_2), when comparisons are made pre- to post-training at a high work rate. (Casaburi, Storer, & Wasserman, 1987; Clanton et al., 1987; Dempsey, Ainsworth, & Forster, 1995). The one study that also examined breathing pattern along with $\dot{V}_E$ found no change in V_T or f_b

following a 4 week cycling endurance training program (McParland et al., 1992). Additionally, this study reported that peak $\dot{V}_E$ at maximal exercise was found to be higher following whole-body endurance training (McParland et al., 1992).

On account of the disagreement between cross-sectional and prospective studies, the effect of endurance training on breathing pattern may warrant further study (*with more comprehensive training programs*). The decrement in $\dot{V}_E$ following endurance training is clear but the cause isn't resolved. It may simply be due to a reduction in the metabolic needs of conditioned respiratory muscles or perhaps it is related to other adaptations such as altered chemosensitivity.

Training Effects on Chemosensitivity

There are some indications that chemosensitivity may adapt to training. Studies have shown both the PC and CC to be affected by endurance training.

A review of the available literature indicates that endurance trained athletes tend to have a reduced hypercapnic ventilatory response at rest ($HCVR_R$) compared to untrained subjects (Mahler, Moritz, & Loke, 1982; Martin et al., 1979; Miyamura, Yamashina, & Honda, 1976; Rebuck & Read, 1971). There seem to be two major problems with these initial investigations linking athletic performance to a reduced chemosensitivity. First, they compared the ventilatory responses while at rest, not during exercise. Based on the principle of specificity, it would seem a response made during exercise would be a better measure of events happening during exercise. Also, it is generally accepted that

the ventilatory responsiveness is increased during exercise (especially in hypoxia) compared to rest. The second potential problem is that this assumption is based on the indirect relationship from research which relied on comparisons between untrained subjects and athletes. Cross-sectional studies of this nature can only provide an indirect link between training and chemosensitivity. Although it is possible that the decreased chemosensitivity is acquired as a result of endurance training, it has been shown that decreased hypoxic drive (and to a lesser extent hypercapnic ventilatory responses) are common in untrained relatives of accomplished athletes (Scoggin, Doekel, Kryger, Zwillich, & Weil, 1978). Thus, a familial or genetic link for the reduced chemoreceptor response found in these studies cannot be ruled out.

Clearly, studies that prospectively examined the effect of a conditioning program on the chemosensitivity of the subjects would be better suited for determining the effect of endurance training on chemosensitivity.

There are five such prospective studies which examined the effects of training on the $HCVR_R$. Two studies showed a decreased $HCVR_R$ following extensive training programs (Blum, Kanarek, Callahan, Braslow, & Kazemi, 1979; Miyamura & Ishida, 1990). One found an increase in CO_2 sensitivity (Kelley, Laufe, Millman, & Peterson, 1984), and two found no change (Bradley, Mestas, Forman, & Unger, 1980; Katayama et al., 1999). The most recent study done by Miyamura and Ishida (1990), is perhaps the best designed and provides the most provocative results. Not only did they show a consistently reduced

chemosensitivity over three years of training, but when the subjects underwent detraining the CO_2 ventilatory responsiveness increased.

A recent study reported a reduced PC sensitivity following endurance exercise training (Katayama et al., 1999). Although another study reported no change (Levine et al., 1992). These investigators measured the hypoxic ventilatory response at rest to test PC sensitivity. However, the hypoxic ventilatory response and thus the PC sensitivity may be increased only during exercise (Weil et al., 1972). Also, it is known that the PC respond to stimuli other than hypoxia (e.g., CO_2, H^+, and K^+) (Nye, 1994) and the hypoxic ventilatory response does not lend insight to the effects of these other natural stimuli on the PC. In conclusion, the effects of endurance training on CC sensitivity, or combined CC and PC sensitivity, have been confounded by several labs reporting increases, decreases, or no change in chemosensitivity following endurance training programs. Therefore, the true effect of endurance training on the complete PC response (and even hypoxic ventilatory response) during exercise has not been thoroughly examined. Finally, the mechanism(s) behind these changes and their relevance to endurance exercise remain unclear.

Summary

A large body of research exists which indicates the human respiratory muscles respond to endurance training in a fundamentally similar fashion as other skeletal muscles (Roussos & Macklem, 1985). In fact, few studies suggest

otherwise. This makes it tempting to speculate that the evidence presented above implies that the human respiratory muscles react to endurance training in a nearly identical way to skeletal muscles (e.g., in a dose-response fashion). However, making such a conclusion is premature because the direct evidence is lacking. Nevertheless, what has been presented certainly shows that there are many similarities between skeletal and respiratory muscle which suggests the potential for such a connection.

RMT and Whole-Body Exercise Performance

In the late eighties and early nineties, several papers directly addressed the effects of RMT on exercise performance using healthy young adults exercising under normal environmental conditions (the default criteria for human exercise studies). Table 1 includes studies that performed some form of voluntary eucapnic hyperpnea endurance training with healthy and sometimes diseased subjects (Ries & Moser, 1986). Studies using inspiratory resistive loading have been omitted.

A review of this research shows a wide disparity between results (Table 1). Some researchers reported substantial improvements in exercise performance (Boutellier et al., 1992; Boutellier & Piwko, 1992; Markov et al., 2001; Sonetti, Wetter, Pegelow, & Dempsey, 2001; Spengler et al., 1999) while others did not find improvements in performance (Fairbarn, Coutts, Pardy, & McKenzie, 1991; Morgan, Kohrt, Bates, & Skinner, 1987), and one study had a

Table 1. Summary of RMT studies

STUDY	SUBJECTS	RMT INTERVENTION	RESPIRATORY CAPACITY Δ	EXERCISE MODE	EXERCISE CAPACITY Δ
Leith & Bradley (1976)	Normal n = 4	3-5 variable duration intervals to exhaustion: 5 d/wk, 5 wk	19% ↑ l/min MSVC (S)	Does Not Apply	Does Not Apply
O'kroy & Coast (1993)	Normal n = 6 other groups too	70% MVV, for 20min 4 d/wk, 4 wk	> 10% ↑ l/min MSVC (S)	Does Not Apply	Does Not Apply
Markov et al. (1996)	Active C: n = 8 E: n = 8	30-45 b/min, ↑ b/min 30min 5 d/wk, 4-5 wk 20-24 total	C: +1% l/min MVV (NS) -1% l/min BET (NS) E: +15% l/min MVV (NS) +350% ↑ l/min BET (S)	Does Not Apply	Does Not Apply
Keens et al. (1977)	C: n = 7 E: n = 4 CF: n = 4	"Maximal" intensity 25 min/d 5 d/wk, 6 wk	C: 0% l/min MSVC (NS) E: +22% ↑ l/min MSVC (S) CF: +57% ↑ l/min MSVC (S)	Does Not Apply	Does Not Apply
Belman & Mittman (1986)	COPD n = 10	2 x 15min MSVC 5 d/wk, 6 wk	+33% ↑ l/min MSVC (S)	12 min walk Legs Cycling Arms Cycling	+12% ↑ m (S) +54% ↑ min (S) +82%↑ min (S)
Ries & Moser (1993)	COPD n = 5	3 x 15min MSVC daily, 6 wk	+29% ↑ l/min MSVC (NS)	12 min walk Endurance walk	+8% m (NS) p < 0.07 +21% min (NS)
Mancini et al. (1994)	Congestive heart failure C: n = 6 E: N = 8	90 min 3d/wk 12wk Endurance: 20 min at MSVC Resistance: 20min 30% Max. inspiratory pressure	C: -6% l/min MSVC (NS) E: +57% ↑ l/min MSVC (S)	6 min walk	C: +2% m (NS) E: +29% ↑ m (S)
Morgan et al. (1987)	Trained Cyclists C: n = 5 E: n = 4	85% MVV, increasing duration, 5 d/wk, 5 wk	C: 0% l/min MVV (NS) 0% min BET (NS) E: +14% ↑ l/min MVV (S) +1575% ↑ min BET (S)	Cycling 95% VO_2max (~8min)	C: -8% min (NS) E: -6% ↓ min (NS)
Belman & Gaesser (1988)	Elderly C: n = 13 E: n = 12	2 x 15min ~90-100% MSVC 4 d/wk, 8 wk	C: +10% l/min MVV (NS) +2% l/min MSVC (NS) E: +17% ↑ l/min MVV (S) +20% ↑ l/min MSVC (S)	12 min walk	No significant changes

Table 1. Summary of RMT studies

STUDY	SUBJECTS	RMT INTERVENTION	RESPIRATORY CAPACITY Δ	EXERCISE MODE	EXERCISE CAPACITY Δ
Fairbarn et al. (1991)	Trained Cyclists C: n = 5 E: n = 5	3 x 8-10min ≥MSVC 3-4 d/wk, 16 total	C: -4% l/min MSVC (NS) E: +12% ↑ l/min MSVC (S)	Cycling 90% Peak Power (~6min)	C: +4% min (NS) E: +25% min (NS) (1 of 5 improved)
Boutellier & Piwko (1992)	Sedentary n = 4	58-63% MVV, ↑ l/min 20-30min 5 d/wk, 4 wk	+2% l/min MVV (NS) +268% ↑ min BET (S)	Cycling, 64% VO_2max, 27min	+50% ↑ min (S) (4 0f 4 improved)
Boutellier et al. (1992)	Trained n = 8	55-68% MVV, ↑ l/min 30min 5 d/wk, 4 wk	+6% ↑ l/min MVV (S) +555% ↑ min BET (S)	Cycling 77% VO_2max (~23min)	+38% ↑ min (S) (7 of 8 improved)
Kohl et al. (1996)	Normal n = 8	50% MVV, ↑ l/min 30min 5 d/wk, 4 wk, 19 total	+360%↑ min BET (S)	Cycling 74% VO_2max (~22min)	-19% ↓ min (S) (6 of 8 worsened)
Spengler et al. (1999)	Trained n = 20	60-85% MVV, 30min 5 d/wk, 4 wk	+19% ↑ l/min MVV (S) +532% ↑ min BET (S)	Cycling 85% Peak Power (~21min)	+27% ↑ min (S) (16 of 20 improved)
Markov et al. (2001)	Trained C: n = 15 E: n = 13	65->79% MVV, 30min for 15 wk, 40 total	C: +7% l/min MVV (NS) -25% min BET (NS) E: +6% l/min MVV (NS) >+770% ↑ min BET (S)	Cycling 70% Peak Power (~33-37min)	C: -1% min (NS) E: +24% ↑ min (S)
Sonetti et al. (2001)	Trained P: n = 9 E: n = 8	50-60% MVV, increased to "Maximal", 30 min 5d/wk 5wk and ~4-5min resistive training 25 total	P: -1% l/min MVV (NS) +78% min 90% BET (NS) E: +1% l/min MVV (NS) +74% min 90% BET (NS)	Cycling A) TT (13min) B) 80-85% Peak Power (~16min)	P: 0% TT (NS) +16% ↑ B (S) E: +1.8% ↑ TT (S) +26% ↑ B (S) 8:9 and 9:9 improved
McMahon et al. (2002)	Trained cyclists C: n = 10 E: n = 10	60->79% MVV, 30min for 4-6 wk, 20 total	C: -1% l/min MVV (NS) +4% min BET (NS) E: +11% ↑ l/min MVV (NS) >+259% ↑ min BET (S)	Cycling 85% Peak Power (~15-20min)	C: -8% min (NS) E: +15% ↑ min (S) (7 of 10 improved)

C = control; E = RMT; P = placebo; CF - cystic fibrosis; COPD - chronic obstructive pulmonary disease; MSVC - maximal sustainable ventilatory capacity for 15 min; MVV - maximal voluntary ventilation; BET - breathing endurance test (~70-75% MVV); MIP - maximal inspiratory pressure; TT - time trial; l - liter; b - breaths; min - minute; d - day; wk - week; NS = not significant; S = significant ($p < 0.05$);

significant decrement in cycling performance (Kohl, Koller, Brandenberger, Cardenas, & Boutellier, 1996). It has been suggested (Sheel, 2002) that the differing results may be explained by differences related to the number and fitness of subjects involved in the studies, the intensity at which the exercise performance tests were performed, the variability or type of the performance measure, the training regime, and whether or not control groups participated.

Several patterns emerge when the impact of these factors on the success of RMT in improving performance is examined more closely. First, RMT improved performance at high-intensity (but sub-maximal) workloads, but did not improve performance at near-maximal or maximal workloads. All the studies used a fixed-rate cycling endurance test (CET) as a measure of performance. These fixed-rate tests have a high variability, with a coefficient of variation ranging from 17 to 40%. One study used a cycling time-trial as the performance measure (Sonetti et al., 2001). Time-trial tests have a very high reliability and more closely resemble actual competitions compared to fixed-rate tests. This makes them a better measure of performance and partially explains why, following RMT, performance improvements by time-trial cycling tests were much smaller (+1.8 ± 1.2%) than for fixed-rate (26.4 ± 18.2%) cycling tests (Sonetti et al., 2001). The one advantage of a fixed-rate test is that the constant work load does allow for direct comparison of physiological markers before and after an intervention (see respiratory muscle training and its physiological effects).

Curiously, performance measurements were always done while cycling, and all studies exhibiting significant improvements following RMT came solely from Boutellier's group. Finally, neither sample size nor the use of a control group seemed to impact performance, as different studies with or without control groups and decidedly small or somewhat larger sample sizes did and did not show significant performance improvements.

The importance of good scientific rigor cannot be overlooked. Using a control group is necessary to a well designed study, optimally a placebo training group also (using a mock device or training protocol). Furthermore, a highly repeatable measure of performance should be employed to increase the statistical power and to help reduce the number of subjects needed to achieve this to a minimum. None of the studies in table 1 met these standards. Sonetti et al. (2001) were closest to meeting these criteria. They used a time-trial (high repeatability; CV range of 1.0% - 3.4%) for a performance measure and a placebo group (sham training), but no control group (Sonetti et al., 2001).

Despite these shortcomings (lack of scientific purity/perfection), it seems reasonable to conclude that RMT is beneficial in improving performance in some subjects. Perhaps RMT may be an example of an intervention for which there are responders and non-responders. Something unique to the "responders" could cause the respiratory system to be a limiting factor for endurance exercise.

RMT and its Physiological Effects

As mentioned in the previous section, some RMT programs have been accompanied by improved cycling performance. Increased exercise performance is generally attributed to improvements to skeletal muscle and the cardiovascular system. The majority of RMT studies have not shown significant changes in indicators of skeletal muscle and cardiovascular function such as maximal $\dot{W}$, $\dot{V}_E$, $\dot{V}_{O_2}$, and heart rate (f_C), during incremental exercise to exhaustion (Boutellier et al., 1992; Fairbarn et al., 1991; Markov, Orler, & Boutellier, 1996; Morgan et al., 1987; Spengler et al., 1999). Sonetti et al., (2001) reported increases in $\dot{W}$, $\dot{V}_E$, but as with other studies no changes with $\dot{V}_{O_2}$ and f_C Also, no study has demonstrated changes for $\dot{V}_{O_2}$ and f_C during sub-maximal fixed-rate exercise (Boutellier et al., 1992; Kohl et al., 1996; Sonetti et al., 2001; Spengler et al., 1999). Furthermore, at sub maximal work rates, respiratory exchange ratio, cardiac stroke volume and output, blood gas concentrations, or oxyhemoglobin saturation were not affected by RMT (Markov et al., 2001; Stuessi, Spengler, Knopfli-Lenzin, Markov, & Boutellier, 2001). Taken together, these results show that cardiovascular conditioning, the hallmark adaptation to the metabolic response of normal whole-body exercise, is not altered by RMT.

However, during sub-maximal tests some studies reported reductions in blood lactate concentrations ([La]) (Boutellier & Piwko, 1992; Kohl et al., 1996; Spengler et al., 1999), while other studies reported no change in [La] (Boutellier

et al., 1992; Sonetti et al., 2001). Not all studies investigating RMT measured [La], but the inconsistency of these findings suggests that [La] is not the likely mechanism for enhanced endurance performance.

The effects of RMT on exercise $\dot{V}_E$ have been conflicting. Studies have reported increases, decreases or no changes in $\dot{V}_E$ following RMT. To help understand the varying results it is important to understand when $\dot{V}_E$ was analyzed. The major sections which can be distinguished for analysis of $\dot{V}_E$ are the quasi-steady state (middle) and the end portions of the CET tests and the peak $\dot{V}_E$ measured at maximal intensity (end) during an incremental test. Generally, the peak $\dot{V}_E$ has been reported to increase during the incremental (maximal) or the final portion of CET (Boutellier et al., 1992; Kohl et al., 1996; Sonetti et al., 2001; Spengler et al., 1999). Kohl et al, (1996) found an increased $\dot{V}_E$ during the middle portion (quasi-steady state) of the CET as well (Kohl et al., 1996). Spengler et al. (1999), and Sonetti et al. (2001), observed no such change in $\dot{V}_E$ during this quasi-steady state of the fixed-rate cycling test. Two other studies reported no change in $\dot{V}_E$ of any type (Fairbarn et al., 1991; Morgan et al., 1987). In other studies, the Boutellier group reported a reduction in $\dot{V}_E$ throughout the CET in trained subjects (Boutellier et al., 1992), and they observed $\dot{V}_E$ to plateau. This plateau was at a lower level of $\dot{V}_E$ after RMT in sedentary subjects (Boutellier & Piwko, 1992). However, a more recent study from the same group reported that the quasi-steady state $\dot{V}_E$ did not change in sedentary subjects after RMT (Stuessi et al., 2001).

A more consistent finding of interest is the relationship between $\dot{V}_E$ during the CET and the end-times of the CET. In trained and untrained subjects alike, the changes in $\dot{V}_E$ during the latter portions of the CET have tended to correlate with changes in the end-times themselves. Subjects that had reduced the exercise $\dot{V}_E$ during the latter portions of the CET also had the tendency to proportionally increase their end-times and those subjects that increased the exercise $\dot{V}_E$ had corresponding decreases in the cycling end-times (Boutellier et al., 1992; Boutellier & Piwko, 1992; Kohl et al., 1996; Spengler et al., 1999). More specifically, the relative decrease in $\dot{V}_E$ (compared at a $\dot{V}_E$ corresponding to the end-time of the pre-training CET) correlated with the relative increase in end-time (Spengler & Boutellier, 2000). Interestingly, the subjects who showed improvements in exercise endurance had a delayed hyperventilatory response to exercise (the increase in breathing frequency often observed at the end of exhaustive exercise; i.e., rapid shallow breathing). Those subjects' whose end-times weren't improved exhibited an exaggerated hyperventilatory response near exhaustion.

Two studies have reported scientific results on perceived respiratory exertion and the results are conflicting. Sonetti et al., (2001) observed no change in dyspnea ratings while Spengler et al., (1998) found a decrease in the sensation of respiratory exertion. Boutellier at al., (1992) mentioned an elimination of breathlessness during uphill running and cycling but this was purely a preliminary observation based on the subjects' personal comments.

A study by Kohl et al., (1996) examined the effect of exercise-induced hyperventilation on airway resistance before and after RMT. They observed no change. Markov et al., [1996 #232] investigated the effect of RMT on the hypoxic ventilatory response at rest. The hypoxic ventilatory response was used as an indication of the peripheral chemoreceptor response (pR_c). They found no alteration in the hypoxic ventilatory response.

The most consistent findings on the effects of RMT are on the functional capacity of the respiratory system. Pulmonary function has been shown to be unaffected by RMT. With near uniformity, pulmonary function measurements (vital capacity, forced expiratory vital capacity, forced expiratory volume in 1 s, and peak expiratory flow) are unchanged (Boutellier et al., 1992; Boutellier & Piwko, 1992; Fairbarn et al., 1991; Markov et al., 1996; 2001; Morgan et al., 1987; Sonetti et al., 2001; Spengler et al., 1999). Two studies have reported slight increases in VC (Leith & Bradley, 1976; McMahon, Boutellier, Smith, & Spengler, 2002). The inconsistency of these small changes makes their relevance questionable (Sonetti et al., 2001).

Measures of the endurance capacity of the respiratory system (MSVC or BET) have shown significant improvements following RMT programs (Boutellier et al., 1992; Boutellier & Piwko, 1992; Fairbarn et al., 1991; Kohl et al., 1996; Markov et al., 1996; Markov et al., 2001; Morgan et al., 1987; Sonetti et al., 2001; Spengler et al., 1999) in all but one instance (Sonetti et al., 2001). Another measure of the respiratory system's functional capacity, the MVV, has not

provided such consistent results. About half of the studies have reported MVV improvements (Boutellier et al., 1992; Morgan et al., 1987; Spengler et al., 1999) and the other half no changes (Boutellier & Piwko, 1992; Markov et al., 1996; Sonetti et al., 2001).

The relationship between $\dot{V}_E$ and the end-times of the CET appears to be the unifying factor associated with RMT. The postponement of the final hyperventilation (increase in f_b) during exhaustive exercise is the one consistent link to explain the improvements in endurance exercise reported in previous RMT studies. Interestingly, a similar rapid shallow breathing pattern has been reported following inspiratory muscle fatigue, either with (Sliwinski, Yan, Gauthier, & Macklem, 1996) or without (Gallagher, Hof, & Younes, 1985; Mador, 1991) concurrent increases in $\dot{V}_E$.

Exercise and Chemoreceptor Response

Overview

Ventilatory control is complex, and ventilatory control during exercise is an even more intricate process. One of the longest-standing and most difficult problems in exercise physiology is understanding and quantifying the major mechanisms controlling the respiratory system during exercise. The respiratory controller must integrate a complex variety of excitatory and inhibitory inputs in regulating the hyperpnea of exercise. These inputs are nonsensory (central command) and sensory (respiratory and nonrespiratory) in nature. An example

of the nonsensory inputs is a feedforward mechanism from the suprapontine brain (central command) and a short-term potentiation of the neural respiratory response to any stimulation (Rowell & Sheperd, 1996). The sensory inputs can be both non-respiratory or respiratory in origin. Non-respiratory feedback includes the chemical and mechanical effects of muscular activity and movement, effects of changes in cardiac output, pulmonary blood flow, and body temperature. Respiratory feedback mechanisms include the effects of reflexes associated with the respiratory muscles and lung movement, and the effects of arterial gas pressures, pH, and other (blood borne) substances on the PC and CC.

Peripheral Chemoreceptor Response

The carotid bodies are located peripherally at the bifurcation of the carotid arteries. They consist of special cells which resemble a chemically sensitive synapse and are receptive to several chemical stimuli (hypoxia, hypercapnia, hyperkalemia, and acidosis). The precise mechanism by which these stimuli activate the PC seems to be by causing an increase in intracellular calcium ions (Nye, 1994).

At natural levels of the major stimuli, the PC exhibit a low-level and irregular pattern of sensory discharge (Nye, 1994). The sensory discharge response curves to the stimuli are known and likely are similar to the changes in ventilation associated with these stimuli in terms of their thresholds, etc. The

changes in $\dot{V}_E$ resemble the changes in PC sensory discharge which resemble the changes in stimuli. The PC response to all natural stimuli can be essentially eliminated by breathing hyperoxic gas (> 50% O_2) (Dejours, 1962).

The contribution of the PC to the ventilatory drive is effectively eliminated by inspiring one to two breaths of 100% O_2 (Dejours, 1962). "The magnitude of the transient ventilatory decline, the nadir of which is reached in 20s after hyperoxic exposure, is a measure of the ventilatory drive arising from the peripheral chemoreceptors before the hyperoxic bout" (Ward, 1994a). A modification of the Dejour's method of hyperoxic suppression is the most common method for estimating the contribution of the carotid bodies to the ventilatory drive (Dejours, 1962; Dejours, 1963). Early electrophysiological studies on animals described by Dejours (Dejours, 1962) confirms that the sensory discharge from the PC is, for all practical purposes, abolished. Thus, we used this test to isolate the effect of RMT on the pR_c.

It has been suggested that exercise itself may lead to an increased chemosensitivity (Weil et al., 1972). In this way a constant level of oxygen or carbon dioxide in the blood (or even a slightly reduced level) could provide a greater stimulus to breathe. Indeed, it is a common observation that ventilation is depressed more by oxygen administration during exercise than while at rest, suggesting the PC contribution to ventilation is enhanced during exercise (Weil et al., 1972). Additionally, some studies have measured the effects of hypoxia

directly during light exercise (Flenley & Warren, 1983; Weil et al., 1972), further confirming this effect of increased chemosensitivity during exercise.

As mentioned above, a previous study by the Boutellier group investigated the resting response to hypoxia following respiratory muscle training (Markov et al., 1996). From those results it appears that the HVR at rest is unaltered by a respiratory muscle training program and is thus an unlikely mechanism for the reported alterations in cycling endurance time. However, the HVR only accounts for one of the stimuli to the carotid bodies and, in addition, the response was measured at rest as opposed to exercise. So, the question of the effect of RMT on the complete peripheral chemoreceptor response (pR_c) still remains, particularly during exercise.

Cross-sectional studies have looked at the effects of exercise and endurance training on the pR_c. Endurance athletes have a lower peripheral chemoreceptor sensitivity than untrained individuals (Weil & Swanson, 1991). Recently, Katayama et al., (1999) demonstrated a reduction in HVR following endurance training. The specific mechanism for this reduced pR_c is unresolved, but the study does demonstrate the role of a training component in the reduced HVR in athletes and also the potential plasticity of the PC to training.

The role of the PC in the control of ventilation during exercise is controversial. Perhaps this is partially because past studies used resting measurements of the pR_c and applied them to exercise or the methods used did not account for the pR_c to all their natural stimuli. In this study, we took these

factors into account and chose to examine the pR_c using a modified Dejours O_2 test during exercise.

Central Chemoreceptor Response

The central respiratory drive comes from within the dorsal and ventral respiratory groups of the medulla oblongata (brain stem) (Mateika & Duffin, 1995). These brain stem respiratory neurons or CC are sensitive to hydrogen ion ([H+]) in the cerebral spinal fluid, which is rapidly altered by arterial PCO_2. Other sites within the brain of cats, e.g., caudal hypothalamus, also respond to CO_2 and may affect $\dot{V}_E$ (Mateika & Duffin, 1995). The CC apparently respond to increasing levels of $[H^+]$ or PCO_2 in the cerebral interstitial fluid by increasing ventilation. During exercise, PCO_2 is believed to be the primary regulator because H^+ does not readily traverse the blood-brain-barrier.

The hypercapnic ventilatory response (HCVR) is a way of measuring the central chemoreceptor response (cR_c) but in conjunction with the pR_c to CO_2. If the HCVR is measured in a background of O_2 (> 200 mm Hg), then the PC activity is essentially abolished and the stimulus:response curve obtained is an index of the cR_c alone, albeit in a hyperoxic background. Ideally, the site of measurement for the stimulus to the increased fractional concentration of inspired carbon dioxide (F_ICO_2) would be the pH near the choroid plexus surface, but this is not feasible in humans, so end-tidal or at best arterial PCO_2 is used. Thus problems may arise given hyperoxia and that the measured stimulus

is not the same as the actual stimulus. Nevertheless, the HCVR is widely accepted and used as a test of cR_c.

The value of S, in the hyperoxic background, is representative of the cR_c. The relationship between the end-tidal partial pressure for CO_2 ($P_{ET}CO_2$) and $\dot{V}_E$ can be analyzed by least squares linear regression with the following equation:

$$\dot{V}_E = S(P_{ET}CO_2 - B)$$

where S is the slope of the best fit line relating a change in $P_{ET}CO_2$ to a change in $\dot{V}_E$ and B is the extrapolated x-axis ($P_{ET}CO_2$) intercept when $\dot{V}_E = 0$. The slope of the CO_2 response lines ($\Delta\dot{V}_E/\Delta P_{ET}CO_2$) are denoted S_R for the slope of the HCVR at rest or S_{EX} for the slope during exercise and have the units l/min/mm Hg. The X - axis intercepts (at theoretical zero $\dot{V}_E$) at rest are denoted by B_R and during exercise by B_{EX} and are expressed with the units mm Hg. By convention, the x - axis intercept is chosen for B because physiologically $\dot{V}_E$ can never be less than zero.

The available literature has been unable to establish a clear and consistent difference in the slope of the HCVR in trained or untrained subjects (either at rest or during exercise). A number of studies have shown increases (Cummin, Alison, Jacobi, Iyawe, & Saunders, 1986; Miyamura et al., 1976; Weil et al., 1972), decreases (Clark & Godfrey, 1969), or no change (Duffin, Bechbache, Goode, & Chung, 1980; Kelley, Owens, & Fishman, 1982) in the HCVR from rest to exercise. These findings are even more confounding when the fact that one laboratory has produced all three variety of results itself (Jacobi,

Iyawe, Patil, Cummin, & Saunders, 1987; Jacobi, Patil, & Saunders, 1989a; Jacobi, Patil, & Saunders, 1989b). This may be due to the different methods used for determining the HCVR. It may also be that the difficulty arises from the estimation of P_aCO_2 from end-tidal measurements, making it advisable to use the $P_{ET}CO_2$ as opposed to estimating P_aCO_2 (J. Dempsey, personal communication, October 1999). The most useful information concerning the ventilatory response to PCO_2 may have to do with very small changes in PCO_2 about its normal set point (Cummin et al., 1986; Jacobi et al., 1987). The only certainty is that the HCVR data on the cR_c are inconclusive and contradictory.

What is clear is the X - axis intercept of the HCVR is shifted to the left when comparing rest to exercise (Koepchen, 1975). The meaning or mechanism for this has not been described in the literature. One possibility is heightened nervous activity (i.e., muscle sympathetic nervous activity) which is thought to play a role in the initial increase of ventilation at the onset of exercise and changes in chemosensitivity between rest and exercise. Muscle sympathetic nervous activity is also described as playing a role, via vasoconstriction, in the competition for blood flow between respiratory and limb muscles during very high-intensity and maximal exercise (Harms & Dempsey, 1999; Harms et al., 1997; Wetter, Harms, Nelson, Pegelow, & Dempsey, 1999).

In addition to a heightened chemosensitivity in exercise compared to rest, a review of the available literature indicates that endurance trained athletes tend to have a reduced chemosensitivity at rest compared to untrained subjects

(Mahler et al., 1982; Martin et al., 1979; Miyamura et al., 1976; Rebuck & Read, 1971). Several studies have noted a correlation between the magnitude of the ventilatory response to exercise ($\Delta\dot{V}_E/\Delta V_{CO_2}$) and the ventilatory sensitivity to inspired carbon dioxide ($\Delta\dot{V}_E/\Delta P_{CO_2}$), both at rest (D'Urzo, Chapman, & Rebuck, 1987; Martin, Weil, Sparks, McCullough, & Grover, 1978; Rebuck & Read, 1971) and during exercise (McConnell & Davies, 1992). McConnell et al. (McConnell & Davies, 1992) noted a stronger correlation between $\Delta\dot{V}_E/\Delta V_{CO_2}$ and CO_2 sensitivity during exercise than at rest. Again, one cannot overlook the potential of a familial (genetic) link to changes in chemosensitivity of endurance athletes when cross-sectional studies are used. However, some prospective studies have demonstrated decreased chemosensitivity as a physiological effect of endurance training as well (Blum et al., 1979; Miyamura & Ishida, 1990). A review of the studies examining the effects of endurance training on cR_c, or combined the CC and PC sensitivity, have reported increases (Kelley et al., 1984), decreases (Blum et al., 1979; Miyamura & Ishida, 1990), or no change (Bradley et al., 1980; Katayama et al., 1999) in chemosensitivity following endurance training programs. These discrepancies are probably do to the different methods and testing procedures used and the high variability of the HCVR (McConnell & Semple, 1989).

In summary, although it is has been shown that the decreased chemosensitivity is acquired as a result of endurance training, it has been shown that decreased hypoxic, and to a lesser extent hypercapnic, ventilatory

responses are common in untrained relatives of accomplished athletes. This strongly suggests a familial or genetic link and this is indeed the most generally accepted view. However, from my review of the literature, it seems that neither possibility can be ruled out. The effect of physical training on the ventilatory response to hypercapnia (and hypoxia) is still open to debate.

The specific cR_c can be assessed by measuring the ventilatory response to changes in CO_2 in a background of hyperoxia. This study employed such a method, using the HCVR measured in a background of 50% oxygen, by a steady-state method during rest and exercise. Perhaps the reduction in exercise $\dot{V}_E$ could be caused by a decreased chemoreceptor response. In this RMT study we simulated exercise hyperpnea while avoiding the affects of an increase in metabolism that accompanies whole-body exercise, to test the hypothesis that the hyperpnea of exercise itself is responsible for lowering the cR_c.

Summary

Many muscles are involved with ventilation. Some of these muscle can have both an inspiratory and expiratory function and others also play a supporting role as postural muscles or even for propulsion during exercise. The interaction of the respiratory muscles during breathing, especially the hyperpnea of exercise, is enormously complex.

The respiratory muscles can be considered to be skeletal muscles which have developed to be specifically well suited for their life sustaining role. In

general, they are fatigue resistant with a high proportion of fatigue resistant fibers (type I and type IIa), are highly vascularized, and have a high oxidative enzyme capacity. These properties enable them to contract rhythmically in the ceaseless process of moving air in and out of the lungs and make them particularly well suited to adaptations in response to training.

There are numerous choices of breathing pattern capable of achieving a given $\dot{V}_E$. Unraveling the interactions of the various respiratory muscles and the numerous breathing patterns involved in maintaining $\dot{V}_E$ over a long duration, especially in meeting the demands of exercise hyperpnea, is a daunting task and may never be completely understood.

Somewhat surprisingly, the respiratory muscles have a relatively low efficiency of about 10%. This suggests the potential for improvement in respiratory muscle efficiency is considerable when compared to the efficiency of other muscle groups (~25%). Small improvements in efficiency could translate into small but important improvements in performance. Changes in the metabolic cost of breathing or in respiratory muscle efficiency (mechanical work of breathing / oxygen cost of breathing) have not been measured in conjunction with a RMT regime.

It is tempting to speculate that the evidence presented above implies that the human respiratory muscles react to endurance training in near identical way to skeletal muscles (e.g., in a dose-response fashion). Indeed, a large body of research exists which indicates the human respiratory muscles respond to

endurance training in a fundamentally similar fashion to other skeletal muscles (Roussos & Macklem, 1985).

A review of RMT studies provides some evidence that RMT is beneficial in improving performance in some subjects, although all affirmative studies came entirely from one lab. Perhaps RMT may be an example of an intervention for which their are responders and non-responders. For these "responders", the respiratory system may be endurance exercise limiting factor to some extent.

The relationship between $\dot{V}_E$ and the end-times of the CET appears to be the unifying factor associated with many RMT studies. The postponement of the final hyperventilation (increase in f_b) during exhaustive exercise could be the finding which unlocks the explanation for the improvements in endurance exercise.

Any reductions in $\dot{V}_E$ could be related to improvements in ventilatory efficiency. Improvements in ventilatory efficiency could spare resources that could then be made available to the locomotor muscles. These changes may affect or be mediated by respiratory control mechanisms, such as the PC and CC, which will be the focus of this study.

For instance, a reduction in exercise $\dot{V}_E$ could be caused by an altered chemoreceptor response. The role of the PC and CC in the control of ventilation during exercise is not completely understood and somewhat controversial. Research supports that the pR_c and cR_c are heightened during exercise

compared to rest. There are also some indications that the pR_c and cR_c may be reduced by whole-body endurance training, but the results are ambiguous.

Perhaps this is partially because past studies used resting measurements of the pR_c and applied them to exercise or the methods used did not account for the pR_c to the full range of natural stimuli. In this study, we took these factors into account and chose to examine the pR_c using a modified Dejours O_2 test during exercise. Measurement of the specific cR_c, requires that the PC be inactive. To isolate the cR_c from the pR_c this study employed the HCVR in a background of 50% oxygen, by a steady-state method during rest and exercise.

Based on the principle of specificity of training (Brooks et al., 1996) and because ventilation during exercise is primarily flow dependent, a flow dependent stimulus would best simulate conditions encountered by the respiratory muscles during exercise. Additionally, the training regime itself should facilitate endurance adaptations to the respiratory muscles. This can be done by a mode of respiratory muscle exercise called voluntary eucapnic hyperpnea. The stimulus elicited by voluntary eucapnic hyperpnea is highly specific to the respiratory muscles. When performed in way to emulate an endurance conditioning program, voluntary eucapnic hyperpnea is known as respiratory muscle training (RMT). With RMT it is possible to simulate or even exaggerate the $\dot{V}_E$ achieved during exercise while minimizing or removing the concurrent metabolic and cardiovascular stresses of endurance exercise. By simulating exercise hyperpnea while avoiding the affects of an increase in

metabolism that accompanies whole-body exercise, it is possible to test the hypothesis that the hyperpnea of exercise itself is responsible for lowering the pR_c and cR_c that reportedly occurred following whole-body endurance training in some studies.

Rationale/Statement of Problem

Some very notable similarities exist between respiratory muscles and skeletal muscles and between RMT and whole-body endurance training. The respiratory muscles are functionally, morphologically, and embryologically skeletal muscles (NHLBI, 1990), and they too, can be trained for strength and endurance (Leith & Bradley, 1976; O'Kroy & Coast, 1993; Tzelepis et al., 1994). RMT leads to an improved breathing endurance in sedentary (Belman & Mittman, 1980; Boutellier & Piwko, 1992; Keens et al., 1977; Leith & Bradley, 1976), as well as in physically active subjects (Boutellier et al., 1992; Fairbarn et al., 1991; Morgan et al., 1987). RMT can improve submaximal constant load exercise not only in patients (Belman & Mittman, 1980; Mancini, Henson, La Mancha, Donchez, & Levine, 1995), but also in sedentary (Boutellier & Piwko, 1992) as well as in physically active subjects [Boutellier, 1992 #37; (Sonetti et al., 2001; Spengler et al., 1999) (see Table 1).

Following RMT programs, hyperpnea during exercise has been slightly reduced in some instances, resulting in a lower minute ventilation, corresponding higher $P_{ET}CO_2$ levels (Boutellier & Piwko, 1992) and enhanced cycling

performance (Boutellier et al., 1992; Boutellier & Piwko, 1992; Markov et al., 2001; Spengler et al., 1999). Importantly, the changes in end-time $\dot{V}_E$ during the latter portions of the CET have shown the tendency to be correlated to the changes in the end-times of the CET themselves which implies $\dot{V}_E$ plays an active role in determining performance.

Similarly, the skeletal muscles' adaptations to a whole-body endurance training program is an improved performance in the mode of exercise trained. Whole-body endurance training has also resulted in a reduced exercise $\dot{V}_E$ for a given level of work (Casaburi et al., 1987; Taylor & Jones, 1979) and reductions in the pR_c (Katayama et al., 1999) and cR_c (Miyamura & Ishida, 1990). These findings support the involvement of a training component in the pR_c and cR_c of endurance athletes. The mechanism responsible for the changes in $\dot{V}_E$ and chemosensitivity associated with endurance training are unknown. They may be the specific result of the repeated exposure to the high levels of $\dot{V}_E$ that occur during whole-body endurance training.

RMT provides an ideal way to study this hypothesis. RMT mimics the hyperpnea of exercise while eliminating or reducing the other consequences of the obligatory increase in metabolism which must accompany whole-body exercise. In addition, the load to the respiratory muscles experienced with RMT (high flow, low resistance) also resembles the ventilatory load faced during normal exercise.

In theory, a RMT program could improve ventilatory efficiency, one

example of which could be a reduced $\dot{V}_E$ at a given relative work load during exercise. A reduction in $\dot{V}_E$ would reduce the metabolic requirements of the respiratory muscles, easing the competition for resources (e.g., blood flow) between the respiratory muscles and muscles of propulsion and this could result in an improvement in endurance performance.

Also, it is possible that the ventilatory changes observed in previous studies after endurance or RMT programs are related to changes in chemosensitivity; i.e. reduced exercise ventilation after training may be correlated to decreases in the ventilatory sensitivity of the CC or the PC. Using RMT enables the effect of high levels of $\dot{V}_E$ on the pR_c and cR_c to be studied without the potentially confounding side-effects of whole-body training.

The objective of the present study was to determine whether any change in the pR_c or the cR_c following a RMT program was correlated to changes in $\dot{V}_E$ after RMT, compared to a non-RMT control group.

We hypothesized that simulating the effect of exercise hyperpnea by voluntary eucapnic hypernea endurance training (RMT) over a 4-6 week period would reduce chemosensitivity and that this reduction in chemosensitivity might correlate with improvements in cycling endurance and corresponding reductions in ventilation. The cR_c was assessed by measuring the ventilatory response to step changes in inspired CO_2 at rest and during exercise. The pR_c was measured by repeat pulses of pure O_2 (modified Dejours O_2 test) during exercise.

CHAPTER II METHODS

Procedures and Tests

Subjects

Twenty experienced male cyclists (22-41 years old), who were not taking any medication, participated in this study. The subjects' mean age was 27.8 (± 5.1) years, body mass 69.1 (± 7.1) kg, body height 179.2 (± 5.7) cm, and who routinely underwent endurance training 7.0 (± 3.8) h wk^{-1}. They were randomly assigned by a matched pairs design, based on this anthropometric data and their habitual hourly level of endurance training, into two groups of ten (Table 2). One group performed RMT (see below) and the other group served as a control. Subjects were instructed not to exercise the day of any test and to avoid intensive exercise the day before any test. Subjects did not drink caffeine the day of the test and their last meal before any test preceded the test by a minimum of 2 h. The subjects were interrogated on each test day to ensure these stipulations were fulfilled. In addition, they were requested to keep their individual training constant for at least four weeks prior to and throughout the course of the study. The subjects were required to keep a log (see Appendix A) of their physical activities in which they entered the type, duration, intensity, and perceived exertion for all exercises they participated in, including RMT.

Table 2. Physical characteristics of the subjects

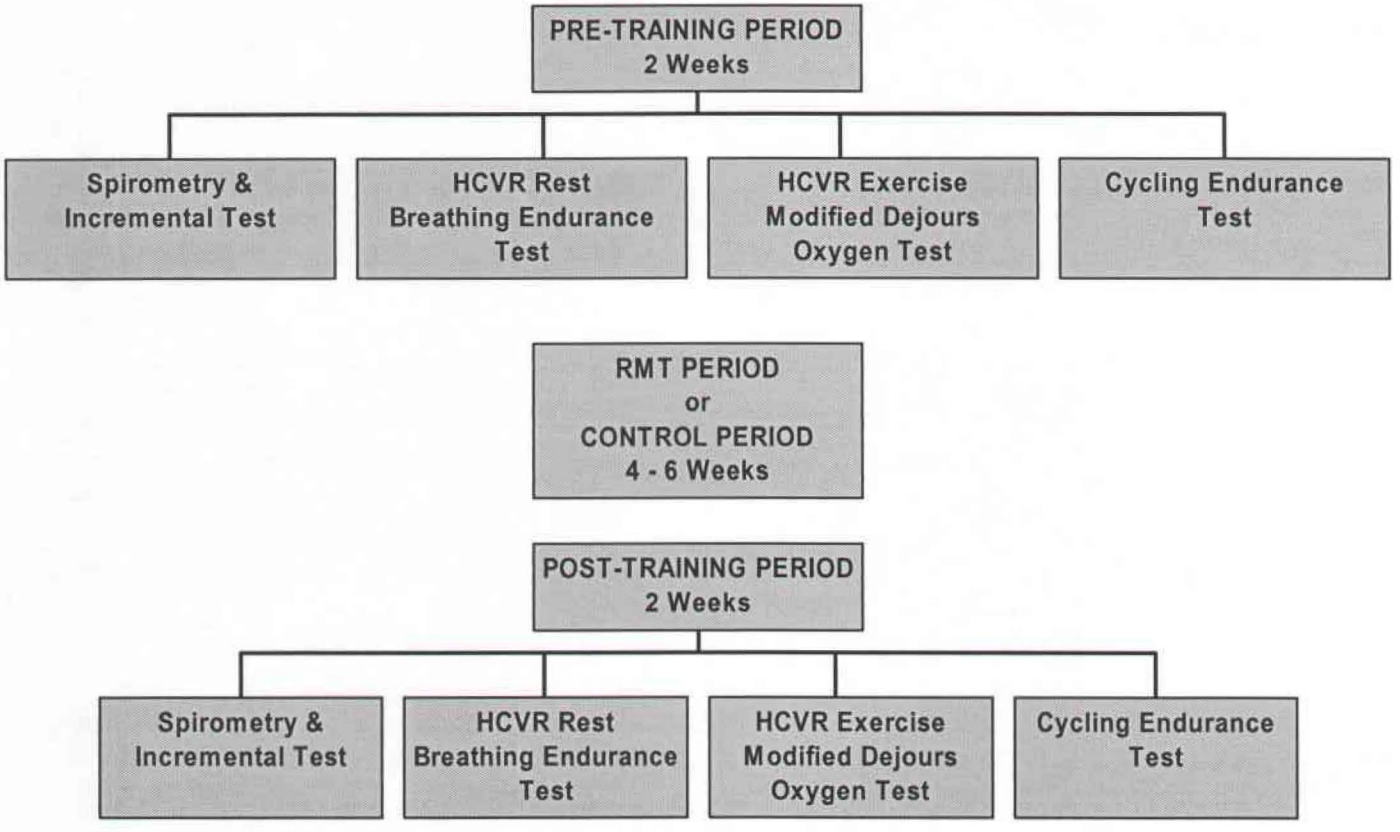
	CONTROL	RMT
Age (years)	28 ± 6	26 ± 4
Height (cm)	180 ± 4	179 ± 7
Weight (kg)	70 ± 9	69 ± 6
Training ($h \times wk^{-1}$)	7 ± 3	7 ± 4

Values are means ± SD.

Before obtaining informed consent (see appendix B), the study requirements, the experimental protocol, and all risks associated with the study were outlined for each subject (see Appendix C). However, the subjects were kept unaware of the intent of the study. The study was approved by the Ethics Committee of Physiology and Pharmacology at the University of Zurich (see appendix D).

Do to the rigors of the initial testing protocol, described in the ethics report (see appendix B), only 8 out of 22 subjects who began that initial protocol (appendix B) were able to complete it as prescribed. Of those eight subjects which remained, four were from the control group and four were from the RMT group. Thus, the protocol was modified (Figure 1) in such a way as to reduce both the number of experimental tests a given subject must undertake and also to reduce the time required for any given test session. These changes resulted in greater compliance with future subjects.

Figure 1. Study protocol



Protocol

Pre-RMT Period

On a separate day, prior to any testing, subjects partook in at least one familiarization session to acquaint themselves with the facility, equipment, and testing procedures. Of particular importance was familiarization with the respiratory training device. Proper familiarization was critical to ensure normocapnic conditions during RMT, to remove any learning effect associated with the use of the respiratory training device, and to select an appropriate ventilatory level for a breathing endurance test (BET). After the familiarization, both groups of subjects underwent four test sessions on four different days (Table 3). The pre-training test sessions consisted of: 1) spirometric measurements and an incremental cycling test to exhaustion; 2) a $HCVR_R$ and a BET; 3) a $HCVR_{EX}$ test followed by a modified Dejours O_2 test during exercise; 4) and a cycling endurance test (CET) to exhaustion. All of the test sessions were performed on separate days with a minimum of two days between each test session. The pre-training lasted approximately 14 days. A 4-6 week RMT or control period followed.

RMT Period

During the RMT period, subjects completed 20 voluntary eucapnic hyperpnea sessions of 30 min each using a special device (see below). The $\dot{V}_E$

of the first training session was 60%-70% of MVV. The intensity of training was increased progressively throughout the 4 - 6 week RMT program until 20 voluntary eucapnic hyperpnea session had been completed

Post-RMT Period

After the RMT or control period, the four test sessions were repeated (post-training). Table 3 provides a graphic representation of the testing protocol.

Test Sessions

Test Session 1

Spirometry

The first test session consisted of spirometry followed in 15 min (minimum) by an incremental cycling test. An additional 15 min after the incremental cycling test a final familiarization using the respiratory training device (described below) was completed. Spirometric variables, i.e., vital capacity (VC), forced expiratory vital capacity, forced expiratory volume in 1 s, peak expiratory flow, and maximum voluntary ventilation in 15 s (MVV), were measured with an ergospirometric device (Oxycon Beta, Jaeger, Wurzburg, Germany).

Incremental Test

The incremental exercise test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800S, Ergoline, Bitz, Germany). This

ergometer can effectively maintain a constant power with pedal revolutions between 30-130 rpm.

The test began at 100 W and the intensity was increased by 30 W every 2 min until the subjects could no longer continue. This allowed determination of peak oxygen consumption ($\dot{V}_{O_2peak}$) and maximal work capacity ($\dot{W}_{max}$). The subjects selected their preferred pedaling frequency and maintained this cadence ± 5 rpm during the test. Ventilatory variables and gas exchange were measured breath by breath with the Oxycon Beta metabolic cart. Cardiac frequency (f_C) was recorded every 5 s (PE 4000, Polar Electro, Kemple, Finland). Blood samples (20 μl), from an earlobe, were taken at the end of each workload step and at the end of the test to analyze the blood lactate concentration ([La]). Analysis of [La] was made with a Biosen 5040 (EKF Industrie, Elektronik GmbH, Barleben, Germany).

Test Session 2

The second test session consisted of the $HCVR_R$ test and the BET. The BET was started a minimum of 15 min after the $HCVR_R$ test.

The Hypercapnic Ventilatory Response at Rest

The $HCVR_R$ was measured with an open-circuit apparatus using a variation of the steady-state method similar to dynamic end-tidal forcing (Bascom, Pandit, Clement, & Robbins, 1992; Swanson & Bellville, 1975). Briefly, this method attempts to maintain the $P_{ET}CO_2$ at higher than normal, but constant

levels, by manually adjusting the F_ICO_2, until ventilation stabilized.

The subjects began with 15 min of seated relaxation, then they breathed air for 5 min through a mouthpiece connected to the gas sampling equipment and wide bore tubing, while wearing a nose-clip. Then the inspired air was switched to 50% O_2 / 50% N_2 for 5 min. This mixture served two purposes: 1) the hyperoxia was used to inhibit the PC contribution to ventilation; and 2) breathing compressed gas prior to measuring the hypercapnic response prevented the subject from detecting when the CO_2 was added. Next, gas from a tank containing 50% CO_2 / 50% O_2 was mixed with the gas from the first tank. This allowed the inspired CO_2 to be adjusted so a step-change in $P_{ET}CO_2$ of 4 mmHg above that of the baseline (from the preceding 50% O_2 / 50% N_2 portion of the test) was reached. The F_ICO_2 (from the two tanks, 50% O_2 / 50% N_2 and 50% CO_2 / 50% O_2) was manually adjusted by a self-built mixing valve (Boutellier et al. 1992) to achieve and maintain the target increase in $P_{ET}CO_2$ and maintain the flow rates required to meet the demands of the increased $\dot{V}_E$. Two further step-increases of approximately 4 mmHg $P_{ET}CO_2$ done in the same manner followed. Each step was maintained for 6 - 10 min which allowed $P_{ET}CO_2$ and $\dot{V}_E$ values to stabilize and remain so over a 30 - 60 s period. Figure 2 is a graphic representation of the setup used to measure the hypercapnic ventilatory response at rest.

Hypercapnic Ventilatory Response Set-Up

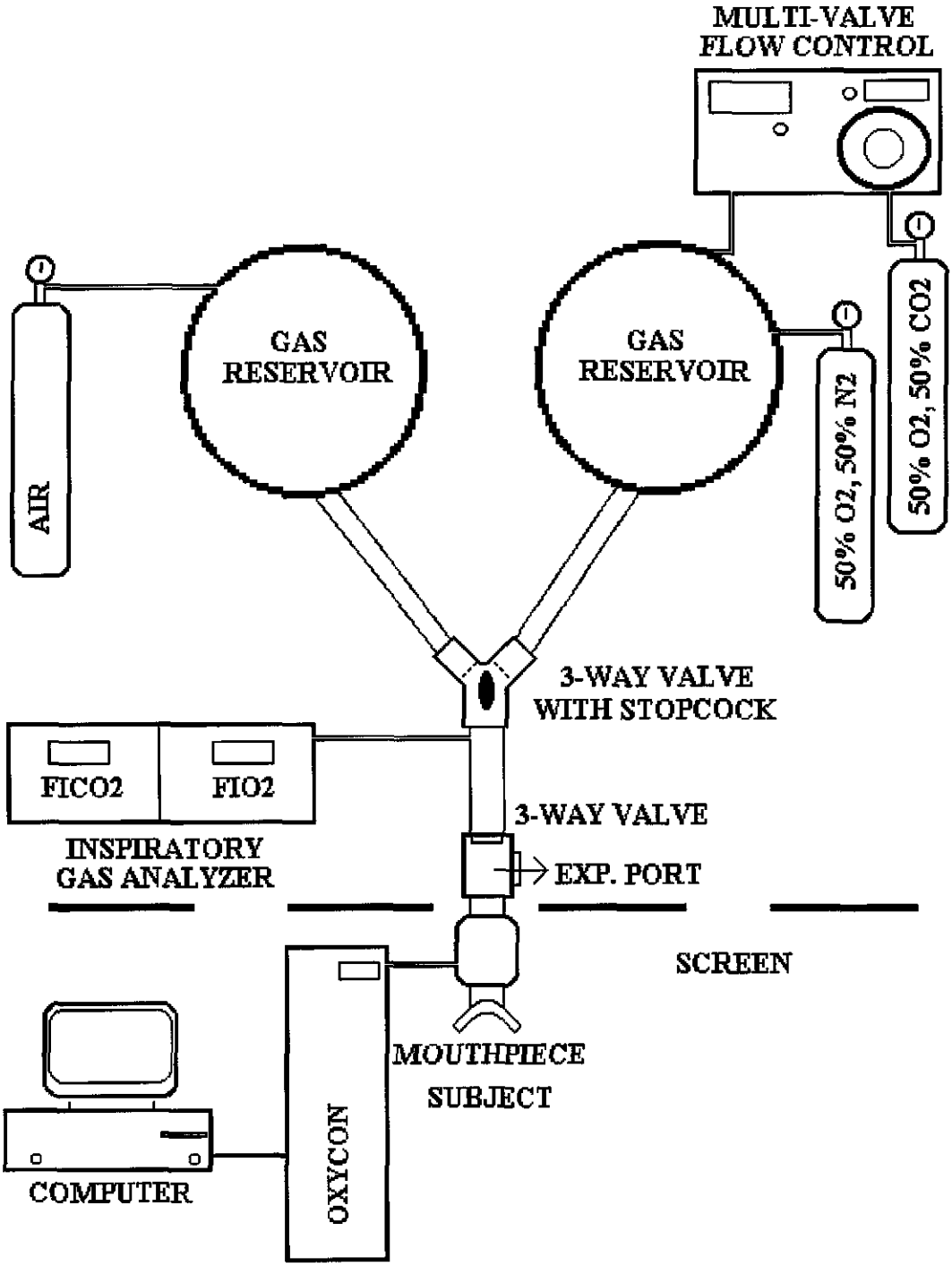


Fig 1. Apparatus used for measuring the hypercanic ventilatory response at rest and during exercise

Breathing Endurance Test

The BET was conducted at a $\dot{V}_E$ corresponding to 74 ± 10% MVV. To assure normocapnia, the RMT device (see below) was used. During familiarization sessions a level of ventilation was chosen by the investigators that ensured the subjects could continue for a minimum of 6 min but not longer than 15 min. Subjects were required to maintain this pre-selected $\dot{V}_E$ while keeping V_T and f_b constant, paced by a metronome (DM-20, Seiko, Tokyo, Japan). The test administrator ensured the fractional end-tidal concentration of CO_2 ($F_{ET}CO_2$) was maintained within the range of 4.6 to 5.8%. The test was terminated when a subject could not maintain the proper level of ventilation, V_T or f_b , gave up, or after 40 min. The 40 min cut-off time was reached only by subjects in the RMT group during the post-training BET tests. The end-time was used as an estimate of respiratory muscle endurance. Ventilatory variables and gas exchange were measured breath by breath and heart rate was sampled every 5 s.

Test Session 3

The Hypercapnic Ventilatory Response during Exercise

During the third test session, the $HCVR_{EX}$ test was completed. The $HCVR_{EX}$ test utilized a similar protocol as described above for the $HCVR_R$ measurements, but was done while seated on a bicycle ergometer. During the steady-state exercise portion of the test the subjects cycled at 40% $\dot{W}_{max}$. Using

a nose-clip and breathing through a mouthpiece the subjects breathed room air for five minutes followed by a mixture of 50% O_2 / 50% N_2 for 5 minutes. This mixture served two purposes: 1) the hyperoxia was used to inhibit the PC contribution to ventilation; and 2) breathing compressed gas prior to measuring the hypercapnic response prevented the subject from detecting when the CO_2 was added. Next, gas from a tank containing 50% CO_2 / 50% O_2 was mixed with the gas from the first tank. This allowed the inspired CO_2 to be manually adjusted so that three 4 mmHg $P_{ET}CO_2$ step increases could be accomplished. This was done in a manner identical the $HCVR_R$ (see earlier, test session 2 and figure 2). Each step was maintained until $P_{ET}CO_2$ and $\dot{V}_E$ values were constant (a minimum of 6 min) for a 30 - 60 s period. The $HCVR_{EX}$ test used the same equipment set-up as the $HCVR_R$ test (Fig. 1)

The Modified Dejour's O_2 Test

The modified Dejour's test which used hyperoxia to suppress ventilation was also completed during the third test session. Following the $HCVR_{EX}$ test, the subjects the began the hyperoxic suppression test. The modified Dejours O_2 test (Dejours, 1963; Dejours, Puccinelli, Armand, & Dicharry, 1965) was completed with the subjects cycling at 40% $\dot{W}_{max}$. First, the subjects rested 15-30 min while sitting in a chair and listening to relaxing music through headphones. Next, the subjects moved to the ergometer and cycled (40% $\dot{W}_{max}$) for 5 min breathing room air followed by another 5 min of breathing compressed air, from a gas

bottle, for 5 min. The subjects' mouthpiece was connected by wide bore low resistance tubing to a three-way valve. The valve was out of the subjects' sight behind a screen. The subjects could not see or hear when the inspired gas was switched. Breathing compressed gas prior to hyperoxia prevented the subjects from sensing when the switch to 100% O_2 occurred. After this 10 min warm-up and acclimatization period, during an exhalation, the compressed air was surreptitiously switched to 100% oxygen, from a gas bottle, for 10-14 breaths (~20-30 s). Immediately, the subjects were switched back to compressed air for 3 minutes. This 3 minutes allowed ample time for $\dot{V}_E$ to return to the same level it had prior to hyperoxia (baseline level). The combination of hyperoxia and compressed air was defined as one trial. Each subject completed 6 hyperoxic trials in this fashion. Figure 3 is a graphic representation of the setup used to measure the hyperoxic suppression of the PC.

Dejours's one to two breath test was slightly modified as preliminary trials showed the most consistent responses occurred while breathing 100% oxygen for ~12 breaths. Within several seconds, hyperoxia resulted in a decrease in ventilation to an eventual nadir within 30 s. This decrease reflects the removal of the carotid bodies' contribution to the ventilatory drive and can be expressed as a percentage of the pre-oxygen breathing ventilation (Dejours, 1962; Dejours, 1963). Blood samples were taken prior to and at the end of the test to measure [La].

Modified Dejours Oxygen Test Set-Up

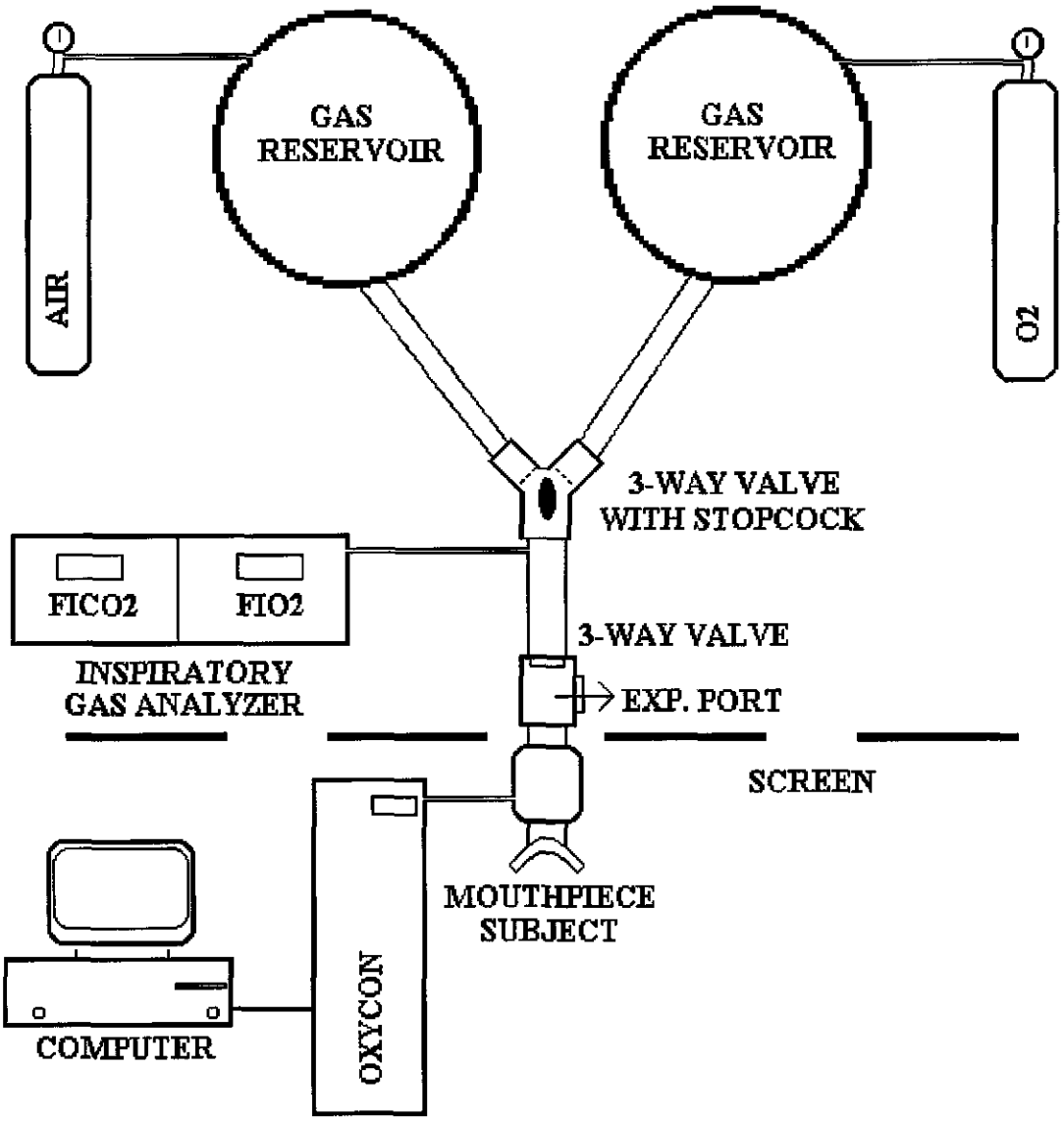


Fig 2. Apparatus used for the modified Dejour's oxygen test during exercise

Test Session 4

During the fourth test session, the CET, at 85% $\dot{W}_{max}$, was performed to exhaustion. This test began with 4 min of sitting quietly on the cycle ergometer, followed by a warm-up consisting of 2 min of unloaded pedaling, 2 min at 40% $\dot{W}_{max}$, 3 min at 60 % $\dot{W}_{max}$, and finally the test load at 85% $\dot{W}_{max}$ until termination of the test. Subjects chose their preferred cadence. This cadence was maintained throughout all CET tests (± 5 rpm). The time at which the subject was unable to maintain the cadence within the proper range, or gave up, was used as the end-time. This time, excluding rest and unloaded pedaling, was used as the measure of cycling endurance. Ventilatory variables and gas exchange were recorded breath by breath (Oxycon Beta) and f_C was sampled every 5 s. Blood samples were taken every 2 min and at the end of the test to measure [La].

RMT

RMT was done using a special device which allowed partial rebreathing of CO_2 to maintain eucapnia. The device consisted of a mouthpiece connected by an inverted "L", (⅂) shaped tubing (inner diameter 19 mm), in turn connected to a rebreathing bag (3-5 l). The middle of the tubing (⫲) contained a side-port (19 mm diameter). This side-port contained a hole (6 mm diameter) and a one-way non re-breathing valve.

This design allowed the subjects to exhale and inhale from the

rebreathing bag while obtaining fresh air through the hole during inspiration and expiring partially through the hole and expiratory valve. Nasal breathing was prevented by the occlusion of the nostrils with a nose-clip.

The size of the rebreathing bag was adjusted to be 50-60% of the subjects' VC. At the beginning of RMT, f_b was chosen so that $\dot{V}_E$ was 60-70% of MVV. Subjects were instructed to fill and empty the rebreathing bag completely at the f_b assisted by the metronome. Additional inspiratory and expiratory flow passed through a small hole in the tubing to avoid an increase in arterial CO_2 partial pressure and a fall in O_2 saturation, i.e., the subjects' V_T was slightly larger than the size of the rebreathing bag. In this way, by increasing or decreasing the size of the V_T, the subjects self-regulated eucapnia. Correct performance, i.e., the maintenance of eucapnia was checked with the training device connected to the Oxycon Beta. If eucapnic conditions were not maintained during the control sessions, the hole in the tube was adjusted in size, or V_T and f_b or both were changed.

The $\dot{V}_E$ of the first training session was about 60-70% of the individual MVV in 15s. Once a subject reached 30 min, the initial RMT session ended. The intensity of other training sessions was progressively increased such that the subjects could hold the voluntary eucapnic hyperpnea $\dot{V}_E$ constant for 30 min, but not longer than 35-40 min. The increases in training intensity were done by increasing $\dot{V}_E$, primarily by increasing breathing frequency. Every training session was performed in this subjective manner, i.e., the subjects judged, by

their own perception, the $\dot{V}_E$, and maintained a $\dot{V}_E$ that they imagined they could have maintained for approximately 5-10 min longer than the prescribed training time of 30 min but no shorter. The theory behind this subjective intensity level was to ensure they never became exhausted and to prevent over-training.

The subjects performed RMT at home, with the exception of every fifth training session which was performed in the laboratory. During these control training sessions the subjects' RMT and habitual training logs were reviewed and then they performed 30 min of voluntary eucapnic hyperpnea connected to a metabolic cart to observe their training technique (constant V_T and f_b) and to ensure eucapnia. Analysis of the four control training sessions performed in the laboratory showed the subjects maintained the $F_{ET}CO_2$ within the range of 4.6% - 5.8%.

Test Standardization

General Procedures

It was important that the influence of environmental factors on the condition of the individual subject was similar for the pre-training and post-training test sessions. To help achieve this, the subjects were informed of the importance of maintaining their habitual training levels, and dietary and sleep habits. They followed strict guidelines for two days prior to testing (see pre-test preparation).

Every attempt was made to ensure the subjects maintained their habitual

training level for the duration of their participation in the study. Beginning a minimum of two weeks prior to testing, subjects were required maintain records of their training in a training log (Appendix A). The mode of exercise, intensity, duration, and distance were recorded as applicable. The training during these two weeks was then reviewed with the subject to determine that it was representative of their normal training regime. If so, the subjects were requested to maintain this training routine and to continue keeping a record in the training log.

It was important that the conditions of the post-training testing replicated the pre-training tests as close as possible. For example, post-training tests were completed at the same time of day as their pre-training counter-part. All tests were begun within 2 hours of the time that the pre-training tests were begun. It is should be pointed out, the chemoreflex control of breathing is affected by circadian rhythms (Stephenson, Mohan, Duffin, & Jarsky, 2000).

To help ensure muscle and liver glycogen as well as blood glucose were maximized and consistent, subjects were instructed to eat the same foods for the two meals which preceded a test session.

It has been shown that verbal encouragement can affect performance and physiological parameters (Moffatt, Chitwood, & Biggerstaff, 1994). Therefore, to avoid externally influencing the test results, no verbal encouragement was given at any time.

To ensure comfort and familiarity, the pedals and seat of the cycling

ergometer could be interchanged with those of the subjects'. The ergometer also allows individualized adjustment of seat height, seat fore and aft positioning, handlebar height, and handlebar distance from the subject. The position most similar to the subjects' own bicycles was chosen and recorded (see Appendix E). This ensured the subjects used the same cycling position for all tests.

Cycling cadence was maintained throughout all tests using the ergometer (± 2-5 rpm). This was done to avoid possible affects of cycling frequency on breathing pattern and other of physiological variables (Lepers, Millet, Maffiuletti, Hausswirth, & Brisswalter, 2001).

After collection, at the end of the test, lactate samples were deep frozen. This allowed all lactate samples for a given test (pre- and post-training) to be analyzed at the same time. During analysis, a [La] sample from a pre-training test would be analyzed alternately with a sample from a post-training test. These procedures were important to negate the affect of any drift occurring during analysis and also ensured the condition of the machine's membrane was consistent between pre and post training tests for a given subject.

Pre-Testing Preparation

Subjects followed the pre-test instructions for a period of 24-48 hours prior to testing (see Appendix C).

Two days prior to testing, the subjects were requested to abstain from any intensive or long training, to eat carbohydrate rich foods, and to sleep for a

minimum of 7 hours. One day prior to testing, the subjects were instructed to train very lightly or not at all, to eat carbohydrate rich foods, and to sleep for a minimum of 7 hours.

On the day of testing, before the test session, subjects were not allowed to do any sports nor were they allowed to drink or eat any products containing caffeine. They were instructed to have their last meal 3-4 hours before the test and only small snacks (e.g., energy bars) were allowed within two hours of the test session.

Upon arriving at the testing facility, the subjects would rest while going over the training logs, especially the last 48 hours. Close attention was also paid to the eating and sleeping habits over the prior 48 hours. In addition the subjects would fill out a subjective ratings questionnaire (see Appendix F). If there were no counter-indications (e.g., instructions were followed and the post training conditions resembled those of the pre-training) the testing protocol for that day would be explained and testing would begin after the 15 min rest period.

HCVR and Modified Dejours O_2 Test

It is evident from studies that have previously measured the ventilatory response to hypercapnia and hyperoxia that environmental and external stimuli can be deleterious to reproducibility. This is especially true during resting measurements because ventilation at rest is easily perturbed. These disruptive stimuli include noise (whispering, unexpected sounds), movement, temperature,

and discomfort. Additional disturbing factors include having a full bladder (CO_2 breathing may cause diuresis), ingested stimulants (e.g. caffeine), muscular movement (crossing of the legs can stimulate ventilation, thus restlessness could void a test), diurnal variations (generally Pco_2 is lowest in the early afternoon, perhaps due to the activity of the reticular activating system), and anticipation of the chemical stimulus (merely thinking that you have been given a gas mixture may alter ventilation (Rebuck, 1976; Severinghaus, 1976). Fortunately, measurements made during exercise minimize the affect of these variables (Jones, 1976). Nevertheless, certain precautions against their effects were taken.

To minimize anxiety during the experiment subjects underwent at least one preliminary test to familiarize themselves with the procedure. External stimuli were minimized by having the subjects first empty their bladders immediately before starting an experiment, then relax for 10-20 minutes while listening to calming music. This was followed by a 10 minute period of quiet breathing on a mouthpiece while listening to white noise. Only after this would an experiment begin. All experiments were performed at the same time of day. Finally, care was taken to prevent the subject from being aware of when gas mixtures where switched or altered. This was done by using a screen between the subject and the researcher. The screen prevented the subject from receiving any feedback about what the researcher was doing, especially in terms of gas mixtures.

Cycling Endurance Test

In addition to eating the same foods for the two meals preceding all tests, subjects were given two doses of a carbohydrate nutritional supplement in powder (Novartis Consumer Health, Switzerland). The subjects were instructed to mix the pre-measured powder dose with 500ml water. This drink provided 100g of carbohydrate and was taken after the last two meals before the CET.

A warm-up was incorporated into the CET. It known that using a warm-up can affect performance, f_C and [La] as well as V_T and f_b (Martin, Robinson, Wiegman, & Aulick, 1975).

Equipment

An ergospirometric device (Oxycon Beta, Jaeger, Wurzburg, Germany) was used to measure spirometric variables (VC, forced expiratory vital capacity, forced expiratory volume in 1 s, and peak expiratory flow), ventilatory variables such as $\dot{V}_E$, V_T and f_b, an gas exchange variables ($\dot{V}O_2$,$\dot{V}CO_2$, $F_{ET}CO_2$, $F_{ET}O_2$) breath by breath during for all test involving such measurements. It utilizes fast responding gas analyzers (paramagnetic for O_2 and infrared absorption for CO_2) to measure the fractional concentrations of O_2 and CO_2. The gas analyzers were calibrated with certified amounts of O_2 and CO_2 before and after each test. The turbine for volume measurements were calibrated with a 3l syringe before and after each test. By convention, all volume measurements are reported as BTPS and gas measurements are reported as STPD.

All cycling tests were performed with an electromagnetically braked bicycle ergometer (ergometrics 800S, Ergoline, Bitz, Germany), connected to the "Oxycon Beta" unit. This ergometer can effectively maintain a constant power with pedal revolutions between 30-130 rpm. The ergometer was calibrated on a regular basis.

All blood lactate concentrations were analyzed enzymatically by Biosen 5040 enzymatic analyzer (EKF Industrie, Elektronik GmbH, Barleben, Germany), which uses 20 μl samples taken from the subject's earlobe. Calibration was done before and after analysis of the whole blood samples with standardized solutions.

All heart rate measurements were recorded on a "PE 4000" heart rate monitor (Polar Electro, Kemple, Finland).

Data Sampling and Analysis

Incremental Test

The $\dot{W}_{max}$ was defined as the highest workload sustained for a minimum of 90 s during the incremental test. $\dot{V}_{O2peak}$ was obtained from the highest $\dot{V}_{O2}$ reached over the highest 30 s average during this test.

Hypercapnic Ventilatory Response

Both the $HCVR_R$ and $HCVR_{EX}$ tests were analyzed in the same way using breath-by-breath measurements of $\dot{V}_E$ and $P_{ET}CO_2$. Mean values for $P_{ET}CO_2$ and

$\dot{V}_E$ were calculated using the last 30 s from each of the three step increases in $P_{ET}CO_2$. The relationship between $P_{ET}CO_2$ and $\dot{V}_E$ was analyzed by least squares linear regression using the following equation:

$$\dot{V}_E = S(P_{ET}CO_2 - B)$$

where S is the slope of the best fit line relating a change in $P_{ET}CO_2$ to a change in $\dot{V}_E$ and B is the extrapolated x-axis ($P_{ET}CO_2$) intercept when $\dot{V}_E = 0$. The slope of the CO_2 response lines ($\Delta\dot{V}_E/\Delta P_{ET}CO_2$) are denoted S_R for the slope of the HCVR at rest or S_{EX} for the slope during exercise and have the units l/min/mm Hg. The X - axis intercepts (at theoretical zero $\dot{V}_E$) at rest are denoted by B_R and during exercise by B_{EX} and are expressed with the units mm Hg. The value of S, in the hyperoxic background, was considered to be representative of the subject's cR_c.

Hyperoxic Suppression

Each modified Dejours O_2 test was analyzed using breath-by-breath measurements. The steady-state $\dot{V}_E$ was obtained from the 30 s mean $\dot{V}_E$ preceding the switching to hyperoxia. The magnitude of the following decrement in $\dot{V}_E$ was determined using a two-breath moving average (Stockley, 1977; Stockley, 1978). Equipment errors, sighs or swallows (single breaths that deviated more than ±2 S.D. from the previous or following breath) were not included in the analysis. These low volume breaths had more than a 60% decrease from the baseline $\dot{V}_E$ or previous breaths and did not exhibit an

appropriate corresponding increase in end-tidal CO_2. Four to six hyperoxic episodes per modified Dejours O_2 test were completed by each subject. The interval between episodes of hyperoxia was 3-5 minutes, the time needed to ensure the subjects' $\dot{V}_E$ had returned to baseline. The average of these 4-6 trials was taken as the subject's pR_c.

Cycling Endurance Test

Breath-by-breath data from the CET were averaged into 15 s segments. For comparison of cardio-respiratory parameters, these 15 s segments were organized into two categories, quasi-steady-state and end-time. The quasi-steady-state included data from the 85% $\dot{W}_{max}$ level, less the first 1 min 45 s and the last 2 min of the shorter test, either the pre- or post-training test. An identical time period was chosen for the longer of the two tests. Thus, the quasi-steady-state of the CET was a comparison of identical cycling times (dictated by the test of shorter duration) for the pre- and post-training test of each subject. The end-time category included the last complete 60 s (i.e., four 15 s segments) of each test irrespective of the other tests duration. The 2 min samples of [La] were grouped into similar quasi-steady-state and end-time categories.

Statistics

Data are reported as means ± SD. Statistical comparisons between groups were made using unpaired t-tests with exceptions for the BET, the $HCVR_R$ and $HCVR_{EX}$ tests for which the non-parametric Mann-Whitney U test

was employed. This was done because the data was not normally distributed. For instance, in some cases the end of the BET was stopped at 40 min. Any within group statistical comparisons were made using a paired t-test. The Pearson product-moment correlation coefficient was used to calculate correlations for selected variables. A Fisher's R to Z test was used for detecting statistical significance. In some instances, the Spearman correlation was used to calculate correlation coefficients and to detect statistical significance. For all tests, statistical significance was defined as a value of $P < 0.05$. Statistical analyses were completed with StatView 4.53 (Abacus Concepts, Berkeley, CA, USA).

For technical reasons, the cardio-respiratory data pertaining to the CET of two subjects, one from each group, was not available for the final analysis. This was because electrical power to the computer was inadvertently disconnected when the subjects dismounted from the ergometer unfortunately before the test results were saved). Additionally, two subjects, one from each group and different from the two mentioned above were unable to complete the HCVR tests, because of extremely erratic ventilatory responses to the hypercapnia and headaches.

CHAPTER III RESULTS

Cycling Endurance Test

RMT increased cycling endurance significantly in the RMT group compared to the control group (Fig. 4). The RMT group improved their cycling endurance by 15% whereas the control group exhibited a slight decrease of-8% (+3.26 ± 4.98 min; -1.46 ± 3.67 min, respectively $p = 0.027$). Ventilatory and cardiovascular parameters of the CET in both the quasi-steady-state and end-time categories are shown in Table 3. $\dot{V}_E$ was increased in the RMT group compared to the control group, but only borderline significantly ($p = 0.059$). However, V_T was increased significantly in the quasi-steady-state category in the RMT group compared to the control group ($p = 0.02$). V_T was the only parameter that changed significantly (Table 3) during quasi-steady state exercise.

For the RMT group, the change in the individual end-times of the CET correlated significantly with changes in the quasi-steady-state $\dot{V}_E$ of the CET (Fig. 5) and the same relationship was borderline significant for the end-time category ($r = -0.61$, $p = 0.061$; $r = -0.54$, $p = 0.14$; RMT and control group, respectively) of the CET. The correlation with cycling end-times and the increased V_T was borderline significant for the quasi-steady state category ($r = -0.66$, $p = 0.054$; $r = -0.19$, $p = 0.63$; RMT and control group, respectively) and significantly for the end-time category ($r = -0.69$, $p = 0.036$; $r = -0.26$, $p = 0.518$;

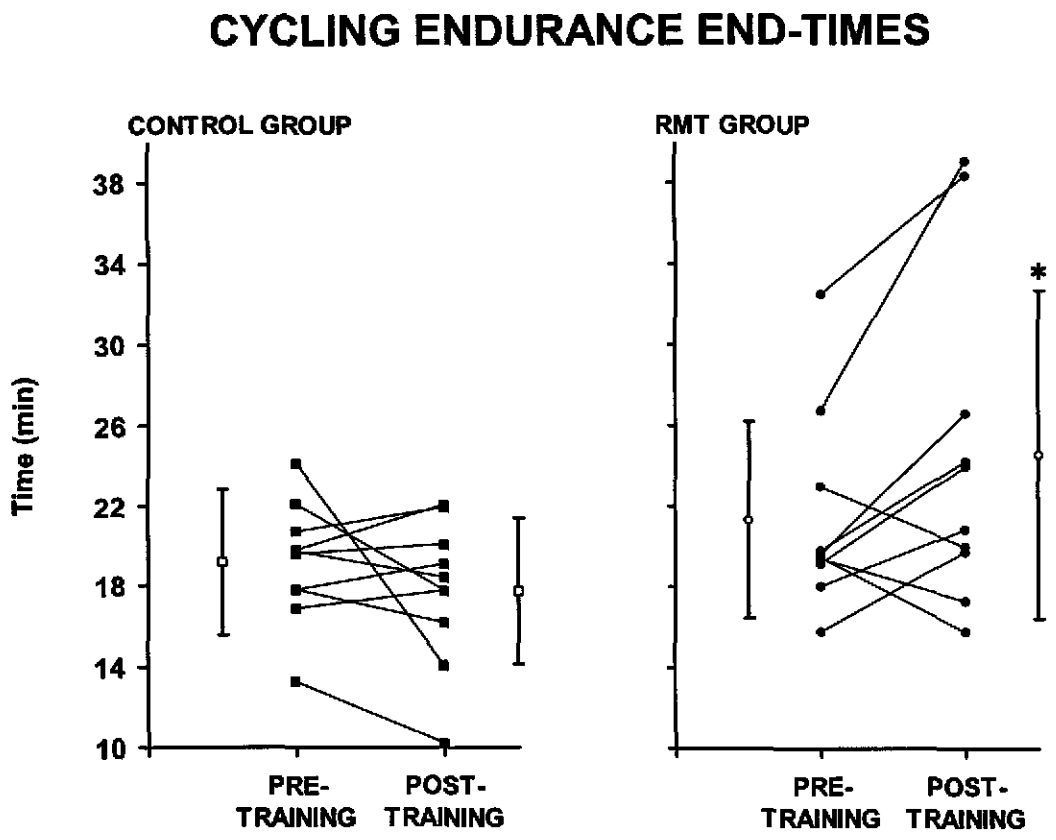


Figure 4. Cycling endurance end-times: the effect of respiratory muscle training (RMT) on cycling endurance end-times for individuals of the control group (n = 10 ■) and the RMT group (n = 10 ●). Open symbols without connecting lines are means ± S.D. * significantly different for between-group difference of end-time, p = 0.027.

Table 3. Cycling endurance test results before and after the respiratory muscle training (RMT) or control period.

		Quasi-steady-state		End-time	
		Pre-training	Post-training	Pre-training	Post-training
$\dot{V}E$ ($l \times min^{-1}$)	Control	121.9 ± 12.6	121.6 ± 12.8	147.1 ± 25.0	143.8 ± 19.8
	RMT	119.3 ± 20.9	124.2 ± 24.4	147.8 ± 24.0	156.2 ± 32.6
f_R (min^{-1})	Control	41.1 ± 8.8	42.6 ± 9.4	53.0 ± 13.7	53.5 ± 14.4
	RMT	36.6 ± 4.0	36.7 ± 4.1	47.4 ± 6.1	48.8 ± 6.7
V_T (l)	Control	3.1 ± 0.6	3.0 ± 0.7	2.9 ± 0.6	2.8 ± 0.8
	RMT	3.3 ± 0.4	3.4 ± 0.4*	3.1 ± 0.4	3.2 ± 0.4
$P_{ET}CO_2$ (mmHg)	Control	39.52 ± 3.39	38.92 ± 3.70	32.39 ± 4.17	33.78 ± 3.77
	RMT	41.76 ± 4.96	40.15 ± 4.53	34.76 ± 3.59	32.71 ± 4.05
$\dot{V}O_2$ ($ml \times min^{-1}$)	Control	4216 ± 419	4157 ± 523	4398 ± 596	4404 ± 655
	RMT	4455 ± 509	4345 ± 576	4755 ± 609	4577 ± 653
$\dot{V}CO_2$ ($ml \times min^{-1}$)	Control	4581 ± 474	4495 ± 608	4694 ± 735	4700 ± 733
	RMT	4701 ± 540	4675 ± 595	4994 ± 700	4847 ± 617
f_C (min^{-1})	Control	170.2 ± 14.8	172.8 ± 14.0	177.1 ± 15.1	178.1 ± 14.2
	RMT	175.8 ± 7.9	174.8 ± 9.4	183.5 ± 8.6	182.6 ± 10.2
[La] ($mmol \times l^{-1}$)	Control	7.54 ± 1.58	7.70 ± 2.22	11.71 ± 3.30	10.97 ± 3.00
	RMT	8.58 ± 1.75	7.85 ± 1.37	11.46 ± 2.28	10.42 ± 4.15

Values are means ± SD (n = 9). $\dot{V}E$ = expired minute ventilation; f_b = breathing frequency; V_T = tidal volume; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production; f_C = cardiac frequency; [La] = blood lactate concentration, * $p < 0.05$.

RMT and control group, respectively) of the CET. The RMT group breathed deeper and these changes were related to the improvements in cycling performance.

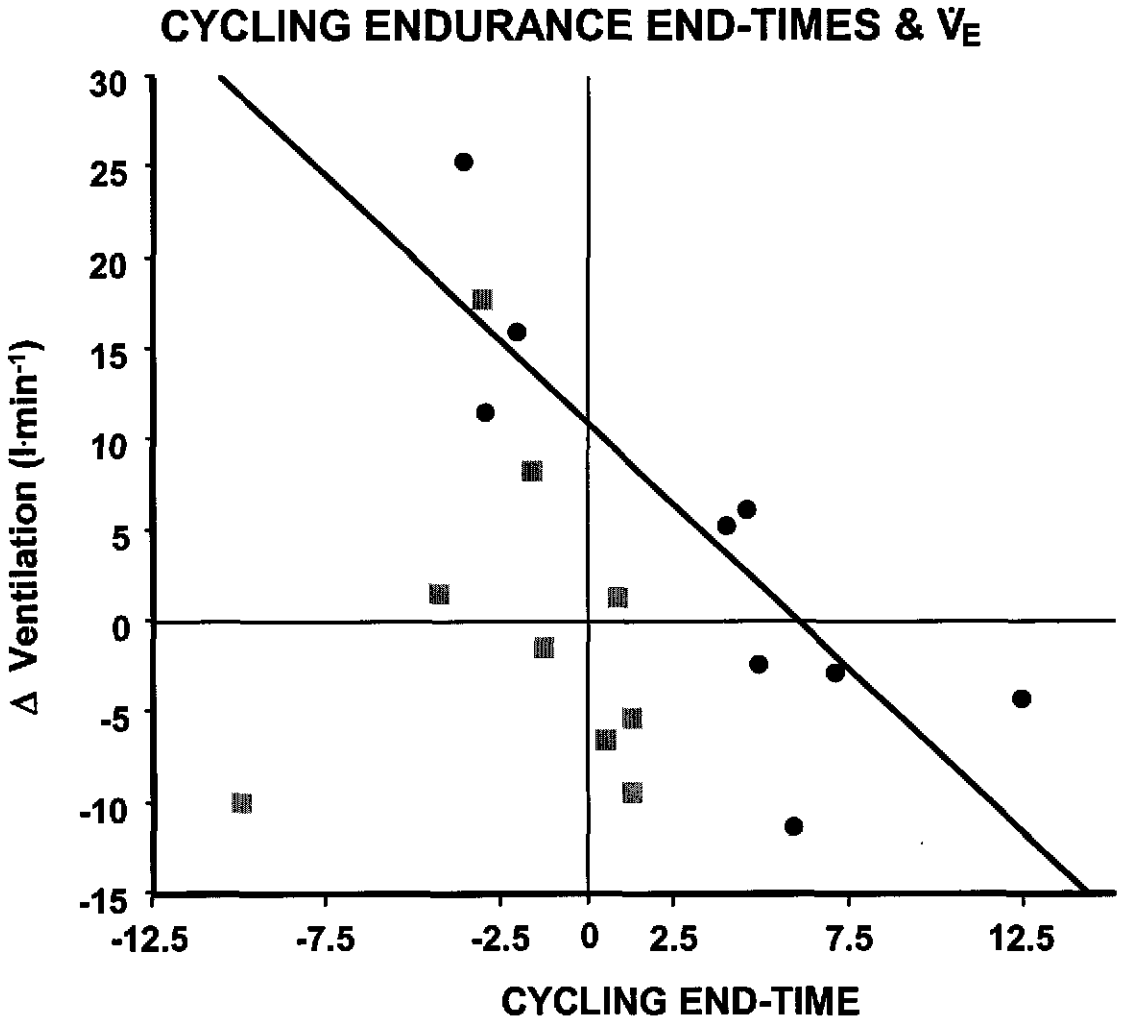


Figure 5. Correlation between cycling ventilation and end-times: the change in quasi-steady-state $\dot{V}_E$ plotted as a function of the change in cycling endurance test (CET) end-times for the control group (■, n = 9, r = 0.00, p = 1.00) and for the respiratory muscle training group (●, n = 9, r = -0.84, p = 0.003). The regression line is for the RMT group.

Peripheral Chemoreceptor Response

RMT reduced the pR_c in the RMT group (-5.8 ± 6.0%) compared to controls (0.1 ± 4.6%; Fig. 6). This result was significant using parametric (unpaired t-test: p = 0.032) or non-parametric (Mann-Whitney U: p = 0.024) statistical analysis. Baseline $\dot{V}_E$, measured prior to beginning the hyperoxic breathing trials during the modified Dejours O_2 test, was not significantly changed after the RMT period in the RMT group (pre: 53.0 ± 3.9 $l \cdot min^{-1}$ vs. post: 54.4 ± 6.5 $l \cdot min^{-1}$) nor the control group (pre: 52.6 ± 3.0 $l \cdot min^{-1}$ vs. post: 52.9 ± 4.0 $l \cdot min^{-1}$). There was no correlation between the changes in pR_c and the baseline $\dot{V}_E$ before the O_2 trials (RMT: r = 0.01, P = 0.974 vs. control: r = -0.37, p = 0.348). Also, end [La] was not significantly changed in the RMT (pre: 0.84 ± 0.26 $mmol \cdot l^{-1}$ vs. post: 1.17 ± 0.37 $mmol \cdot l^{-1}$) nor in the control group (pre: 0.78 ± 0.12 $mmol \cdot l^{-1}$ vs. post: 1.06 ± 0.29 $mmol \cdot l^{-1}$) following RMT. There were no significant correlations between [La] and pR_c (RMT: r = -0.10, P = 0.795 vs. control: r = 0.02, P = 0.956).

Additionally, there were no significant correlations between tests. The changes in pR_c did not correlate significantly with the change in end-time of the CET in either group (RMT: r = 0.11, P = 0.79; control: r = 0.13, P = 0.74). Figure 7 shows plots the the changes in the pR_c vs. the changes in the quasi-steady-state $\dot{V}_E$ of the CET; these correlations were not significant (RMT: N=8, r = -0.43, P = 0.31; control: N=8, r = 0.64, P = 0.087)

Peripheral Chemoreceptor Response

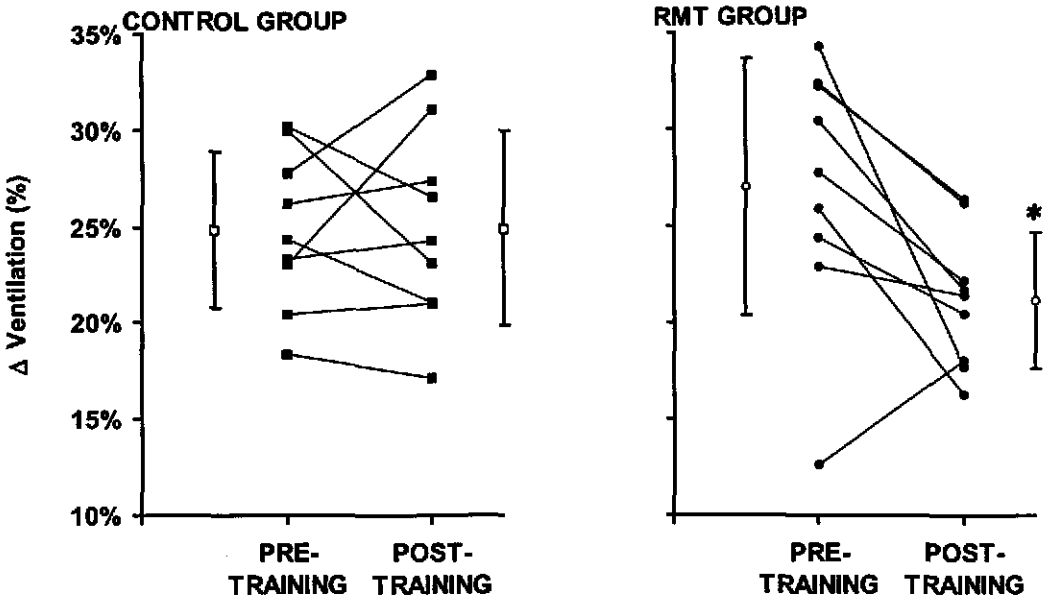


Figure 6. Peripheral chemoreceptor response, the effect of respiratory muscle training (RMT) on the peripheral chemoreceptor response to hyperoxia for individuals of the control group (■) and the RMT group (●). Open symbols without connecting lines are means ± S.D. * significantly different for between-group difference of response, ($p = 0.032$).

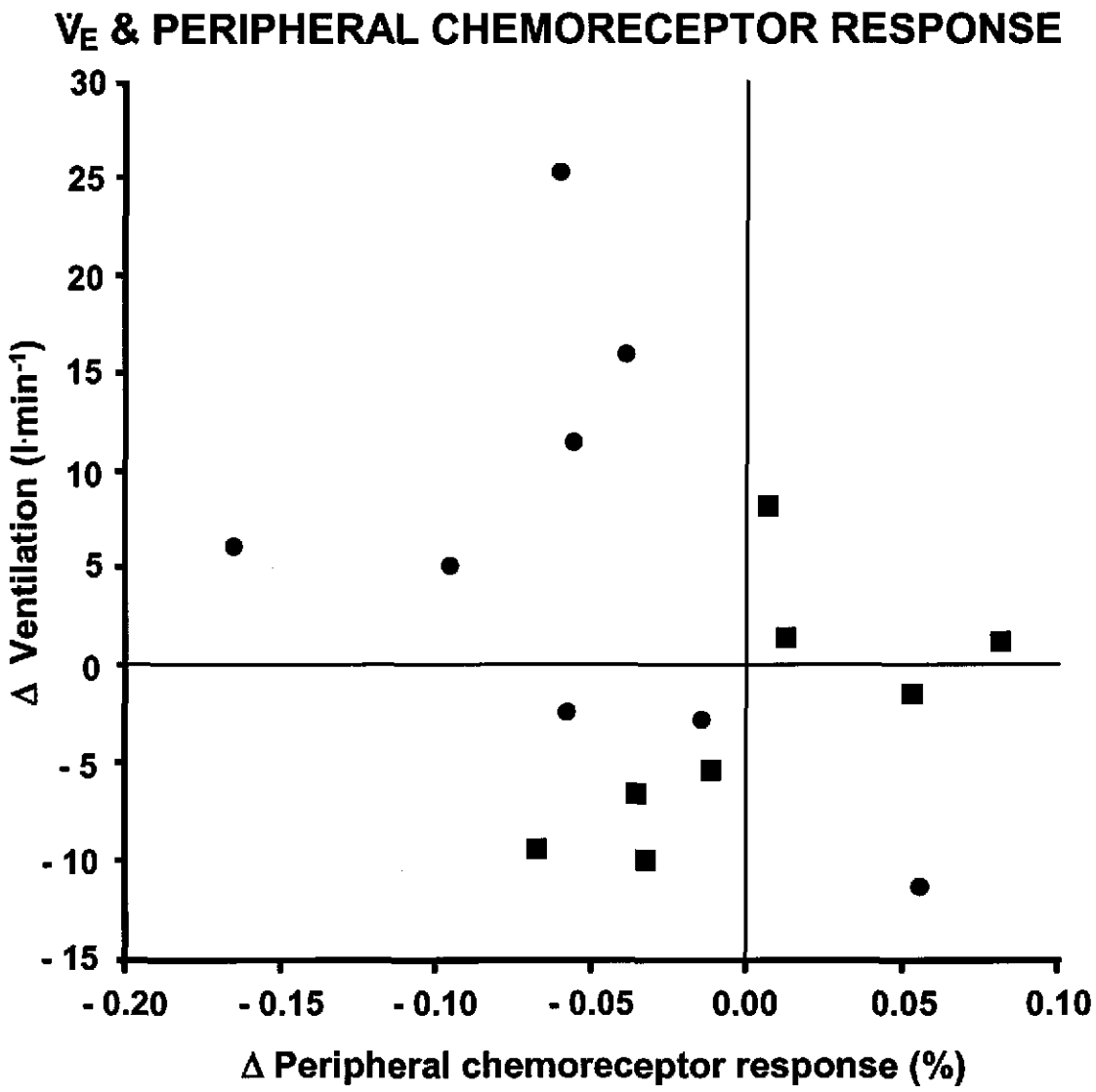


Figure 7. Relationship between ventilation and the peripheral chemoreceptor response during the cycling endurance test for the control group (■, n = 8, r = 0.64, p = 0.087) and for the respiratory *muscle training group* (●, *n* = 8, *r* = -0.43, *p* = 0.313).

Central Chemoreceptor Response: Rest and Exercise

The results of the $HCVR_R$ and the $HCVR_{EX}$ are shown in Figure 8 (control group)and in Figure 9 (RMT group). The $HCVR_R$ did not exhibit any significant changes in either the RMT group or the control group following the RMT training period, ; S_R (0.57 ± 0.56 and 0.90 ± 1.31, RMT and control group, respectively) nor B_R (+1.31 ± 2.05 and -0.36 ± 2.22, RMT and control group, respectively). In the $HCVR_{EX}$ test S_{EX} (0.19 ± 0.51 and -0.21 ± 0.72, RMT and control group, respectively) was not changed significantly for either group; however in the RMT group B_{EX} was increased significantly after RMT (+5.83 ± 10.68 and -0.38 ± 2.48, P=0.047, RMT and control group, respectively). Control values were (-0.21 ± 0.72) for S_{EX} and (-0.38 ± 2.48) for B_{EX}. The baseline ventilation preceding the $HCVR_{EX}$ did not significantly change after RMT in either the RMT group (pre: 53.0 ± 3.9 $l \cdot min^{-1}$ vs. post: 54.4 ± 6.5 $l \cdot min^{-1}$) or the control group (pre: 52.6 ± 3.0 $l \cdot min^{-1}$ vs. post: 52.9 ± 4.0 $l \cdot min^{-1}$). There was no correlation between the changes in S or B for the $HCVR_{EX}$ and the baseline $\dot{V}_E$ before and after RMT (B, RMT group: r = 0.01, P = 0.974 vs. control: r = -0.37, P = 0.348). Also, the mean end [La] values of the $HCVR_{EX}$ were not significantly changed (RMT group, pre: 0.84 ± 0.26 $mmol \cdot l^{-1}$ vs. post: 1.17 ± 0.37 $mmol \cdot l^{-1}$; Control group, pre: 0.78 ± 0.12 $mmol \cdot l^{-1}$ vs. post: 1.06 ± 0.29 $mmol \cdot l^{-1}$) following RMT. There were no significant correlations in [La] and S or B for the $HCVR_{EX}$ (B, RMT: r = -0.10, P = 0.795 vs. control: r = 0.02, P = 0.956).

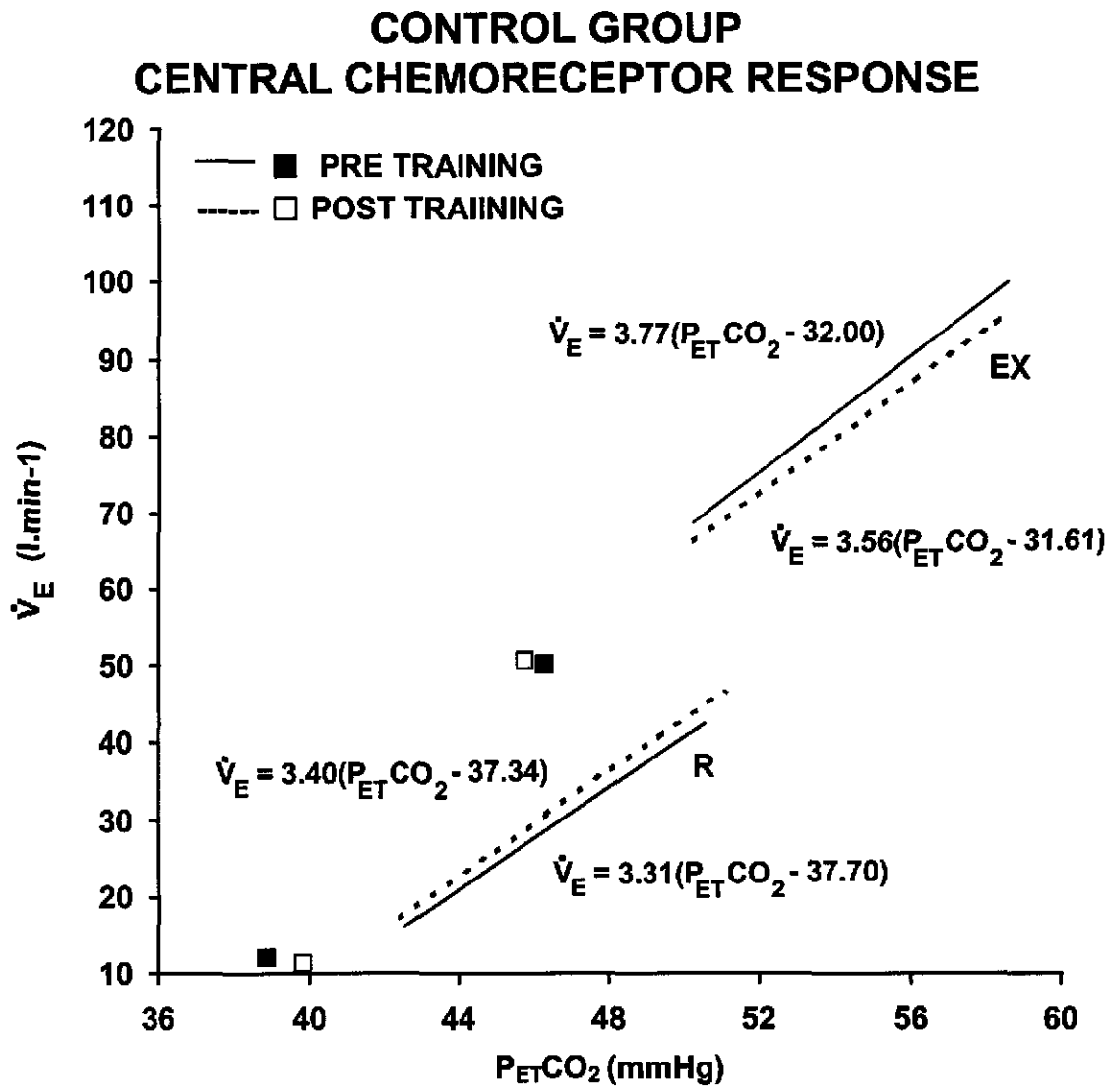


Figure 8. Hypercapnic ventilatory response for the control group at rest (R) and exercise (E). The squares are baseline values prior to hypercapnia (■ and solid line pre-training; □ and dashed line post-training (n = 9).

RMT GROUP
CENTRAL CHEMORECEPTOR RESPONSE

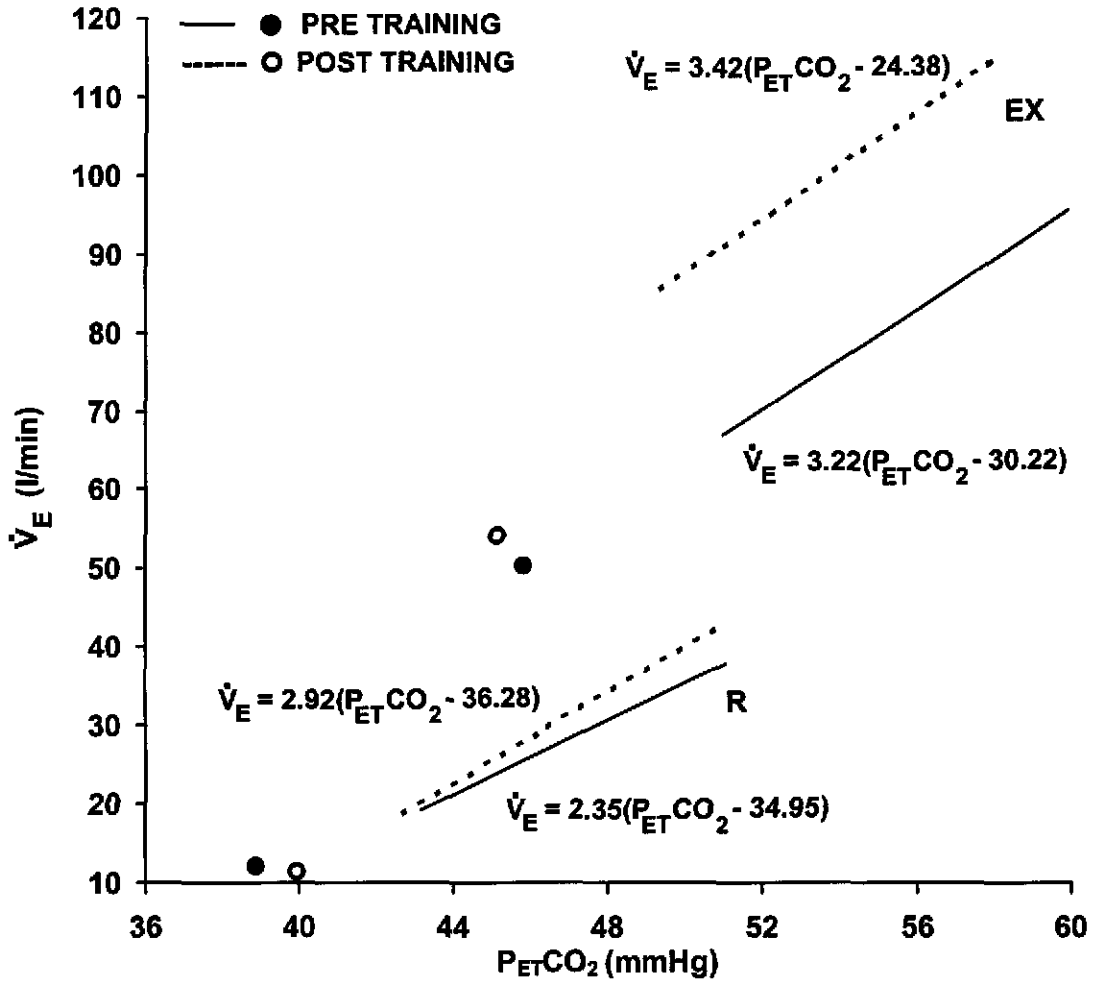


Figure 9. Hypercapnic ventilatory response for the respiratory muscle training group at rest (R) and exercise (E). The circles are baseline values prior to hypercapnia (● and solid line pre-training; O and dashed line post-training (n = 9).

Spirometry

RMT significantly improved MVV in the RMT group (+20 l/min) compared to the control group (-3 l/min) (Table 4). VC was also significantly improved (+0.2 l and -0.1 l RMT and control group, respectively; Table 4). Other spirometric variables, i.e., forced expiratory vital capacity, forced expiratory volume in 1s, and peak expiratory flow, were not significantly changed (Table 4).

Table 4. Pulmonary function before and after respiratory muscle training (RMT); RMT versus the control group.

		Pre-training	Post-training
VC	CONTROL	6.1 ± 1.0	6.0 ± 1.0
(l)	RMT	5.9 ± 0.6	6.1 ± 0.6*
FEVC	CONTROL	5.8 ± 0.9	5.8 ± 0.5
(l)	RMT	5.8 ± 1.0	5.9 ± 0.6
FEV_1	CONTROL	4.7 ± 0.6	4.6 ± 0.8
(l)	RMT	4.7 ± 0.6	4.7 ± 0.5
PEF	CONTROL	10.4 ± 2.0	10.4 ± 3.1
($l \cdot s^{-1}$)	RMT	10.2 ± 1.2	10.7 ± 1.7
MVV	CONTROL	203 ± 31	200 ± 34
($l \cdot min^{-1}$)	RMT	182 ± 31	202 ± 29**
BET	CONTROL	11.12 ± 2.86	11.61 ± 4.40
(min)	RMT	8.30 ± 2.00	29.80 ± 13.60**

Values are means ± SD (N = 10). VC = vital capacity; FEVC = forced expiratory vital capacity; FEV_1 = forced expiratory volume in one second; PEF = peak expiratory flow; MVV = maximum voluntary ventilation in 15 s; BET = breathing endurance test. * $p < 0.05$; ** $p < 0.01$ for between group differences of response.

Breathing Endurance Test

Following the RMT period, the time to exhaustion during the BET was significantly improved in the RMT group (+259%) compared to the control group (+4%; Table 4). This improvement (RMT group: +21.49 ± 13.52 min; control group: +0.49 ± 4.41 min, P=0.004) occurred despite the fact that the BET was stopped at the 40 min cut-off time for 5 of the 10 subjects in the RMT group but not for any of the 10 subjects in the control group. Only one of the ten subjects from the RMT group didn't improve after completing the RMT program. Analysis of the 4 observed RMT laboratory training sessions showed the subjects maintained normocapnia, range 4.6 % to 5.8% $F_{ET}CO_2$ (Figure 10).

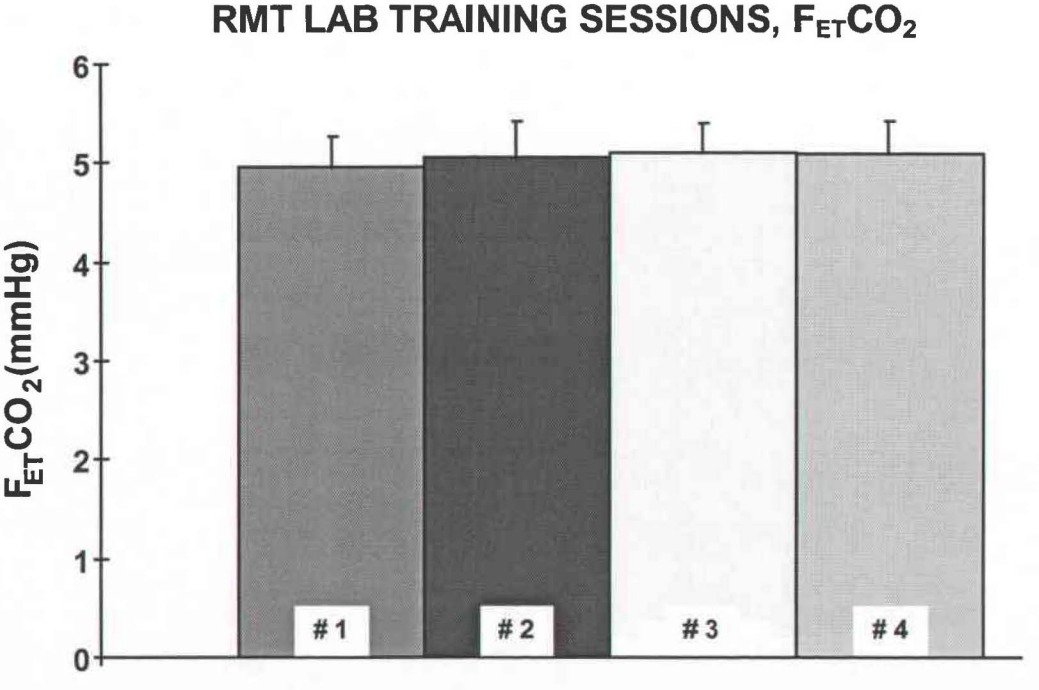


Figure 10. Respiratory muscle training (RMT) lab training sessions; fractional end-tidal CO_2 concentrations ($F_{ET}CO_2$) from every fifth training in the respiratory muscle training group (n = 10).

Also, the levels of $\dot{V}_E$ during the training period rose progressively, as seen from the results of weekly control sessions (Figure 11).

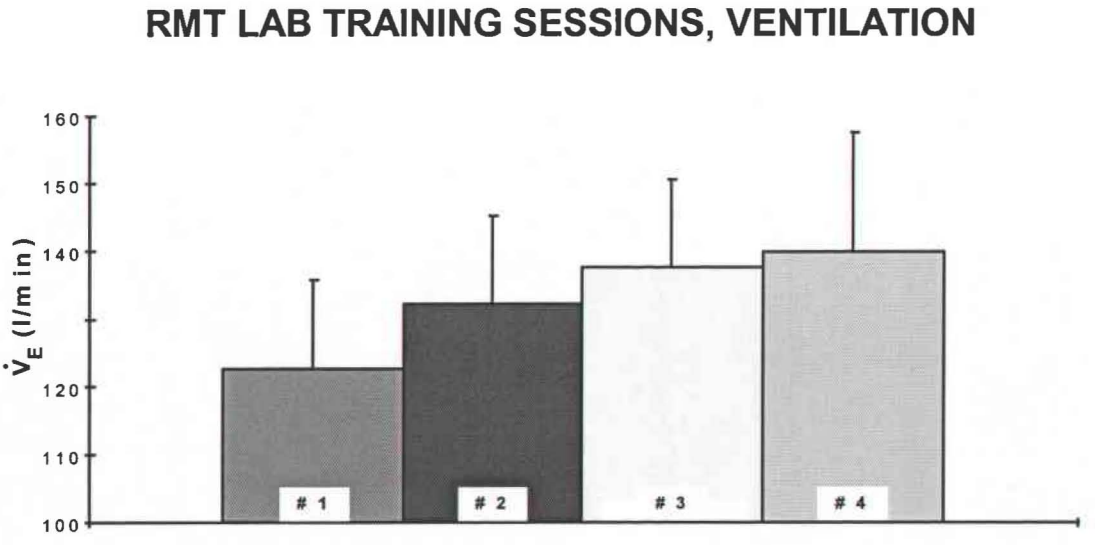


Figure 11. Respiratory muscle training (RMT) lab training sessions, ventilation ($\dot{V}_E$) from every fifth training in the respiratory muscle training group (n = 10).

These mean values increased from ~120 l/min during the first supervised training session in the laboratory (#1) to ~140 l/min in session #4.

Incremental Test

The cardiovascular, and metabolic indicators of aerobic fitness (i.e., $\dot{V}_{O_2}$, f_C, [La], and $\dot{W}_{max}$) measured during the incremental test, did not change significantly following the RMT or control period for either group as shown in Table 5. In addition, the respiratory variables (i.e., $\dot{V}_E$, V_T, or f_b), were not changed significantly. This lack of statistical significance was constant through all work levels of the incremental test.

Table 5. Incremental cycling test results before and after the respiratory muscle training (RMT) or control period.

		280 W		End-time	
		Pre-training	Post-training	Pre-training	Post-training
$\dot{V}_{O_2}$	Control	52.62 ± 3.64	53.50 ± 3.78	70.10 ± 12.63	69.38 ± 10.19
($l \cdot min^{-1} \cdot kg^{-1}$)	RMT	51.58 ± 4.06	53.08 ± 2.73	73.56 ± 14.98	73.23 ± 13.08
f_C	Control	160.1 ± 16.7	162.1 ± 16.5	186.3 ± 11.7	183.8 ± 10.3
(min^{-1})	RMT	158.9 ± 11.3	160.2 ± 7.4	187.1 ± 5.6	184.1 ± 6.3
[La]	Control	4.39 ± 1.65	4.54 ± 1.40	11.74 ± 2.45	10.66 ± 1.88
($mmol \cdot l^{-1}$)	RMT	4.64 ± 1.74	4.14 ± 1.92	11.65 ± 1.88	11.26 ± 1.79
$\dot{W}_{max}$	Control			360 ± 38	350 ± 47
(W)	RMT			368 ± 36	371 ± 31

Values are means ± SD (N = 10). 280 W represents the highest common power output completed by all subjects. $\dot{V}_{O_2}$ = oxygen consumption; f_C = cardiac frequency; [La] = blood lactate concentration; $\dot{W}_{max}$ = maximum work capacity.

CHAPTER IV DISCUSSION

Primary Findings

The assumptions made at the beginning of this study have led to some interesting findings. Simulating the high levels of exercise $\dot{V}_E$ with RMT (30 min sessions of voluntary eucapnic hyperpnea over 4-6 weeks) improved endurance cycling performance (+ 15%) and shifted the CO_2 response curve during exercise to the left (B increased for $HCVR_{EX}$; 19%). RMT also caused a significant decrease in the pR_c (-5.8 ± 6.0%) without changing $\dot{V}_E$ during any portion of the tests involving cycling. However, there was a consistent and significant increase in V_T for the quasi-steady state category of the CET.

General Considerations

Training Protocol and Study Design

All studies with human subjects are inherently difficult because many factors cannot be controlled. The potential influence of external factors increases the number of test the subjects need to perform, the complexity of the requirements they are expected to meet, and the longer the duration of the study (e.g., prospective/longitudinal training studies). We attempted to minimize the number of external influences by standardizing the testing procedure. Guidelines were set for the subjects concerning their habitual training prior to

and throughout the study. These guidelines were reviewed with the subjects on a regular basis to make sure they were clearly understood and to serve as a reminder. This was done by verbally questioning the subjects about their compliance with the study protocol and by reviewing the training logs (Appendix A) and subjective rating handout (Appendix F). These procedures seemed to work. An example of this comes from the initial stages of the study. These procedures, helped us to determine that a large number of the initial subjects weren't complying or weren't able to meet certain of the study's requirements. We were then able to reduce the rigors of the testing procedures without impacting the most important parts of the study. This proved effective in increasing the level of compliance in the new subjects.

As in any study which investigates humans, in the end we were reliant on the subjects' self-accountability in fulfilling these requirements and giving us honest feedback. The Swiss subjects seemed to be truthful and accountable and they gave us no reason to believe otherwise. Certainly, the pre- and post-training metabolic data (Table 5) indicate no change in overall fitness during the course of this study.

Effectiveness of RMT

The effectiveness of the RMT program was supported by the significant increases in VC, MVV, and BET (Table 4). These results are indicative of a successful respiratory muscle training program and, with the exception of VC,

are in agreement with a vast majority of previous RMT studies (Boutellier et al., 1992; Boutellier & Piwko, 1992; Leith & Bradley, 1976; Spengler et al., 1999).

Normally, improvements in endurance performance are related to adaptations of the heart and cardiovascular system to meet the metabolic demands of the exercising muscle and changes within the muscles themselves. The lack of significant changes of cardiovascular, and metabolic indicators of aerobic fitness, i.e., f_C, [La], $\dot{V}_E$, $\dot{V}_{O2peak}$, and $\dot{W}_{max}$, during the incremental test (Table 5) and CET (Table 3) indicated that the general and cardiovascular fitness of all the subjects (control and RMT groups) had not changed during the study. As definitively shown by Markov et al., (2001) by direct measurement of stoke volume and heart rate, changes in cardio-respiratory factors as a result of RMT are not expected. The above results, combined with examination of the subjects' training logs, show that the subjects' physical fitness was the same throughout the study. Therefore, RMT was the likely factor responsible for the improved cycling endurance, the decreased pR_c as assessed by the modified Dejours O_2 test, and the leftward shift in the CO_2 response curve during exercise (B decreased for $HCVR_{EX}$) and the significant effects on VC and breathing pattern (increased V_T) during exercise.

Explanation of Results

By necessity , indirect measures rely on assumptions about variables and confounding factors that often cannot be rigorously controlled. Some of the

assumptions and other pitfalls that were associated with the methods used in this study were discussed above. The modified Dejours O_2 test and the HCVR tests used in this study provided indirect assessment of the pR_c and cR_c. Therefore, at best, we can only report our observations with certainty. Any mechanistic inferences made from these observations, especially insights derived using correlations, are speculative in nature.

RMT

Findings

RMT protocol consisted of 20 sessions of voluntary eucapnic hyperpnea lasting 30 min over a 4-6 week period. This protocol was designed to promote an endurance conditioning stimulus for the respiratory muscles. Voluntary eucapnic hyperpnea was chosen because it allows the respiratory system to achieve rates of $\dot{V}_E$ similar to exercise without the other concurrent responses of whole-body exercise. The levels of $\dot{V}_E$ during the training period rose progressively, as seen from the results of weekly control sessions (see Figure 11). These values (~120 - 140 l/min) correspond to $\dot{V}_E$ levels attained during the CET (~120 l/min during quasi-steady state) and even near the end-time of exercise (~140 - 155 l/min). This indicates that RMT did simulate the level of $\dot{V}_E$ during exercise. Exercise which achieves such high ventilatory rates places a tremendous metabolic and cardiovascular load on the body which requires considerable time for regeneration. Thus, attaining these levels of $\dot{V}_E$ more than

a couple times per week through exercise is prohibitive, yet possible nearly everyday with voluntary eucapnic hyperpnea.

In terms of respiratory muscle functional capacity, RMT significantly improved VC, MVV and BET. Respiratory muscle trained subjects also significantly increased V_T throughout the CET suggesting that the breathing pattern during exercise became deeper. This showed that the anticipated respiratory muscle endurance conditioning response, resultant from high levels $\dot{V}_E$ being repeated in an endurance training regime, had occurred. The lack of any concurrent metabolic or cardiovascular changes (Table 5), indicates that these adaptations occurred without changes in whole-body fitness

These improvements in respiratory functional capacity were greater than those reported to occur following whole-body endurance training. This was to be anticipated given the specific nature of the RMT stimulus on the respiratory muscles and the non-specific stimulus created by whole-body endurance training on the respiratory muscles. Although not measured, it seems likely that other aspects of the respiratory muscles, such as structural and biochemical changes, would have also adapted more in response to RMT than with whole-body endurance training.

Critique of the Methods

We did not measure cardiac output or blood gases directly, but instead relied on the results from past studies (Markov et al., 2001; Stuessi et al., 2001)

and indirect measures from the CET (Table 3) and incremental test (Table 5) to infer that whole-body cardiovascular changes did not occur. Furthermore, the lack of any data contrary to this makes us confident that no whole-body metabolic or cardiovascular changes occurred in the subjects.

For this study, the goal of the RMT protocol was to ensure subject compliance while providing a sufficient endurance training stimulus. Although the results indicate this was achieved, the endurance training protocol of this study was very rudimentary. Such a training program was not likely to have provided the optimal training stimulus; therefore, the endurance conditioning responses were likely less than maximal.

It seems logical that an optimal RMT program should contain the time tested components of an comprehensive endurance training program. Such training programs include a variety of components (duration, intensity, strength, technique, rest, and taper) which are responsible for responses that together maximize endurance adaptations and performance. An ideal RMT program would have begun more gradually (recovery) by having fewer training sessions the first couple of weeks, followed by incorporation of a mix of less intensive longer sessions with more intensive shorter sessions (intervals), and some inspiratory resistive loading (strength), and even controlled breathing pattern by varying V_T and f_b (technique). If such a program were done over a longer period of time (8-12 weeks) and then incorporated a reduction in the overall level of RMT before testing (taper) even more pronounced and definitive changes could

have occurred to the respiratory system and its components. The importance of such a comprehensive program is likely to increase with trained subjects whose respiratory muscles have already undergone nonspecific training. Two previous studies did use RMT programs slightly more involved than the protocol in this study. The subjects of Sonetti et al., (2001) performed inspiratory resistive loading in conjunction with RMT. Spengler et al, (1998) used two different methods of RMT. One increased the training load by increasing f_b and keeping V_T constant and the other by increasing V_T and keeping f_b constant. Both studies reported results similar to this and other RMT studies which used slightly more rudimentary RMT protocols. This is not surprising because these studies failed to incorporate too many of the other components of an advanced endurance training program to have expected more.

Conclusion

The progressive improvements observed in the weekly RMT control training sessions and the improvements in VC, MVV, BET, and exercise V_T without the obvious cardiovascular changes that normally accompany whole-body endurance training show clearly that the objective of simulating the $\dot{V}_E$ of exercise in an endurance training fashion was successful.

In addition to the training components of a RMT program, the timing of the voluntary eucapnic hyperpnea exercises to other modes of exercise warrants exploring. The effects of voluntary eucapnic hyperpnea done immediately prior

to or following or even during whole-body exercise are not known. Future research is important and necessary to answer the many questions that remain regarding the optimization of a RMT program.

Cycling Endurance Test

Findings

The 15% increase in CET for the RMT group was less than the range of performance improvements (24 - 50%) from previous studies that reported improvements (see Table 1). Nevertheless, this result was statistically significant, although partially due to the slight performance decrement in the control group (-8%). If a paired t-test were performed (i.e., if the study design had not incorporated a control group) the 15% improvement would have only been borderline significant ($p = 0.068$). The paired t-test result for the control group would have been non significant ($p = 0.24$).

Although the majority of respiratory parameters were unchanged by RMT, V_T was increased significantly (quasi-steady state and end-time categories) throughout the CET. Increases in V_T during exercise can occur by decreasing the EELV or increasing the EILV. Normally during exercise, the EELV decreases first and then plateaus, after which, increases in V_T are accomplished by increases in the EILV (see Introduction, exercise breathing pattern). The initial increases in the EELV are accomplished by contraction of the expiratory muscles which is indicative of greater abdominal muscle activation, at least in running

(Henke et al., 1988). This increased expiratory muscle work would tend to minimize the load on the inspiratory muscles by the contribution of the chest wall static recoil to inspiration when lung volumes fall below the passive functional residual capacity. Reflecting on the properties of the expiratory intercostals (see earlier under Introduction, fiber size, capillarization, and summary of the respiratory muscles) they are well suited for this role, perhaps due to expiratory flow limitation considerations. However, the requirements to meet further increases in V_T cannot be met by the internal intercostals and abdominal muscle group alone. At this point V_T increases occur by increases in the EILV and the increasing respiratory load must be endured by the inspiratory muscles, albeit shared among many. In so doing, the elastic recoil of the chest wall and lung can be utilized to minimize the load on the internal intercostal during expiration at the higher lung volumes. In fact, the beginning of expiration in exercise can be passive at elevated lung volumes, because of the compliance of the chest wall and lung, making these changes in V_T efficient. However, at high EILV further it becomes more efficient to increase f_b. At very high levels of $\dot{V}_E$ increasing either the EILV or f_b may minimize expiratory flow limitation, but by increasing f_b instead of EILV inspiratory muscle work is reduced. So as the inspiratory muscles tire and are unable to sustain the "pre-fatigue" EILV they resort to increasing f_b in an attempt to meet the ventilatory demands.

EELV and EILV were not measured in this study so it is not known how V_T increased. Given that RMT likely trains both the inspiratory and expiratory

muscles, we could speculate that V_T was increased by changes in both EELV and EILV. However, because EELV normally plateaus followed by increases in EILV during exercise, and because the inspiratory muscle load is shared among many muscles, we speculate that the increased V_T could occur more effectively by increasing the EILV. If this is so, the deeper V_T suggests the RMT subjects elected to increase the elastic work of inspiration, by ventilating less compliant lung volumes, in order to minimize the active expiratory work (i.e., a breathing pattern was adopted that better utilized the passive elastic recoil of the chest wall and lung at high lung volumes which would allow the load on the internal intercostal muscles to be decreased by increasing the demand on the many inspiratory muscles).

The changes in the end-times of the CET correlated significantly with the changes in $\dot{V}_E$ during the quasi-steady state phase of the CET for the RMT group (Fig. 5). RMT group's V_T also correlated strongly with the change in end-times ($p = 0.054$, quasi-steady state; $p = 0.028$, iso-end time). The correlation with $\dot{V}_E$ is consistent with past results that also showed the relationship between $\dot{V}_E$ and the end-times of the CET, and appears to be the common factor associated with RMT. Figure 12 shows the graph for $\dot{V}_E$ during the CET for the RMT group, subdivided into those subjects that increased their end-times (responders) and those that decreased their end-times (non-responders).

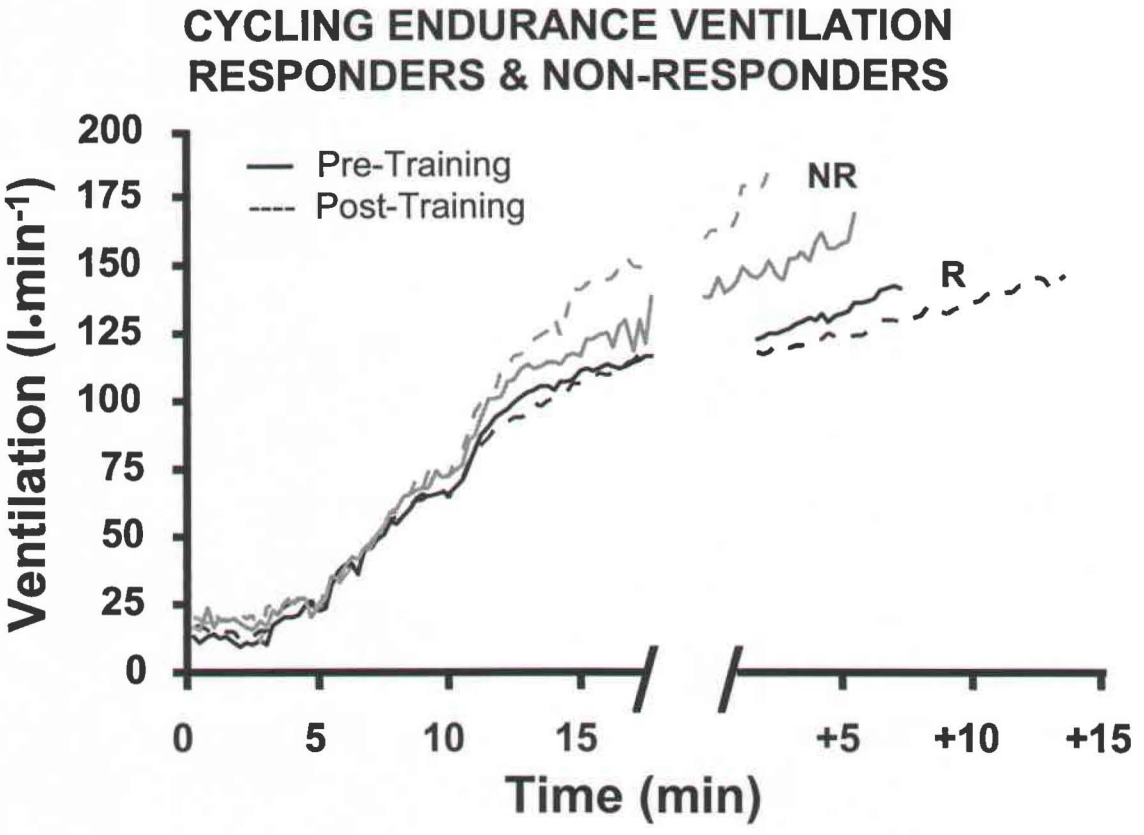


Figure 12. Cycling endurance test ventilation in the respiratory muscle training group, subdivided into responders (R, n = 6) and non-responders (NR, n = 3). The difference between R and NR is significant for ventilation (p = 0.005) and end-time (p = 0.002)

The change in $\dot{V}_E$ folowing RMT was significantly different in the responders (decreased) compared to the non-responders (increased). The f_b was decreased in the responders but only borderline significantly ($p = 0.059$). This supports the theory that the postponement of the final hyperventilation, or tachypnea, during exhaustive exercise seems to explain the improvements in endurance exercise. Indeed, V_T was increased significantly in the RMT group compared to controls throughout the CET. This change in V_T was increased in more in the non-responders than the responders (+231 ± 220 ml; 37 ± 100 min, respectively), but these increases were not significantly different ($p = 0.1$).

The delayed tachypnea in the responders compared to non-responders (see fig. 12) could be the result of improved ventilatory efficiency (perhaps linked to the prior increases in exercise V_T) or delayed inspiratory muscle fatigue. In fact, increased ventilation, with a rapid shallow breathing pattern, has been reported following inspiratory muscle fatigue (Sliwinski et al., 1996). This suggests two things: 1) the non-responders exhibited a pattern of breathing similar to that seen with inspiratory muscle fatigue; and 2) the responders were resistant to or were able to delay the onset of inspiratory muscle fatigue.

Whether by improved ventilatory efficiency (e.g. because of improved mechanics that led to increases in V_T) or delayed fatigue, the changes in $\dot{V}_E$ seen in figure 12 would reduce the metabolic requirements of the respiratory muscles. A reduction in the metabolic requirements of $\dot{V}_E$ at a given workload could ease the competition for resources (e.g., blood flow) between the respiratory muscles

and leg muscles involved in cycling and this could result in an improvement in cycling performance.

This rational is supported by recent studies by Harms et al., (1997) which showed that the respiratory muscles are in competition with the leg muscles for blood flow. In fact, increasing the work of breathing required of the respiratory muscles caused a reduction in blood flow to the working locomotor muscles do to sympathetically mediated vasoconstriction. Furthermore, it was demonstrated that the normal load on the respiratory muscles influences exercise performance. Reducing the work of breathing improved cycling performance presumably via decreased muscle sympathetic nerve activity which reduces vasoconstriction thus increasing leg blood flow (Harms et al., 2000). In the present study, it appears that the RMT subjects adopted a breathing pattern that better utilized the passive elastic recoil of the chest wall and lung which would allow the load on the internal intercostal muscles to be decreased by increasing the demand on the many inspiratory muscles. By delaying the onset of fatigue or through an improvement in the efficiency of the respiratory muscles, the sympathetic nerve activity to the locomotor muscles may have been reduced compared to pre-training CET. More blood flow available to the leg muscles may have resulted in the increase in CET end times.

Critique of the Methods

Fixed-rate endurance tests use exercise time to exhaustion as the measure of performance. When performed at a submaximal level of exercise, near or below the $\dot{V}_E$, [La], or cardiac threshold, use of a fixed-work rate allows a steady-state to be achieved because the work rate is fixed. This is the primary advantage of a fixed-work rate test such as the CET because it allows for a convenient comparison of physiological markers over a variety of time intervals (iso-times) and even after an intervention (e.g., RMT). However, if a steady-state is not achieved, comparisons between tests become more tenuous. The assumption that the magnitude of the physiological processes occurring during a given time period of a test should be the same between tests weakens. If exercise is not homeostatic then perhaps the CET should be looked at as a *single unified process consisting of characteristic components* (i.e., a beginning, middle portion(s) and an end) which might represent physiological phases that would best be compared in relative terms (e.g., various percentages of time from the end of the tests) as opposed to absolute (e.g., iso-times). For the quasi-steady state category of the CET test, we used an iso-time approach. The iso-times were based on the shorter of the pre-training and post-training test for each subject individually. The quasi-steady state consisted of the average values for the middle portion of the shorter test (i.e., the entire shorter test less the beginning 1.75 min and end 2 min values). This time was then applied to the longer test. Done in this way the quasi-steady state was an intra-subject iso-time

based on their shorter test. The generality of this middle phase could have masked subtle effects happening within a smaller portion of this phase. A few specific iso-time measures were selectively chosen for analysis but did not yield results differing from the quasi-steady state for any parameters. End-time comparisons made from the last 60s of each individual CET, independent of the duration of a given subject's other CET test.

There are some major disadvantages to the CET. The first is that fixed-rate tests in general, have a high variability. Studies have shown the coefficient of variation ranges from 17 - 40% (Jeukendrup, Saris, Brouns, & Kester, 1996). The coefficient of variation of CET tests performed in the Boutellier lab is about 17.8% (McMahon unpublished results). There also seems to be a familiarization effect not related to the researcher or to the equipment.

In unpublished results, the reliability of the CET was improved by 41% after three familiarization trials (in addition to the initial familiarization procedures performed before studies begin). These results are based on 14 subjects who completed a series of 6 CET. The coefficient of variation for the first 3 CET trials (familiarization) was 22% and this decreased to 13% for the last 3 CET trials (McMahon unpublished results).

Another disadvantage of CETs, which may be linked to the variability and familiarization problems, is that the fixed rate tests to exhaustion do not have an objectively measured fixed endpoint. Exhaustion is a subjective measure as is intensity (perceived). This is very different from exercises or races done in

everyday life. Competitive situations almost always use objective measures (distance or sometimes time) as a fixed endpoint. While no laboratory test can truly mimic competitive situations, tests with distance as a fixed endpoint that are completed in as quick a time possible (time trials) create a situation more typical of racing. Even though the intensity chosen by the subjects is subjective and variable during time trials they have a surprisingly high reliability (coefficient of variation 1.0 to 3.1%; Jeukendrup et al., 1996). Perhaps the variability would be lower for the fixed-rate tests if our everyday activities used exhaustion as a fixed endpoint.

Conclusion

CET end-times were increased by RMT. The significance of the improvements in CET were influenced by the decrease in the pre- to post-training CET end-times of the control group. When analyzed without the control group, the RMT group CET improvements would have been on the borderline of significance. In the future, a more reliable and realistic measure of performance should be used, or if a CET is used to measure performance, more subjects would be recommended.

For this study, the CET provided the best choice to measure both performance and physiological variables. The Initial subjects were overloaded by the requirements of the study (protocol and design) and the testing protocol needed to be reduced to increase subject compliance. Adding CET

familiarization trials or another measure of performance (e.g., a time trial test), although potentially informative and beneficial, would not have been an option.

Peripheral Chemoreceptor Response

Explanation

After the RMT period, the PC contribution to $\dot{V}_E$ was significantly reduced (parametric and non-parametric analysis) by 5.8%. *This supports the hypothesis* that RMT, which simulated exercise hyperpnea, can itself cause reductions in pR_c. As the PC are generally believed to contribute by augmenting $\dot{V}_E$ during exercise (Dejours, 1963; Weil & Swanson, 1991), one would expect that a reduction in pR_c would be related to concomitant reductions in hyperpnea during exercise for the RMT group. However, we found a negative correlation ($r = -0.43$), although not significant ($p = 0.31$), between the change in quasi-steady-state $\dot{V}_E$ during the CET and the change in pR_c among the RMT group (Fig. 6).

The control group exhibited a borderline significant correlation suggesting a relationship between pR_c and $\dot{V}_E$. This relationship was removed, even reversed, in the RMT group. Such a negative trend might suggest that the PC could have had an inhibitory, rather than an excitatory, effect on $\dot{V}_E$ during exercise. Previously, Dempsey and Smith (1994) have proposed this possibility, that the carotid chemoreceptors may inhibit $\dot{V}_E$ in some species of animals; one other study (Pianosi & Marchione, 1995) has reported that the HVR (a measure of the pR_c) was inversely related to degree of exercise hyperventilation. This

may have occurred in the subjects of this study but no conclusion is warranted given that the data do not support a statistically significant correlation between pR_c and $\dot{V}_E$.

Critique of the Methods

Measuring the pR_c can be done using several different techniques. In humans, all of these techniques provide indirect measurements of the PC. The most common is the HVR which relies on the hypoxic affects of low O_2 (below P_AO_2 of 60 Torr) to which the PC respond by increasing $\dot{V}_E$. Another method is a special modification of the HCVR. This method relies on the effect of increasing hypercapnia on $\dot{V}_E$ and also on the time course of changes to $\dot{V}_E$. Both the hypoxic and hypercapnic methods give an indirect measure of the pR_c to the particular stimulus (low O_2, high CO_2) used. However, hypoxia and hypercapnia only reflect a portion of the natural stimuli of the PC. Hyperoxia, on the other hand, abolishes the nervous output of the carotid bodies from all the natural stimuli (low O_2, or increased CO_2, H^+, and K^+) (Dejours, 1962; Dejours, 1963). Therefore, it provides indirect assessment of the complete PC contribution to $\dot{V}_E$ during normoxic exercise.

The importance of the hyperoxic effect on the PC deserves closer examination. The validity of the modified Dejours O_2 test is based on the assumption that the inspiration of pure O_2 physiologically denervates the PC to these stimuli. This has been confirmed in cats under a variety of arterial PCO_2

and pH conditions (Fitzgerald & Parks, 1971; Pokorski & Lahiri, 1983). Also, two studies (Burger, Estavillo, Kumar, Nye, & Paterson, 1988; Paterson & Nye, 1991) showed that hyperoxia suppressed the pR_c to K^+. In humans, differences in the timing of ventilatory changes to increases in end-tidal PCO_2 at rest (Miller, Cunningham, Lloyd, & Young, 1974) and moderate-intensity exercise (Ward & Bellville, 1983) suggest that the pR_c is abolished with hyperoxia (Cunningham, 1987). Recently, McLoughlin (1993) suggested that the pR_c to metabolic acidosis is not eliminated by 100% O_2 during heavy exercise. This reflected earlier findings by Rausch et al. (1991) but contradicts those of Cunningham (1987) and MacFarlane and Cunningham (1992) which showed that the pR_c was abolished by hyperoxic breathing during heavy exercise. Due to these equivocal findings, we logically chose moderate-intensity exercise for the modified Dejours O_2 test to ensure the most accurate interpretation of the pR_c. While the Dejours O_2 test may not provide an exact measure of the pR_c under all conditions (Ward, 1994b), during the light to moderate intensity (40% $\dot{W}_{max}$) at which we performed this test it was reasonable to assume PC input was abolished (McLoughlin et al., 1993; Ward & Bellville, 1983).

Other methodological considerations warrant discussion. An alteration of any of the known natural stimuli or their counterparts following RMT could affect the PC contribution to $\dot{V}_E$. If the level of a given stimulus was increased, the PC nervous output would be increased and this would presumably contribute to an increase in $\dot{V}_E$. In this example, $\dot{V}_E$ would be reduced less by hyperoxia after

RMT than before RMT. This smaller decrement would be interpreted as a reduced pR_c. It is important to note that the levels of indirect stimuli to the PC were likely to be unchanged after RMT, i.e., [La] as well as end-tidal PCO_2, were not significantly changed by RMT during the modified Dejours O_2 test. Given that the pre- and post-training baseline $\dot{V}_E$ immediately preceding the hyperoxic trials were the same, it is also unlikely that RMT altered the levels of the known stimuli to the carotid bodies.

An additional methodological consideration was whether the magnitude of the ventilatory decline to hyperoxia was complete before its secondary stimulating effects on $\dot{V}_E$ occurred. These secondary effects include the acidifying influence of sustained hyperoxia via cerebral vasoconstriction, the Haldane effect, and the hypercapnia that results from the primary fall in $\dot{V}_E$ (Dejours, 1962). The use of 100% O_2 ensured that the end-tidal PO_2 was greater than 300 mmHg within the first breath (Griffiths, Henson, & Whipp, 1986) which would silence the PC within the first few breaths (Dejours, 1962; Ward, 1994b). This would result in suppression of the pR_c within ~5 s, corresponding to the circulatory time from the pulmonary capillaries to the carotid bodies. Becker et al. (1995) found a significant increase in $\dot{V}_E$ only after 7 min of hyperoxia. Thus, the transient administration of O_2 for 10-12 breaths, while longer than some studies, was likely short enough to minimize the complications (the stimulation of ventilation) due to acidification. Finally, the secondary central stimulation of $\dot{V}_E$ by CO_2 was likely insignificant during the period in which the

ventilatory decline was measured (~30 s) in this study (Bellville et al., 1979; Dahan, DeGoede, Berkenbosch, & Olievier, 1990).

Conclusion

The reduced $\dot{V}_E$ following brief bouts of 100% O_2 indicated that the PC contribution to submaximal exercise hyperpnea was reduced (5.8%). This reduction likely reflects reduction in the PC sensitivity, given the low likelihood of the potential problems mentioned above. Despite the suggestion that RMT caused a disassociation of the PC control of $\dot{V}_E$ (Fig. 6) the lack of significant correlations of the pR_c with $\dot{V}_E$ in any of the exercise tests, tells us the PC contributed little to the regulation of ventilation during aerobic exercise. This is consistent with the modest change in blood gases during moderate exercise and the theory that the pR_c only serve to "fine-tune" the ventilatory response to exercise (Dempsey et al., 1995).

Central Chemoreceptor Response: Rest and Exercise

Findings

First, it is easy to describe the effect of RMT on the cR_c at rest; as there was none. Both S and B were unchanged by RMT during $HCVR_R$. During the $HCVR_{EX}$, S was also unchanged. Technically, this would mean there was no change in central chemosensitivity during exercise. However, the X - axis intercept, B, was increased. The mathematical meaning of B is clear, it is the X -

axis intercept of a line which in the case of this study refers to the theoretical level of $P_{ET}CO_2$ when $\dot{V}_E$ would be zero. The physiological meaning of a change in B is unclear. The relationship between CO_2 and $\dot{V}_E$ which the literature primarily focuses on is the changes in chemosensitivity, S, with little mention, let alone an explanation for a change in the X - axis intercept.

Perhaps the increase in B can be described as an additive increase in the response of the cR_c. It appears this would be a good way to describe the leftward shift in the CO_2 response curves of the RMT group (Fig. 8). Such a leftward shift is expected from rest to exercise. It is actually a vertical upward shift (Duffin & McAvoy, 1988). We observed such a shift in comparing the pre-training resting and exercise HCVR of our subjects (fig 11). After endurance training an increased change in the intercept could be due to a further vertical shift because of an added drive to breathe or a leftward shift because of a decrease in CC threshold. However, the latter would have also been expected to occur during the $HCVR_R$ (J. Duffin personal communication, March 6, 2000).

A change in bicarbonate during exercise after the RMT program could also effect the B. Lower bicarbonate levels, would lower pH and this could cause a leftward shift in the cR_c. Such a change could lower the threshold, but this appears unlikely in our study, because stimuli for a reduction in bicarbonate levels, such as hypoxia, were absent.

Several phenomena are known to cause a shift in the CO_2 response curve for $\dot{V}_E$ similar to that which we observed. It has been suggested (Koepchen,

1975) that this leftward shift from rest to exercise may be in response to an increase in epinephrine concentration which is known to occur during single bouts of exercise (Brooks et al., 1996). Furthermore, epinephrine levels have been shown to increase significantly during progressive exercise following whole-body endurance training (Brooks et al., 1996). Thus, one could speculate that the RMT group's upward shift in B for $HCVR_{EX}$ may have been induced by increases in circulating epinephrine during exercise. These changes in HCVR may be due to direct adrenergic effect of the catecholamines on the reticular activating system in the brain stem or indirectly through changes in blood or cerebral spinal pH. However, increases in the blood concentration of catecholamines not only augments ventilation (shift B left) but would also increase the steepness of the CO_2 response curve (increases S). In this study, neither the control or the RMT group exhibited any significant changes in S. This weakens the case for a catecholamine induced augmentation of ventilation during $HCVR_{EX}$. Additionally, epinephrine and norepinephrine levels have been shown to be lower at identical work rates after endurance training (Casaburi et al., 1987). Thus, we would more likely expect RMT to produce decreases in their levels. Although catecholamines were not measured in this study, it is unlikely they played a role in shifting the cR_c.

Comparing figures 9 and 13, the leftward shift we observed in the CO_2 response curve during exercise following RMT (figure 9) resembles the shift in the response curve that occurred between rest and exercise in our subjects

(figure 13). Such an increase in B is also similar to the shift occurring during metabolic acidosis (Koepchen, 1975). In a similar way as with adaptations to altitude, hypoxia or acidosis induced by the rebreathing of CO_2 (increased F_ICO_2) during RMT could have lead to changes in the acid-base balance, decreasing levels of bicarbonate in the blood, and importantly, cerebral spinal fluid. Such a slight metabolic acidosis would result in a shift of the CO_2 response curve to the left (Koepchen, 1975). However, this shift should have also been detectable at rest but we observed no changes in B for $HCVR_R$. Nevertheless, this is unlikely because previous studies from our lab have shown normal levels of oxyhemoglobin saturation during voluntary eucapnic hyperpnea. However, O_2 concentrations were not measured during the RMT sessions. This is due to technical limitations of the gas measuring equipment (metabolic cart) which did not permit measurement of inspired or expired O_2 concentrations during RMT. Thus, these values are not known. Therefore, although it seems unlikely, we can not rule out that a slight hypoxia occurring with voluntary eucapnic hyperpnea may have caused a slow cumulative cerebral spinal fluid acidosis in the subjects but only during the RMT sessions themselves.

Several studies have shown respiratory muscle fatigue and or respiratory loading lead to decreases in HCVR at rest (Clague, Carter, Pearson, & Calverley, 1996; Mador & Tobin, 1992). One study in which the respiratory muscles had an elevated resistive load showed a rightward shift in the CO_2 response curve during rest (Clague et al., 1996). The effects of respiratory

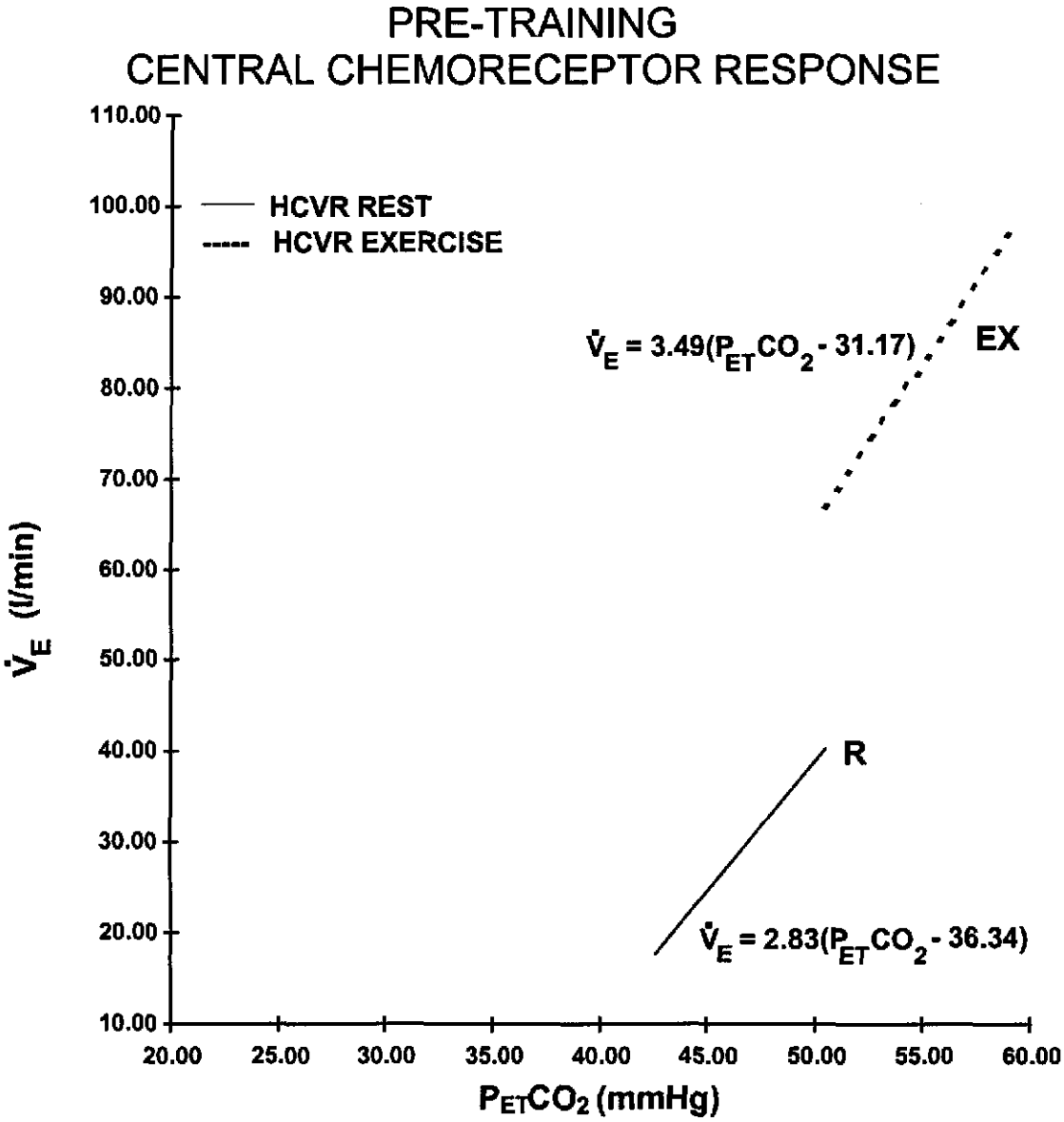


Figure 13. Central chemoreceptor response, rest compared to exercise, the pre-training hypercapnic ventilatory response at rest (R) and exercise (E) for all subjects (n = 18).

muscle fatigue on $HCVR_{EX}$ are not known. One can imagine that exercise might magnify the reductions in the CO_2 response at rest. The improved endurance of the respiratory muscles (BET, Table 2) may have made the respiratory muscles more resistant to fatigue. Perhaps, the respiratory muscles being less susceptible to fatigue resulted in an opposite effect as fatigue and shifted HCVR to the left. We only observed a leftward shift during $HCVR_{EX}$ and no shifts with $HCVR_R$. The endurance trained respiratory muscles likely have an enhanced resistance to fatigue which may not be evident at the $\dot{V}_E$ levels reached during the $HCVR_R$, however, during the increased ventilatory levels experienced with $HCVR_{EX}$ were high enough that the proposed fatigue resistance became apparent as the leftward shift in the CO_2 response curve experienced by the RMT group.

Critique of the Methods

In humans, indirect measurements must be relied on to provide information about the response of the chemoreceptors to hypercapnia. There are many different techniques for measuring the chemosensitivity to hypercapnia All rely on the excitatory nature of high levels of CO_2 on the chemoreceptors to stimulate $\dot{V}_E$. The two most common are the rebreathing and steady-state techniques. Both methods have advantages and disadvantages. For this study, a steady-state method was chosen because it allowed the measurement of the cR_c during rest and exercise more effectively than rebreathing.

Use of the $HCVR_R$ and $HCVR_{EX}$ to measure cR_c control mechanisms is based on the assumptions that the inspiration of high concentration of O_2 physiologically eliminated the peripheral chemoreceptor contribution to $\dot{V}_E$ (Dejours, 1962; Dejours, 1963) and that under these conditions the ventilatory response to CO_2 is mediated solely through the CC (Cunningham, 1987). While evidence suggests these assumptions to be accurate, the indirect nature of the measurements (a necessity because of the invasive nature of more direct assessment of chemoreceptor status) does not permit us to ascertain this with certainty. This method also assumes that increasing the $P_{ET}CO_2$ via increasing F_ICO_2 is physiologically the same as increasing the $P_{ET}CO_2$ by increasing $\dot{V}_{CO_2}$. Nevertheless, a large number of publications (Casey, Duffin, & McAvoy, 1987; Duffin et al., 1980; Mohan, Amara, Cunningham, & Duffin, 1999) have used similar methodology and assumptions to obtain isolated information about the response/sensitivity of the CC.

Although the hyperoxia removes the PC ventilatory drive, inhalation for more than a minute will have secondary stimulating effects on $\dot{V}_E$. As mentioned above, this occurs by cerebral vasoconstriction, the haldane effect, and hypercapnia. These secondary stimulating effects were likely unchanged before and after RMT and their effects on the results if any were minimal. Also, other peer reviewed studies performed HCVR in a background of O_2 to measure the cR_c (Casey, Duffin, & McAvoy, 1987; Duffin et al., 1980; Mohan, Amara, Cunningham, & Duffin, 1999).

Conclusion

The meaning of the increase in B during the $HCVR_{EX}$ is unclear. The likelihood that a change in bicarbonate or catecholamines levels is responsible for the shift in the CO_2 response curve seems low. This leaves a change in threshold or an added drive to breathe (neural) as candidates for the increase in B. A change in threshold should have caused a shift in the $HCVR_R$, but because none occurred, this too seems unlikely. Thus, a sympathetically mediated added drive to breathe is the best assumption for this observation. However, recent research has shown that delaying respiratory muscle fatigue reduces the muscle sympathetic nerve activity. This suggests the sympathetic drive to breathe would be reduced, so this option also loses some merit. There is one last possibility, that the RMT respiratory muscles were less susceptible to fatigue which resulted in the opposite effect that fatigued respiratory muscles normally have on the HCVR and shifted $HCVR_{EX}$ to the left. Finally, it may be that the increase in B has an unknown (or no) physiological meaning.

Spirometry

As in many other instances when RMT was performed, we found improvements in the MVV of the training group but not in the controls. Somewhat surprisingly, VC was also slightly improved (3.4%). However, the FEVC was not changed significantly. Because the FEVC is dependent on small airway compression (which masks the expiratory muscle capacity) and the slow

VC is not flow limited, the increased VC may be indicative of increased expiratory muscle capability. A significant but also small change (+ 3.7%) in VC has only been found in two other studies (Belman & Gaesser, 1988; Leith & Bradley, 1976). Like the total lung capacity, the VC of healthy adults is generally believed to be fixed. It could be the change in VC was a result of the very specific nature of RMT on chest wall and lungs. In this way it could be a marker for a very successful RMT program. Perhaps a more intensive RMT program, which would examine the breathing patterns used during the RMT (perhaps using large V_T) and which took place over a longer duration (to allow more complete adaptations) would be necessary to determine the effects of RMT on VC accurately.

Other measures of spirometry (forced expiratory vital capacity, forced expiratory volume in 1 s, and peak expiratory flow) were unchanged by RMT. All of the spirometric measurements were made in accordance to the criteria outlined by the American Thoracic Society (American Thoracic Society, 1995).

Breathing Endurance Test

Explanation

Breathing performance was significantly improved (259%) in 9 of the 10 subjects. Given the specificity of RMT on the respiratory muscles it seems initially surprising that improvements in BET performance were not uniform.

Critique of the Methods

Problems similar to those discussed for the CET (a high variability and not having a fixed end-point) are also inherent to the BET. Furthermore, a very large learning effect is associated with BET as a whole. This could be due the fact that voluntary hyperpnea is such an unfamiliar task. With this in mind, both the control and RMT groups underwent a minimum of two familiarization sessions until they were comfortable performing the voluntary eucapnic hyperpnea exercises.

Conclusion

As anticipated, RMT resulted in large improvements in the BET. The lack of complete uniformity within the subjects is likely due to the low reliability of tests which use a subjective endpoint such as exhaustion.

Incremental Test

The major purposes of the incremental test were to determine the work rate for the other tests ($HCVR_R$ and $HCVR_{EX}$, modified Dejours test and CET) used in this study and to serve as an indirect means of monitoring metabolic cardiovascular changes related to the subjects' habitual training program. The results indicate this was successful and that the subjects of this study kept their level of fitness constant. There were no significant changes in any of the cardiovascular values (i.e., $\dot{V}_{O_2}$, f_C, [La], and $\dot{W}_{max}$; Table 6) or respiratory values

measured ($\dot{V}_E$ V_T or f_b) during the incremental test for the control or RMT group. It might have been expected to see changes in breathing pattern similar to the CET (*increase in* V_T). *However, the relatively short length (2 min) of the* incremental increases in workload (30 W) may not have been long enough for the respiratory variables to stabilize enough to provide accurate measurement of V_T or f_b at the higher intensities. It may also be a factor that only a small portion of the test is spent at these higher workloads. Although high levels of $\dot{V}_E$ are reached, the duration they are endured may not be long enough to initiate the conditions (e.g., respiratory muscle fatigue) that cause a change in breathing pattern. At the lower workloads, where the respiratory muscles are working *within their capacity, there was no impetus to breathe deeper.*

Even though the incremental test uses exhaustion to determine the endpoint of the test, the reliability is better than other tests using this subjective end-point. Perhaps this is because the work rate is progressively increased (in a step like fashion) and exhaustion is reached in large part do to the physical inability to maintain an ever increasing load. *This may minimize the influence of* the subjects' subjective feeling of exhaustion on determining when the end-point is reached.

Given the design of the test protocol, the high and maximal intensity portions of the test are short in duration. This makes it a poor measure of endurance performance which needs a longer time component; however, this was not the purpose the incremental test in this study. Increasing the step length

to 3-4 min (at least at lower workloads) could increase the test's value as a measure of endurance along with providing more a consistent measure (steady-state) for data sampled at the end of each step.

Conclusion

RMT improved endurance cycling performance (+ 15%) and shifted the CO_2 response curve during exercise to the left (B increased for $HCVR_{EX}$; 19%). It also caused a significant decrease in the PC sensitivity (-5.8 ± 6.0%) without changing $\dot{V}_E$ during any portion of the tests involving cycling. However, there was a clearly significant increase in V_T for the quasi stead-state category of the CET and a borderline significant increase in $\dot{V}_E$ for the end-time category ($p = 0.59$). Also, there were significant negative correlations between cycling end-time and the quasi-steady-state $\dot{V}_E$ of the CET (Fig. 4) and with the end-time category for V_T ($r = -0.69$, $p = 0.036$). In summary, the RMT group's pR_c and cRc were altered although not correlated to $\dot{V}_E$, increased V_T, or performance, but these changes in $\dot{V}_E$ and V_T were related to the improvements in cycling *performance.*

The specific stimulus of $\dot{V}_E$ incurred with RMT causes a specific training stimulus for the respiratory system. This allows a greater conditioning response than that which occurs when $\dot{V}_E$ is elevated in the non-specific manner of exercise in conjunction with a whole-body endurance training program. This view is supported by the comparatively greater and significant increases in VC,

MVV, and BET observed in this and other RMT studies when compared with the sometimes inconsistent findings from whole-body endurance training studies (Clanton et al., 1987; Markov et al., 2001; Robinson & Kjeldgaard, 1982). In a similar way, the inconsistent findings of exercise and whole body endurance training on chemosensitivity (Blum et al., 1979; Bradley et al., 1980; Katayama et al., 1999; Kelley et al., 1984; Levine et al., 1992; Miyamura & Ishida, 1990) may be related to the non-specific effect of whole-body endurance training on the respiratory system.

Interestingly, the non-specific stimulus of RMT on the whole-body influenced exercise performance. This indicates that the specific effects of high levels of $\dot{V}_E$ on the components of the respiratory system (e.g., the respiratory muscles or chemoreceptors) may have a non-specific influence over the actions of the whole-body (e.g., cardiovascular reflexes). In this way, the high level of $\dot{V}_E$ occurring during RMT is in part responsible for the improvements in exercise performance that accompany whole-body endurance training and thus the respiratory muscles could be considered as an exercise limiting factor.

It is clear from other studies (Dempsey et al., 1984; Harms et al., 1997) that the respiratory system can be an exercise-limiting factor and our results suggest the high levels of $\dot{V}_E$ or the pattern of breathing occurring in exercise could be an exercise limiting factor. This is supported by research that showed decreasing the work of breathing, by pressure assisted ventilation, increased exercise times (Harms et al., 2000).

The high levels of ventilation achieved during exercise, as simulated by RMT in this study, significantly reduced the PC sensitivity during moderate-intensity exercise and extended the time to exhaustion during high-intensity fixed-rate cycling exercise. Nevertheless, the lack of a significant correlation between the decrease in pR_c and exercise $\dot{V}_E$ suggests the role of the PC in the control of $\dot{V}_E$ during normoxic exercise is minimal. This does not rule out changes in respiratory muscle usage or changes in breathing pattern or both. Also, RMT significantly shifted the CO_2 response curve to the left during moderate-intensity exercise. The implications of this shift on ventilation and endurance performance requires further investigation.

These results suggest that RMT augmented the cR_c, in a similar fashion as exercise shifts the resting $HCVR_R$, but attenuated the pR_c. A satisfactory explanation for the combined decrease in pR_c and the increased CC drive is difficult to find. There were no significant correlations between the chemoreceptors and exercise $\dot{V}_E$., V_T, or other breathing pattern characteristics. This leads to the simple conclusions that the chemoreceptors' role and physiological relevance on $\dot{V}_E$ during exercise is minimal following RMT.

In conclusion, the high levels of ventilation achieved during exercise, as simulated by RMT in this study, appear to be accompanied by a reduction in PC sensitivity and a leftward shift in the hypercapnic ventilatory response during exercise along with improved cycling performance; however, the role of the PC and CC in the control of ventilation during exercise seems to be minor.

APPENDIX A

TRAINING LOG

Date	MONTAG	DIENSTAG	MITTWOCH	DONNERS.	FREITAG	SAMSTAG	SONTAG	WOCHE:
SWIM	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	Durchscnitt DIST: ZEIT: INT 1 2 3 4 5
BIKE	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	Durchscnitt DIST: ZEIT: INT 1 2 3 4 5
RUN	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	DIST: ZEIT: INT 1 2 3 4 5	Durchscnitt DIST: ZEIT: INT 1 2 3 4 5
VER	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5	1 2 3 4 5

APPENDIX B

Informed Consent
Einverständniserklärung

Ich, ..., erkläre mich hiermit zur freiwilligen Teilnahme an der **Studie von Mike McMahon** bereit. Ich wurde eingehend über die Studie informiert und bin mit den Versuchsbedingungen einverstanden. Besonders habe ich die folgenden, wichtigen Punkte zur Kenntnis genommen:

- Sowohl ich als Versuchsperson als auch die Versuchsleitung haben jederzeit das Recht, die Studienteilnahme ohne Angabe von Gründen zu beenden.
- Ich erkläre mich bereit, meinen Trainingsumfang 2 Wochen vor und während der Studiendauer nicht zu verändern.
- 2 Tage vor dem jeweiligen Test absolviere ich keine intensiven Trainingseinheiten mehr und ernähre mich kohlenhydratreich.
- 1 Tag vor dem Testtag: bitte nur leichtes oder gar kein Training.
- Am Testtag trinke ich keine koffeinhaltigen Getränke vor dem Test.
- Es ist mir bewusst, dass ich im Verlaufe der verschiedenen Fahrradtests bis zur Erschöpfung bzw. bis zur Leistungsgrenze belastet werde und dadurch ein kardiales Risiko nie vollständig ausgeschlossen werden kann.
- Es ist mir ebenfalls bekannt, dass ich bei gewissen Tests möglicherweise Kopfweh oder ein Gefühl von Atemnot kriegen könnte. Dies ist zwar unangenehm, birgt aber keinerlei Risiken. Für die Studienleiter ist es sehr wichtig, dass ich Ihnen mitteile, falls ich Kopfweh oder Atemnot bekomme.
- Es ist mir auch bewusst, dass es durch wiederholte Blutentnahme am Ohrläppchen zu einer vorübergehenden, lokalen Entzündung kommen kann.
- Erkrankungen und die Einnahme von Medikamenten können die Studie verfälschen und das Risiko erhöhen. Ich weiss, dass ich in einem solchen Fall den Versuchsleiter unbedingt orientieren muss, damit die Testfähigkeit abgeklärt werden kann.

Ort,Datum: ..... Unterschrift:.....

APPENDIX C

Subject Information
Probanden Information

Da es für die folgenden Tests, wie für alle Tests in unserem Labor, wichtig ist, dass Du möglichst wenig über die genaue Fragestellung weisst, erklären wir Dir hier nur das Allernötigste. Gerne werden wir Dir am Schluss der Testserie alle Details der Tests und Deine Resultate genau erklären.

Danke für Dein Verständnis!

Versuchsanordnung:

Angewöhnung 2 Wochen:

1. Angewöhnungstag	2. Angewöhnungstag
• Vorstellung der Studie • Erklärung des Trainingstagebuches • Üben des AAT	• Üben des Gas Einatmens • Üben auf dem Fahrrad Ergometer • Üben des AAT

Vortests 2 Wochen:

1. Testtag	2. Testtag	3. Testtag	4. Testtag
•Spirometrie • VO2 max	• Gas Einatmen: Ruhe •Atmungsausdauertest	• Gas Einatmen: Fahrrad #1 • Gas Einatmen: Fahrrad #2	•Fahrradausdauertest • Erklärung des Atmungs-ausdauer-Trainings

<-- 4 Testtage (Sessionen) innerhalb von 10-15 Tagen

Atmungstrainings-Periode 4-6 Wochen:

* sobald als möglich soll mit dem Atmungstraining (AT) begonnen werden
* das AT umfasst 20 Einheiten à 30min während 4-6 Wochen
* Kontrolle jeder fünften Einheit im Labor

AT	AT	AT	AT

<-- 4 Kontrolltrainings innerhalb von 4-6 Wochen >

Nachtests2 Wochen:

1. Testtag	2. Testtag	3. Testtag	4. Testtag
• Spirometrie • VO2 max	• Gas Einatmen: Ruhe •Atmungsausdauertest	• Gas Einatmen: Fahrrad #1 • Gas Einatmen: Fahrrad #2	•Fahrradausdauertest

<-- 4 Testtage (Sessionen) innerhalb von 10-

Allgemeines:

Während einiger der folgenden Tests wirst Du verschiedene Gasgemische atmen, die sich aber alle aus Komponenten unserer normalen Ein- und Ausatmungsluft zusammensetzen. Wir werden sämtliche Konzentrationen dauernd überwachen, sodass die Gase sicher nicht schädlich sind. Es kann aber passieren, dass Du einen trockenen Mund, Kopfweh oder ein Gefühl der Atemnot bekommst. Dies ist zwar unangenehm, aber nicht schädlich. Es ist jedoch wichtig, dass Du dies den Versuchsleitern mitteilst.

Ablauf an den Versuchstagen:

Angewöhnungssessionen: (2 Wochen)

- **1. Session:** (1 h); (5-9 Tage Pause zwischen 1. und 2. Session)
- **2. Session:** ($1^1/_2$ h); (5-9 Tage Pause zwischen 2. Session und 1. Testsession)

Vortests: (Mindestens 10 Tage und Maximal 15 Tage)

- **1. Testsession:** (2 h)
  Wir messen Deine Lungenfunktion (Spirometrie) und Du wirst Dich auf den Fahrrad maximal ausbelasten. Anschliessend wird noch geübt für zwei an einem anderen Tag folgende Tests. (2-4 Tage Pause zwischen 1. und 2. Testsession)
- **2. Testsession:** (2 h)
  Du kannst Dich etwa 15 min entspannen bei beruhigender Musik (bring bitte eine Kassette mit und etwas zum lesen).
  Du wirst in einem Stuhl sitzen und durch ein Mundstück atmen (Deine Nase wird mit einer Nasenklemme verschlossen sein) bis zum Ende des Tests. Wir werden Deine Einatmungsluft auf die Gasmischung umstellen. Während dieser Phase wirst Du im Kopfhörer 'white noise' hören und brauchst Dich nur zu entspannen.
  Ein Atmungsausdauertest folgt. (2-4 Tage Pause zwischen 2. und 3. Testsession)
- **3. Testsession:** (2 h)
  Du kannst Dich etwa 15 min entspannen bei beruhigender Musik (bring bitte eine Kassette mit und etwas zum lesen).
  Du wirst auf dem Fahrrad sitzen, durch ein Mundstück atmen (Deine Nase wird mit einer Nasenklemme verschlossen sein) und bis zum Ende des Tests "leicht" pedalen. Wir werden Deine Einatmungsluft auf die Gasmischung umstellen, während Du im Kopfhörer 'white noise' hören wirst und leicht pedalst.
  Der Test dauert zweimal etwa 45 min mit einer kurzen Pause dazwischen. (2-4 Tage Pause zwischen 3. und 4.Testsession)
- **4. Testsession:** (1 h)
  Ein Fahrradausdauertest (Direkt auschliessend Beginn des Trainings).

Atmungstrainingsphase:

- **Trainings Kontrollen**: (1h)
  Jedes 5. Training wird kontrolliert im Labor durchgeführt. Dies gilt natürlich nur für die Trainingsgruppe (0-2 Tage Pause zwischen letztem Training und 1. Nachtest-Session).

Nachtests: (Mindestens 10 Tage und Maximal 15 Tage)
Die Testsessionen 1-4 werden wiederholt

.

"Reminder" für die Studie

Wir bitten Dich, folgende Punkte nicht zu vergessen:

Während der gesamten Studiendauer:

- Training konstant halten (Tests im Labor einberechnen als Training).
- "Trainingstagebuch" jeden Tag ausfüllen.

Vor dem Testtag:

- 2 Tage vor dem Testtag: *Du solltest Dich kohlenhydratreich ernähren, genug* schlafen (mind. 7 Stunden) und kein angstrengendes Training absolvieren (nicht intensiv und nicht lang trainieren). Training und Ernährung sollst Du im Tagebuch festhalten.
- 1 Tag vor dem Testtag: bitte nur leichtes oder gar kein Training. Du solltest Dich kohlenhydratreich ernähren und genug schlafen (mind. 7 Stunden).

Am Testtag:

- Kein Sport
- Keine koffeinhaltigen Getränke (Kaffee, Schwarztee, Cola etc.) vor dem Test.
- Nimm die letzte, richtige Mahlzeit etwa 4 Stunden vor dem Test ein.
- Keine Mahlzeiten mehr während der 2 Stunden vor dem Test (höchstens ein Snack-Riegel).
- Bring eine Kassette mit ruhiger Musik zum Versuch und etwas zu lesen mit.

APPENDIX D

Ethics Report
Unterlagen für die Mitglieder der Ethikkommission Physiologie-Pharmakologie der Universität Zürich

Changes in lactate metabolism and chemoresponsiveness after respiratory endurance training

A. **Principle Investigators:** Dr. C.M. Spengler 23-H-45 635-5007
Prof. Dr. med. U. Boutellier 23-K-50 635-5078
Responsible MD: Prof. Dr. med. U. Boutellier 23-K-50 635-5078
Experimenters: Mike McMahon, BA 23-L-94 635-5092
Karsten Kulik, stud. biol.23-L-94 635-5092

B. **Aim and Background**

Aim

The present application contains two hypotheses which shall be addressed in one single study:

1) The reduction in blood lactate concentration seen during cycling exercise after respiratory endurance training is caused by changes in respiratory muscle metabolism (increased lactate uptake or decreased lactate production).

2) Chemoresponsiveness to CO_2 and peripheral chemosensitivity are unchanged after respiratory endurance training, during exercise as well as at rest.

Background

It has generally been accepted that ventilation does not limit exercise performance in healthy humans (Dempsey,1986; Leith and Bradley, 1976). More recently Johnson et al. (1993) and Mador et al. (1993) showed that the diaphragm fatigues during constant work exercise at an intensity of at least 80% of maximal oxygen consumption ($\dot{V}_{O_2,max}$). Also, after long lasting competitions, e.g. triathlon, respiratory muscle function is impaired (Chevrolet et al., 1993; Hill et al., 1991). Exercise performance, in turn, is limited when respiratory muscle are previously fatigued (Mador et al., 1993; Martin et al., 1982). Whether respiratory muscle fatigue occurring during exercise is indeed limiting performance, we can only prove by training respiratory muscles and measure exercise performance afterwards.

Since the respiratory muscles are functionally, morphologically, and embryologically skeletal muscles (NHLBI Workshop summary, 1990), they too, as with other skeletal muscles, can be trained for strength and endurance (Leith and Bradley, 1976). Isolated respiratory muscle endurance training (RMT;

isocapnic hyperpnea) leads to an improved breathing endurance in sedentary (Belman and Mittman, 1980; Boutellier and Piwko, 1992; Keens et al., 1977; Leith and Bradley, 1976), as well as in physically active subjects (Boutellier et al., 1992; Fairbarn et al., 1991; Morgan et al., 1987). RMT can improve submaximal constant load exercise not only in patients (Belman and Mittman, 1980; Mancini et al., 1995), but also in sedentary (Boutellier and Piwko, 1992) as well as in physically active subjects (Boutellier et al., 1992; Spengler et al., 1996).

After RMT, blood lactate concentration is significantly reduced during cycling exercise (Kohl et al., 1997; Spengler et al., 1996; Spengler et al., unpublished data). This reduction could be due to changes in metabolism of respiratory muscles (less lactate production, increased lactate uptake and oxidation) or due to a shift in blood supply towards leg muscles thus decreasing their lactate production.

After RMT, hyperpnea during exercise was slightly reduced in some instances, resulting in a lower minute ventilation, higher end-tidal CO_2 partial pressures ($P_{ET}CO_2$) levels and increased cycling time (Boutellier et al., 1992; Boutellier and Piwko, 1992). In contrast, Kohl et al. (1996) found that in a majority of subjects (6 of 8) minute ventilation was higher following RMT and endurance cycling time was reduced. The reason for the different results may be that respiratory muscles of the subjects in the study of Kohl et al. (1996) did not recover fully from the stresses of RMT before exercise testing while those of Boutellier and Piwko (1992) had mostly recovered.

Also whole body endurance training programs have been shown to result in a reduced ventilatory response at a given level of work (Casaburi et al., 1987; Taylor and Jones, 1979). Several studies have noted a correlation between the magnitude of the ventilatory response to exercise ($\Delta V_E/\Delta VCO_2$) and the ventilatory sensitivity to inspired carbon dioxide ($\Delta V_E/\Delta PCO_2$), both at rest (D'Urzo et al., 1987; Martin et al., 1978; Rebuck and Read, 1971) and during exercise (McConnell and Davies,1992). McConnell et al. (1992) noted a stronger correlation between $\Delta V_E/\Delta VCO_2$ and CO_2 sensitivity during exercise than at rest. A review of the literature indicates that endurance trained athletes tend to have a reduced hypercapnic ventilatory response at rest compared to untrained subjects (Mahler et al., 1982; Martin et al., 1979; Miyamura et al., 1976; Rebuck and Read, 1971). These differences seem to be related to both familial (genetic) aspects as well as induced by the physiological effects of the endurance training (Blum et al., 1979; Miyamura and Ishida, 1990).

It is possible that ventilatory changes observed after RMT may be related to changes in sensitivity to CO_2, i.e. reduced exercise ventilation after RMT may be correlated to decreases in the ventilatory sensitivity to CO_2 and vice versa. These changes in ventilation and the ventilatory sensitivity to carbon dioxide may be related to fatigue.

Objective #1 of the present study is to determine whether the reduced blood lactate concentration during cycling after RMT is the result of a changed metabolism (decreased lactate production or increased lactate uptake) by respiratory muscles.
-> For this purpose, blood lactate concentration will be increased by a brief (20 sec) and very intense bout of exercise followed by 30 min of isocapnic hyperpnea. Blood lactate kinetics during hyperpnea will be compared before and after a RMT period.
-> If respiratory muscle metabolism is changed after respiratory endurance training, then blood lactate concentrations during hyperpnea should be lower after the training period.

Objective #2 of the present study is to determine whether a change in chemoreceptor response to CO_2 and chemosensitivity of peripheral chemoreceptors after RMT is correlated to changes in ventilation after RMT.
-> For this purpose, step changes in inspired CO_2 will be employed at rest and during exercise and ventilatory responses will be measured. Also, a hyperoxic suppression test (suppression of peripheral chemoreceptor activity) will be performed during exercise to exam changes in peripheral chemoreceptor responsiveness.
-> As RMT is unlikely to affect chemoreceptor responsiveness (hypoxic ventilatory response is unchanged; Markov et al., 1996), no change is expected. Nevertheless this mechanism needs to be ruled out as a possible factor affecting ventilation and cycling endurance after respiratory endurance training.

C. Methods

1. Subjects

This study will be performed with 24 healthy, male subjects, 18-35 years old and physically active, i.e. about 3 hours of exercise per week. People taking any medication, having any chronic disease and smokers will be excluded from the study. Twelve subjects will be randomly assigned to a respiratory training group, and 12 subjects will serve as controls. The subjects will be informed about the study and will give their written informed consent (see "Einverständniserklärung").

2. Measurements

Measurements performed before and after the respiratory training period:

Spirometric variables, such as vital capacity (VC), forced expiratory vital capacity (FEVC), forced expiratory volume in 1 sec (FEV_1), peak flow (PEF), maximal voluntary ventilation in 15 sec (MVV), will be determined.

An *incremental exercise test* on a cycling ergometer ($\dot{V}_{O_2,\,max}$-test; starting at 100 W, increase by 30 W every 2 min) will be performed. Ventilation, gas exchange, and heart rate will be measured continuously. A blood sample from an earlobe will be taken at the end of each workload step to analyze blood lactate concentration.

Respiratory muscle endurance will be determined at a $\dot{V}_E$ corresponding to about 70% MVV, until either tidal volume (V_T) or breathing frequency (f_R) can not be maintained anymore. Also, heart rate will be measured and blood samples will be taken every 2 min to measure lactate concentration.

A *cycling endurance test* at 85% of the maximal workload achieved during the $\dot{V}_{O_2,\,max}$-test will be performed to exhaustion. Ventilation, gas exchange and heart rate will be measured continuously. Blood samples will be taken every 2 min to measure lactate concentration.

'20 sec - test': Subjects will cycle for 20 sec at very high intensity (approximately 200% $\dot{V}_{O_2,\,max}$) holding their breath. Immediately afterwards they start with isocapnic hyperpnea at 50% MVV which will be continued for 30 min. Isocapnia will be maintained by continuously adjusting inspired CO_2. Every 2 min blood samples will be taken from an earlobe to analyze blood lactate concentration. Heart rate will be measured continuously.

Hypercapnic ventilatory response at rest: Subjects will be sitting with headphones listening to calming music for 15 min. Then music will be switched to white noise and the subjects will breathe air through a mouthpiece and the gas sampling equipment. Then the inspirate will be switched to 50% O_2 / 50% N_2 for 5 minutes. Next the inspired CO_2 (from a tank containing 50% CO_2 / 50% O_2) will be increased to achieve a step-change in $P_{ET}CO_2$ of 2.5 - 5 mmHg. This level will be held for 5 min. Two to three further step-changes of 2.5 - 5 mmHg will follow. The appropriate steps will be determined in preliminary experiments.

Hypercapnic ventilatory response during exercise The same procedure will be applied as described above for resting measurements, except that subjects will be exercising at 40% $\dot{V}_{O_2,peak}$. Increases of inspired CO_2 will start after 10 min cycling at 40% $\dot{V}_{O_2,peak}$ breathing a mixture of 50% O_2 / 50% N_2.

Hyperoxic suppression test:
After cycling at 40% $\dot{V}_{O_2,peak}$ for 10 minutes breathing air (from compressed air tank), the inspirate will be surreptitiously switched to 100% oxygen first for 2-6 breathes and then continuously for up to 2.5 minutes. This results in an almost immediate decrease in ventilation (within one to two breaths). By continuously monitoring ventilation breath by breath, the magnitude of the decrement in ventilation will be measured. Preliminary trials will be used to find the most adequate duration breathing of 100% oxygen and the number of repetitions.

Respiratory training period:

During 4-6 weeks, subjects will complete 20 respiratory training sessions of 30 min each (normocapnic hyperpnea). The subjects will breathe with 60% - 80% MVV depending on their initial evaluation. Subjects will perform their training at home except for every fifth RMT which will be performed in the laboratory to control for correct performance, i.e. to ensure normocapnia.

3. Techniques

Respiratory training will be performed with a device allowing isocapnic hyperpnea by partial rebreathing of CO_2 which maintains normocapnia. For further details see Markov et al. (1996).

Ventilatory variables, such as minute ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f_R), oxygen consumption ($\dot{V}_{O_2}$), CO_2 production ($\dot{V}_{CO_2}$), and end tidal partial pressures of CO_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$), *spirometric variables* such as, VC, forced vital capacity (FVC), FEVC, PEF, FEV1, and MVV will be measured with an ergospirometric device (Oxycon Beta, Mijnhart, Bunnik, Netherlands).

$\dot{V}_{O_2,max}$ *-tests and bicycle endurance tests* will be performed on an electromagnetically braked bicycle ergometer (ergometrics 800S, Ergoline, Bitz, Germany), connected to the "Oxycon Beta" unit.

The *20 sec-test* will be performed on an electromagnetically braked bicycle ergometer (ergometrics 800S, Ergoline, Bitz, Germany). A device described by Boutellier et al. (1992) will be used to keep $P_{ET}CO_2$ constant during the 30 min of hyperpnea after the 20 second-test by adjusting inspired CO_2.

The blood lactate concentration will be analyzed by "ESAT 6661" (Eppendorf, Hamburg, Germany), which uses 20 micro liter samples taken from the subjects earlobe.

Heart rate will be recorded on a "PE 4000" heart rate monitor (Polar Electro, Kemple, Finland)

Hypercapnic ventilatory response: Subjects are breathing gas from two tanks of medically certified gas (50% O_2 / 50% N_2; 50% CO_2 / 50% O_2). Gas from the two tanks will be mixed by a self-built mixing valve (Boutellier et al. 1992).

Hyperoxic suppression test: Inspired gas will be mixed from two tanks, one containing air and the other containing 100% O_2.

Data analysis

Lactate data will be analyzed by a mathematical model developed by Feund and Zouloumian (Freund and Zouloumian, 1981a; Freund and Zouloumian, 1981b; Oyono-Enguelle et al., 1989; Zouloumian and Freund, 1981a; Zouloumian and Freund, 1981b) to determine lactate kinetics.

Average blood lactate levels, the slope of the hypercapnic ventilatory response as well as the result of the hyperoxic suppression test will be compared by an analysis of variance.

4. Schedule

For detailed schedule, please refer to enclosed diagram. The study will start as soon as possible and data collection will be finished within 6-9 months.

D. Special remarks

none

E. Ethical Aspects

1. Risks and Measures of Precaution

Sickness and taking medication could elevate any risks and compromise the results of the study. We will ask our subjects according to these points before each session, and if indicated we will not allow them to perform the test.

$\dot{V}_{O_2, max}$ - *test, 20 sec test and cycling endurance tests:* During these tests, subjects will cycle to their limits. Even with healthy, physically active subjects we can not fully exclude a cardiac incident. These tests are standard procedure and are performed daily in our laboratory. We never had any incident. With high blood lactate levels achieved, subjects may start to feel sick. If that occurs, the testing will be discontinued.

Blood sampling at the ear lobe: Repetitive blood sampling at an earlobe may cause local inflammation.

Hypercapnic ventilatory response: Breathing CO_2 at the proposed levels is a common respiratory physiology technique. $P_{ET}CO_2$ is monitored continuously, keeping it within safe limits (below 70 mmHg). No serious risk is associated with this level of CO_2 in healthy subjects. However, a sensation of shortness of breath is likely and a brief headache or dizziness is possible.

Hyperoxic suppression test This test is commonly used in respiratory physiology. It is well known that breathing high concentrations of oxygen for prolonged periods of time can be toxic and can potentially cause apnea. The technique employed in this study requires that the subjects only be exposed to brief periods of hyperoxia, thus circumventing the possible hazards.

Emergency procedures: MDs and medical students are working in the exercise physiology group. Should those or the emergency MDs of the University be unavailable, the ambulance would be called. A defibrillator is at hand. The experimenters participated in a locally organized resuscitation course (12/12/1997), where they practiced cardiopulmonary resuscitation.

Safety of subjects' data: All information and data will be only used in relation to this research, and will be handled anonymously.

2. Subjects' Reimbursement

The subjects will not be paid for this study. There is no dependence of any kind, towards anyone - in particular principal investigators - involved in this study. The subjects will not have any advantages participating in this study nor disadvantages if refusing to participate.

Subjects will have the opportunity to stop any test at any time and to discontinue participation in the study without giving a reason.

3. Insurance

Insurance is covered by: Haftpflichtversicherung des Kantons Zürich.

F. Enclosures

Einverständniserklärung

Protocol

G. References

Belman M.J. and C. Mittman: Ventilatory muscle training improves exercise capacity in chronic obstructive pulmonary disease patients. Am Rev Respir Dis 121: 273-280, 1980

Blum J., D. Kanarek, B. Callahan, N. Braslow and H. Kazemi: The effect of training on CO_2 ventilatory responsiveness in normal subjects. Am Rev of Resp Dis 119: 291, 1979
Boutellier U., R. Buchel, A. Kundert and C. Spengler: The respiratory system as an exercise limiting factor in normal trained subjects. Eur J Appl Physiol 65: 347-353, 1992
Boutellier U. and P. Piwko: The respiratory 1system as an exercise limiting factor in normal sedentary subjects. Eur J Appl Physiol 64: 145-152, 1992
Casaburi R., T.W. Storer and K. Wasserman: Mediation of reduced ventilatory response to exercise after endurance training. J Appl Physiol 63: 1533-1538, 1987
Chevrolet J.C., J.M. Tschopp, Y. Blanc, T. Rochat and A.F. Junod: Alterations in inspiratory and leg muscle force and recovery pattern after a marathon. Med Sci Sports Exerc 25: 501-507, 1993
D'Urzo A.D., K.R. Chapman and A.S. Rebuck: Effect of inspiratory resistive loading on control of ventilation during progressive respiratory exercise. J Appl Physiol 62: 134-140, 1987
Dejours P.: Chemoreflexes in breathing. Physiol Rev 42: 335-358, 1962
Dejours P.: Control of respiration by arterial chemoreceptors. Annals of the New York Academy of Sciences. 109:682-695, 1963
Dempsey J.A.: J.B. Is the lung built for exercise?. Med Sci Sports Exerc 18:143-155, 1986
Donovan C.M. and G.A. Brooks: Endurance training affects lactate clearance, not lactate production. Am J Physiol 244: E83-E92, 1983
Donovan C.M. and M.J. Pagliassotti: Endurance training enhances lactate clearance during hyperlactatemia. Am J Physiol. 257: E782-E789, 1989
Donovan C.M. and M.J. Pagliassotti: Enhanced efficiency of lactate removal after endurance training. J Appl Physiol 68: 1053-1058, 1990
Fairbarn M.S., K.C. Coutts, R.L. Pardy and D.C. McKenzie: Improved respiratory muscle endurance of highly trained cyclists and the effects on maximal exercise performance. Int J Sports Med 12: 66-70, 1991
Freund H. and P. Zouloumian: Lactate after exercise in man: I. Evolution kinetics in arterial blood. Eur J Appl Physiol 46: 121-133, 1981a
Freund H. and P. Zouloumian: Lactate after exercise in man: IV. Physiological observations and model predictions. Eur J Appl Physiol 46: 161-176, 1981b
Fukuba Y., M.L. Walsh, B.J. Cameron, R.H. Morton, C.T. Kenny and E.W. Banister: The clearance rate of exercise-elevated blood lactate following physical training. Ann Physiol Anthropol. 11: 369-376, 1992
Hill N.S., C. Jacoby and H.W. Farber: Effect of an endurance triathlon on pulmonary function. Med Sci Sports Exerc 23: 1260-1264, 1991
Keens T.G., I.R. Krastins, E.M. Wannamaker, H. Levison, D.N. Crozier and A.C. Bryan: Ventilatory muscle endurance training in normal subjects and patients with cystic fibrosis. Am Rev Respir Dis 116: 853-860, 1977
Kohl J., E.A. Koller, M. Brandenberger, M. Cardenas and U. Boutellier: Effect of exercise-induced hyperventilation on airway resistance and cycling endurance. Eur J Appl Physiol 75: 305-311, 1997
Leith D.E. and M. Bradley: Ventilatory muscle strength and endurance training. J Appl Physiol 41: 508-516, 1976
MacRae H.S., S.C. Dennis, A.N. Bosch and T.D. Noakes: Effects of training on lactate production and removal during progressive exercise in humans. J Appl Physiol 72: 1649-56, 1992
Mador M.J., U.J. Magalang, A. Rodis and T.J. Kufel: Diaphragmatic fatigue after exercise in healthy human subjects. Am Rev Respir Dis 148: 1571-1575, 1993
Mahler D.A., E.D. Moritz and J. Loke: Ventilatory responses at rest and during exercise in marathon runners. J Appl Physiol 52: 388-392, 1982
Mancini D.M., D. Henson, J. La Mancha, L. Donchez and S. Levine: Benefit of selective respiratory muscle training on exercise capacity in patients with chronic congestive heart failure. Circulation 91: 320-329, 1995
Markov G., R. Orler and U. Boutellier: Respiratory training, hypoxic ventilatory response and

acute mountain sickness. Respir Physiol 105: 179-186, 1996
Martin B.J., J.V. Weil, K.E. Sparks, R.E. McCullough and R.F. Grover: Exercise ventilation correlates positively with ventilatory chemoresponsiveness. J Appl Physiol 45: 557-564, 1978
Martin B.J., K.E. Sparks, C.W. Zwillich and J.V. Weil: Low exercise ventilation in endurance athletes. Med Sci Sports 11:181-185, 1979
Martin B., M. Heintzelman and H.I. Chen: Exercise performance after ventilatory work. J Appl Physiol 52: 1581-1585, 1982
McConnell A.K. and C.T. Davies: A comparison of the ventilatory responses to exercise of elderly and younger humans. J Gerontol 47: B137-B141, 1992
Messonnier L., H. Freund, M. Bourdin, A. Belli and J.R. Lacour: Lactate exchange and removal abilities in rowing performance. Med Sci Sports Exerc 29: 396-401, 1997
Miyamura M., T. Yamashina and Y. Honda: Ventilatory responses to CO_2 rebreathing at rest and during exercise in untrained subjects and athletes. Jpn J Physiol. 26: 245-254, 1976
Miyamura M. and K. Ishida: Adaptive changes in hypercapnic ventilatory response during training and detraining. Eur J Appl Physiol 60: 353-359, 1990
Morgan D.W., W.M. Kohrt, B.J. Bates and J.S. Skinner: Effects of respiratory muscle endurance training on ventilatory and endurance performance of moderately trained cyclists. Int J Sports Med 8: 88-93, 1987
NHLBI Workshop summary: Respiratory muscle fatigue. Am Rev Respir Dis 142: 474-480, 1990
Oyono-Enguelle S., M. Gartner, J. Marbach, A. Heitz, C. Ott and H. Freund: Comparison of arterial and venous blood lactate kinetics after short exercise. Int J Sports Med 10: 16-24, 1989
Oyono-Enguelle S., J. Marbach, A. Heitz, C. Ott, M. Gartner, A. Pape, J.C. Vollmer and H. Freund: Lactate removal ability and graded exercise in humans. J Appl Physiol 68: 905-911, 1990
Phillips S.M., H.J. Green, M.A. Tarnopolsky and S.M. Grant: Increased clearance of lactate after short-term training in men. J Appl Physiol 79: 1862-1869, 1995
Rebuck A.S. and J. Read: Patterns of ventilatory response to carbon dioxide during recovery from serve asthma. Clin Sci 52: 388-392, 1971
Robbins P.A., J. Conway, D.A. Cunningham, S. Khamnei and D.J. Paterson: A comparison of indirect methods for continuous estimation of arterial PCO_2 in men. J Appl Physiol 68: 1727-1731, 1990
Spengler C.M., S.M. Laube, M. Roos and U. Boutellier: The effect of breathing pattern during respiratory training on cycling endurance. In: The Physiology and Pathophysiology of Exercise Tolerance; Plenum Press, New York. pp 315-319, 1996.
Swanson G.D. and J.W. Bellville: Step changes in end-tidal CO_2: methods and implications. J Appl Physiol 39: 377-385, 1975
Taylor R. and N. Jones: The reduction by training of CO_2 output during exercise. Eur J of Cardiol 9: 53-62, 1979
Zouloumian P. and H. Freund: Lactate after exercise in Man: II. Mathematical model. Eur J Appl Physiol 46: 135-147, 1981a
Zouloumian P. and H. Freund: Lactate after exercise in man: III. Properties of the compartment model. Eur J Appl Physiol 46: 149-160, 1981b

APPENDIX E

Data & Test Sheets

Name:		**Vorname:**	
Adresse:			
Telefon privat:		Geschäft:	
Geb.-Datum:			
Grösse:	cm	Gewicht:	kg
Sportliche Aktivitäten:			
Training:	Std./Woche		

Spirometrische Daten:

	predicted:	1. Versuch	2. Versuch	3. Versuch
VC				
MVV_{20}				
FEV_1				
Peak flow				

FEVC

Sattel Ht.:

Fore/Aft:

Bar Ht.:

Bemerkungen:

Cycling Endurance Test Sheet
Datenblatt CET

Name: **Vornam:**

ID-Nummer: **Datum:**

Zeit: MIN.	Borg-Skala AA	AN	BN	Lactat	Zeit: MIN.	Borg-Skala AA	AN	BN	Lactat
Ruhe	0	0	0		26				
1					27				
2					28				
3					29				
4					30				
5					31				
6					32				
7					33				
8					34				
9					35				
10					36				
11					37				
12					38				
13					39				
14					40				
15					41				
16					42				
17					43				
18					44				
19					45				
20					46				
21					47				
22					48				
23					49				
24					50				
25					ABBRUCH:				

Gewählte Umdrehungszahl:

Abbruchgrund:

Bemerkungen:

Sattel Height:
Fore/Aft:
Lenker Height:

END-TIME:
PEAK POWER:
85% PEAK POWER:

VO2max Test Sheet
Datenblatt VO2max

Name: **Vorname:**

ID-Nummer: **Datum:**

Zeit: MIN.	Borg-Skala AA	AN	BN	Lactat	Zeit: MIN.	Borg-Skala AA	AN	BN	Lactat
Ruhe	0	0	0		26				
1					27				
2					28				
3					29				
4					30				
5					31				
6					32				
7					33				
8					34				
9					35				
10					36				
11					37				
12					38				
13					39				
14					40				
15					41				
16					42				
17					43				
18					44				
19					45				
20					46				
21					47				
22					48				
23					49				
24					50				
25					ABBRUCH:				

Gewählte Umdrehungszahl:

Sattel Height:

Fore/Aft:

Abbruchgrund:

Lenker Height:

Bemerkung:

END-TIME:

PEAK POWER:

Gas Inhalation Rest & Exercise

Name:	**First Name:**	**Test # :**
ID-Number:	**Date:**	**Time of Day:**
Saddle Height:	**Fore/Aft:**	**Handlebars:**
Peak Power at VO_{2Peak}:	**40% Peak Power VO_{2Peak}:**	**RPM's:**

Time	V_E	$P_{ET}CO_2$	F_ICO_2	$P_{ET}O_2$	F_IO_2
End Air					
End O_2/N_2					

STEPS	PROPOSED STEPS	ACTUAL STEPS	STEP LENGTH	V_E	$P_{ET}CO_2$	F_ICO_2
End Air						
End O_2/N_2						
+ 4 mmHg						
+ 8 mmHg						
+12 mmHg						

Previous Exercise:

Previous Diet:

Remarks:

Gas Inhalation Dejours Test in Exercise

Name: **First Name:** **Test # :**

ID-Number: **Date:** **Time of Day:**

Saddle Height: **Fore/Aft:** **Handlebars :**

Peak Power at VO_{2Peak}: **40% Peak Power VO_{2Peak}:** **RPM's:**

Time	Time	V_E	T_V	F_R	$P_{ET}CO_2$	$P_{ET}O_2$
End Air						
End Press. Air						
O_2 12 Breaths # 1						
O_2 12 Breaths # 2						
O_2 12 Breaths # 3						
O_2 12 Breaths # 4						
O_2 3'. or 12 B # 5						
O_2 3'. or 12 B # 6						
BASELINE						

Previous Exercise:

Previous Diet:

Remarks:

BREATHING ENDURANCE TEST SHEET
ATMUNGSAUSDAUERTEST

Name: **Vornam:**

ID-#: **Datum:**

MVV= **%MVV=** **TV=** **f_b=**

Zeit: MIN.	Borg-Skala AA	Borg-Skala AN	Lactat	Zeit: MIN.	Borg-Skala AA	Borg-Skala BN	Lactat
Ruhe	0	0					
1				21			
2				22			
3				23			
4				24			
5				25			
6				26			
7				27			
8				28			
9				29			
10				30			
11				31			
12				32			
13				33			
14				34			
15				35			
16				36			
17				37			
18				38			
19				39			
20				40			
				ABBRUCH			

Gewählte Umdrehungszahl: **AVERAGE V'e:**

Abbruchgrund: **END-TIME:**

Bemerkungen: **$P_{ET}CO_2$:**

APPENDIX F

Pre-Test Subjective Ratings

	RATING	Health	Sleep Quality	Mood	Muscle Soreness	Stress	Workout Quality	Fatigue
DATE:	Very,Very Low or GOOD	1	1	1	1	1	1	1
WEIGHT:	Very Low	2	2	2	2	2	2	2
PULSE:	Low	3	3	3	3	3	3	3
SLEEP:	Medium or Average	4	4	4	4	4	4	4
HEALTHY: Y N	High	5	5	5	5	5	5	5
INJURIES: Y N	Very High	6	6	6	6	6	6	6
REMARKS:	Very,Very High or BAD	7	7	7	7	7	7	7
	RATING	Health	Sleep Quality	Mood	Muscle Soreness	Stress	Workout Quality	Fatigue
DATE:	Very,Very Low or GOOD	1	1	1	1	1	1	1
WEIGHT:	Very Low	2	2	2	2	2	2	2
PULSE:	Low	3	3	3	3	3	3	3
SLEEP:	Medium or Average	4	4	4	4	4	4	4
HEALTHY: Y N	High	5	5	5	5	5	5	5
INJURIES: Y N	Very High	6	6	6	6	6	6	6
REMARKS:	Very,Very High or BAD	7	7	7	7	7	7	7
	RATING	Health	Sleep Quality	Mood	Muscle Soreness	Stress	Workout Quality	Fatigue
DATE:	Very,Very Low or GOOD	1	1	1	1	1	1	1
WEIGHT:	Very Low	2	2	2	2	2	2	2
PULSE:	Low	3	3	3	3	3	3	3
SLEEP:	Medium or Average	4	4	4	4	4	4	4
HEALTHY: Y N	High	5	5	5	5	5	5	5
INJURIES: Y N	Very High	6	6	6	6	6	6	6
REMARKS:	Very,Very High or BAD	7	7	7	7	7	7	7

BIBLIOGRAPHY

Aaron, E. A., Johnson, B. D., Seow, C. K., & Dempsey, J. A. (1992a). Oxygen cost of exercise hyperpnea: mesurment. *J Appl Physiol, 72(5)*, 1810-1817.

Aaron, E. A., Seow, K. C., Johnson, B. D., & Dempsey, J. A. (1992b). Oxygen cost of exercise hyperpnea: implications for performance. *J Appl Physiol, 72*(5), 1818-25.

American Thoracic Society. (1995). Standardization of spirometry. *Am J Respir Crit Care Med, 152*, 1107-1136.

Bascom, D. A., Pandit, J. J., Clement, I. D., & Robbins, P. A. (1992). Effects of different levels of end-tidal PO2 on ventilation during isocapnia in humans. *Respir Physiol, 88*(3), 299-311.

Becker, H., Polo, O., McNamara, S. G., Berthon-Jones, M., & Sullivan, C. E. (1995). Ventilatory response to isocapnic hyperoxia. *J Appl Physiol, 78*(2), 696-701.

Bellville, J. W., Whipp, B. J., Kaufman, R. D., Swanson, G. D., Aqleh, K. A., & Wiberg, D. M. (1979). Central and peripheral chemoreflex loop gain in normal and carotid body-resected subjects. *J Appl Physiol, 46*(4), 843-853.

Belman, M. J., & Gaesser, G. A. (1988). Ventilatory muscle training in the elderly. *J Appl Physiol, 64(3)*, 899-905.

Belman, M. J., & Mittman, C. (1980). Ventilatory muscle training improves exercise capacity in chronic obstructive pulmonary disease patients. *Am Rev Respir Dis, 121*(2), 273-280.

Berne, R. M., & Levy, M. N. (1993). *Physiology*. (3rd ed.). St. Louis: Mosby-Year Book.

Blum, J., Kanarek, D., Callahan, B., Braslow, N., & Kazemi, H. (1979). The effect of training on CO_2 ventilatory responsiveness in normal subjects. *Am Rev Respir Dis, 119*(Suppl), 291.

Boussana, A., Galy, O., Hue, O., Matecki, S., Varray, A., Ramonatxo, M., & Le Gallais, D. (2003). The effects of prior cycling and a successive run on

respiratory muscle performance in triathletes. *Int J Sports Med, 24*(1), 63-70.

Boutellier, U., Büchel, R., Kundert, A., & Spengler, C. (1992). The respiratory system as an exercise limiting factor in normal trained subjects. *Eur J Appl Physiol, 65*, 347-353.

Boutellier, U., & Piwko, P. (1992). The respiratory system as an exercise limiting factor in normal sedentary subjects. *Eur J Appl Physiol, 64*, 145-152.

Bradley, B. L., Mestas, J., Forman, J., & Unger, K. M. (1980). The effect on respiratory drive of a prolonged physical conditioning program. *Am Rev Respir Dis, 122*(5), 741-746.

Brooks, G. A., Fahey, T. D., & White, T. A. (1996). *Exercise Physiology: Human Bioenergetics and its Applications*. (2nd ed.). Mountain View: Mayfield Publishing Company.

Burger, R. E., Estavillo, J. A., Kumar, P., Nye, P. C., & Paterson, D. J. (1988). Effects of potassium, oxygen and carbon dioxide on the steady-state discharge of cat carotid body chemoreceptors. *J Physiol, 401*, 519-531.

Bye, P. T. P., Farkas, G. A., & Roussos, C. (1983). Respiratory factors limiting exercise. *Ann Rev Physiol, 45*, 439-451.

Carey, C., Schaefer, K., & Alvis, H. (1956). Effect of skin diving on the lung volumes. *J Appl Physiol, 8*, 519-523.

Casaburi, R., Storer, T. W., & Wasserman, K. (1987). Mediation of reduced ventilatory response to exercise after endurance training. *J Appl Physiol, 63*(4), 1533-1538.

Casey, K., Duffin, J., & McAvoy, G. V. (1987). The effect of exercise on the central-chemoreceptor threshold in man. *J Physiol, 383*, 9-18.

Clague, J. E., Carter, J., Pearson, M. G., & Calverley, P. M. (1996). Effect of sustained inspiratory loading on respiratory sensation and CO_2 responsiveness in normal humans. *Clin Sci, 91*(4), 513-518.

Clanton, T. L., Dixon, G. F., Drake, J., & Gadek, J. E. (1987). Effects of swim training on lung volumes and inspiratory muscle conditioning. *J Appl Physiol, 62*(1), 39-46.

Clark, T. J., & Godfrey, S. (1969). The effect of CO2 on ventilation and breath-holding during exercise and while breathing through an added resistance. *J Physiol, 201*(3), 551-566.

Coast, J. R., Clifford, P. S., Henrich, T. W., Stray-Gundersen, J., & Johnson, R., Jr. (1990). Maximal inspiratory pressure following maximal exercise in trained and untrained subjects. *Med Sci Sports Exerc, 22*(6), 811-815.

Cummin, A. R., Alison, J., Jacobi, M. S., Iyawe, V. I., & Saunders, K. B. (1986). Ventilatory sensitivity to inhaled carbon dioxide around the control point during exercise. *Clin Sci, 71*(1), 17-22.

Cunningham, D. J. C. (1987). Studies on arterial chemoreceptors in man. *J Physiol (Lond), 384*, 1-26.

Dahan, A., DeGoede, J., Berkenbosch, A., & Olievier, I. C. (1990). The influence of oxygen on the ventilatory response to carbon dioxide in man. *J Physiol, 428*, 485-499.

De Troyer, A., & Estenne, M. (1984). Coordination between rib cage muscles and diaphragm during quiet breathing in humans. *J Appl Physiol, 57*(3), 899-906.

De Troyer, A., Estenne, M., Ninane, V., Van Gansbeke, D., & Gorini, M. (1990). Transversus abdominis muscle function in humans. *J Appl Physiol, 68*(3), 1010-1016.

De Troyer, A., S., K., & Zin, W. A. (1983). Mechanical action of the intercostal muscle on the ribs. *Science, 120*, 87-88.

De Troyer, A., Sampson, M., Sigrist, S., & Macklem, P. T. (1981). The diaphragm: two muscles. *Science, 213*(4504), 237-238.

De Troyer, A., & Sampson, M. G. (1982). Activation of the parasternal intercostals during breathing efforts in human subjects. *J Appl Physiol, 52*(3), 524-529.

Dejours, P. (1962). Chemoreflexes in breathing. *Physiol Rev, 42*(3), 335-358.

Dejours, P. (1963). Control of respiration by arterial chemoreceptors. *Ann N Y Acad Sci, 109*, 682-695.

Dejours, P., Puccinelli, J., Armand, J., & Dicharry, M. (1965). Concept and measurement of ventilatory sensitivity to carbon dioxide. *J Appl Physiol, 20*(5), 890-897.

Dempsey, J. A., Ainsworth, D. M., & Forster, H. V. (1995). Regulation of hyperpnea, hyperventilation, and respiratory muscle recruitment during exercise. In J. A. Dempsey & A. I. Pack (Eds.), *Regulation of Breathing* (pp. 1065-1134). New York: Marcel Dekker.

Dempsey, J. A., Hanson, P. G., & Henderson, K. S. (1984). Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *J Physiol (Lond), 355*, 161-175.

Dempsey, J. A., & Smith, C. A. (1994). Do carotid chemoreceptors inhibit the hyperventilatory response to heavy exercise. *Can J Appl Physiol, 19*(3), 350-359.

Duffin, J., Bechbache, R. R., Goode, R. C., & Chung, S. A. (1980). The ventilatory response to carbon dioxide in hyperoxic exercise. *Respir Physiol, 40*(1), 93-105.

Duffin, J., & McAvoy, G. V. (1988). The peripheral-chemoreceptor threshold to carbon dioxide in man. *J Physiol, 406*, 15-26.

D'Urzo, A. D., Chapman, K. R., & Rebuck, A. S. (1987). Effect of inspiratory resistive loading on control of ventilation during progressive respiratory exercise. *Journal of Applied Physiology, 62*, 134-140.

Epstein, S. K. (1994). An overview of respiratory muscle function. *Clin Chest Med, 15*(4), 619-639.

Fairbarn, M. S., Coutts, K. C., Pardy, R. L., & McKenzie, D. C. (1991). Improved respiratory muscle endurance of highly trained cyclists and the effects on maximal exercise performance. *Int J Sports Med, 12*(1), 66-70.

Fee, L. L., Smith, R. M., & English, M. B. (1997). Enhanced ventilatory and exercise performance in athletes with slight expiratory resistive loading. *J Appl Physiol, 83*(2), 503-510.

Fenn, W. O. (1963). A comparison of respiratory and skeletal muscles. In C. F. Cori, V. G. Foglia, L. F. Leloir, & S. Ochoa (Eds.), *Perspectives in Biology* (pp. 300). Amsterdam: Elsevier.

Fitts, R. (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev, 74*(1), 49-94.

Fitzgerald, R. S., & Parks, D. C. (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. *Respir Physiol, 12*(2), 218-229.

Flenley, D. C., & Warren, P. M. (1983). Ventilatory responses to O2 and CO2 during exercise. *Annu Rev Physiol, 45*, 415-426.

Freedman, S. (1970). Sustained maximum voluntary ventilation. *Respir Physiol, 8*(2), 230-244.

Gaesser, G. A., & Poole, D. C. (1986). Lactate and ventilatory thresholds: disparity in time course of adaptations to training. *J Appl Physiol, 61*(3), 999-1004.

Gallagher, C. G., Hof, V. I., & Younes, M. (1985). Effect of inspiratory muscle fatigue on breathing pattern. *J Appl Physiol, 59*(4), 1152-1158.

Gilson, J., & Hugh-Jones, P. (1949). The measurement of the total lung volume and breathing capacity. *Clin Sci, 7*, 185 - 216.

Green, H., Plyley, M., Smith, D., & Kile, J. (1989). Extreme endurance training and fiber type adaptation in rat diaphragm. *J Appl Physiol, 66*(4), 1914-1920.

Green, M., & Moxham, J. (1985). The respiratory muscles. *Clin Sci, 68*(1), 1-10.

Griffiths, T. L., Henson, L. C., & Whipp, B. J. (1986). Influence of inspired oxygen concentration on the dynamics of the exercise hyperpnoea in man. *J Appl Physiol, 380*, 387-403.

Grimby, G., Saltin, B., & Wilhelmsen, L. (1977). Pulmonary flow-volume and pressure-volume relationship during submaximal and maximal exercise in young well-trained men. *Bull Eur Physiopathol Respir, 7*, 157-167.

Grinton, S., Powers, S. K., Lawler, J., Criswell, D., Dodd, S., & Edwards, W. (1992). Endurance training-induced increases in expiratory muscle oxidative capacity. *Med Sci Sports Exerc, 25(5)*, 551-555.

Hards, J. M., Reid, W. D., Pardy, R. L., & Pare, P. D. (1990). Respiratory muscle fiber morphometry. Correlation with pulmonary function and nutrition. *Chest, 97*(5), 1037-1044.

Harms, C. A., & Dempsey, J. A. (1999). Cardiovascular consequences of exercise hyperpnea. *Exerc Sport Sci Rev, 27*, 37-62.

Harms, C. A., Wetter, T. J., McClaran, S. R., Pegelow, D. F., Nickele, G. A., Nelson, W. B., Hanson, P., & Dempsey, J. A. (1998). Effects of respiratory muscle work on cardiac output and its distribution during maximal exercise. *J Appl Physiol, 85*(2), 609-618.

Harms, C. A., Wetter, T. J., St Croix, C. M., Pegelow, D. F., & Dempsey, J. A. (2000). Effects of respiratory muscle work on exercise performance. *J Appl Physiol, 89*(1), 131-138.

Harms, C. C., Babcock, M. A., McClaran, S. R., Pegelow, D. F., Nickele, G. A., Nelson, W. B., & Dempsey, J. A. (1997). Respiratory muscle work compromises leg blood flow during maximal exercise. *J Appl Physiol, 82*(5), 1573-1583.

Henke, K. G., Sharratt, M., Pegelow, D., & Dempsey, J. A. (1988). Regulation of end-expiratory lung volume during exercise. *J Appl Physiol, 64*(1), 135-146.

Hesser, C. M., Linnarsson, D., & Fagraeus, L. (1981). Pulmonary mechanics and work of breathing at maximal ventilation and raised air pressure. *J Appl Physiol, 50*(4), 747-753.

Hill, N. S., Jacoby, C., & Farber, H. W. (1991). Effect of an endurance triathlon on pulmonary function. *Med Sci Sports Exerc, 23(119)*, 1260-1264.

Hyatt, R. E., & Flath, R. E. (1966). Relationship of air flow to pressure during maximal respiratory effort in man. *J Appl Physiol, 21*(2), 477-482.

Jacobi, M. S., Iyawe, V. I., Patil, C. P., Cummin, A. R., & Saunders, K. B. (1987). Ventilatory responses to inhaled carbon dioxide at rest and during exercise in man. *Clin Sci, 73*(2), 177-182.

Jacobi, M. S., Patil, C. P., & Saunders, K. B. (1989a). The transient ventilatory response to carbon dioxide at rest and in exercise in man. *Respir Physiol, 77*(2), 225-237.

Jacobi, M. S., Patil, C. P., & Saunders, K. B. (1989b). Transient, steady-state and rebreathing responses to carbon dioxide in man, at rest and during light exercise. *J Physiol, 411*, 85-96.

Jeukendrup, A., Saris, W. H., Brouns, F., & Kester, A. D. (1996). A new validated endurance performance test. *Med Sci Sports Exerc, 28*(2), 266-270.

Johnson, B. D., Babcock, M. A., Suman, O. E., & Dempsey, J. A. (1993). Exercise-induced diaphragmatic fatigue in healthy humans. *J Physiol, 460*, 385-405.

Johnson, B. D., Saupe, K. W., & Dempsey, J. A. (1992). Mechanical constraints on exercise hyperpnea in endurance athletes. *J Appl Physiol, 73*(3), 874-886.

Jones, N. L. (1976). Use of exercise in testing respiratory control mechanisms. *Chest, 70*(1 Suppl), 169-173.

Katayama, K., Sato, Y., Morotome, Y., Shima, N., Ishida, K., Mori, S., & Miyamura, M. (1999). Ventilatory chemosensitive adaptations to intermittent hypoxic exposure with endurance training and detraining. *J Appl Physiol, 86*(6), 1805-1811.

Keens, T. G., Krastins, I. R. B., Wannamaker, E. M., Levison, H., Crozier, D. N., & Bryan, A. C. (1977). Ventilatory muscle endurance training in normal subjects and patients with cystic fibrosis. *Am Rev Respir Dis, 116*, 853-860.

Kelley, M. A., Laufe, M. D., Millman, R. P., & Peterson, D. D. (1984). Ventilatory response to hypercapnia before and after athletic training. *Respir Physiol, 55*(3), 393-400.

Kelley, M. A., Owens, G. R., & Fishman, A. P. (1982). Hypercapnic ventilation during exercise: effects of exercise methods and inhalation techniques. *Respir Physiol, 50*(1), 75-85.

Ker, J. A., & Schultz, C. M. (1996). Respiratory muscle fatigue after an ultra-marathon measured as inspiratory task failure. *Int J Sports Med, 17*(7), 493-496.

Koepchen, H. (1975). Atmmungsregultion. In K. K & J. R (Eds.), *Physiologie des Menschen* (Vol. 6: Atmung, pp. 163-310). Munich: Urban and Scwarzenberg.

Kohl, J., Koller, E. A., Brandenberger, M., Cardenas, M., & Boutellier, U. (1996). Effect of exercise-induced hyperventilation on airway resistance and cycling endurance. *Eur J Appl Physiol & Occup Physiol, 421*(5), 1-7.

Krishnan, B., Zintel, T., McParland, C., & Gallagher, C. G. (1991). The evolution of respiratory motor output during constant load heavy exercise. *FASEB J, 5*, A1480.

Leak, L. (1979). Gross and ultrastructural morphological features of the diaphragm. *Am Rev Respir Dis, 119*(2 Suppl, Pt 2), 3-21.

Leith, D. E., & Bradley, M. (1976). Ventilatory muscle strength and endurance training. *J Appl Physiol, 41*(4), 508-516.

Lepers, R., Millet, G. Y., Maffiuletti, N. A., Hausswirth, C., & Brisswalter, J. (2001). Effect of pedalling rates on physiological response during endurance cycling. *Eur J Appl Physiol, 85*(3-4), 392-395.

Levine, B. D., Friedman, D. B., Engfred, K., Hanel, B., Kjaer, M., Clifford, P. S., & Secher, N. H. (1992). The effect of normoxic or hypobaric hypoxic endurance training on the hypoxic ventilatory response. *Med Sci Sports Exerc, 24*(7), 769-775.

Loring, S., & DeTroyer, A. (1985). Actions of the respiratory muscles. In C. Roussos & P. Macklem (Eds.), *The Thorax* (pp. 327-349). New York: Marcel Dekker Inc.

Loring, S. H. (1992). Action of human respiratory muscles inferred from finite element analysis of rib cage. *J Appl Physiol, 72*(4), 1461-1465.

MacFarlane, D. J., & Cunningham, D. J. (1992). Dynamics of the ventilatory response in man to step changes of end-tidal carbon dioxide and of hypoxia during exercise. *J Physiol, 457*, 539-557.

Mador, M. J. (1991). Respiratory muscle fatigue and breathing pattern. *Chest, 100*(5), 1430-1435.

Mador, M. J., & Acevedo, F. A. (1991). Effect of respiratory muscle fatigue on subsequent exercise performance. *J Appl Physiol, 70*(5), 2059-65.

Mador, M. J., Maglang, U. J., Rodis, A., & Kufel, T. J. (1993). Diaphragmatic fatigue after exercise in healthy human subjects. *Am Rev Respir Dis, 148*, 1571-1575.

Mador, M. J., & Tobin, M. J. (1992). The effect of inspiratory muscle fatigue on breathing pattern and ventilatory response to CO2. *J Physiol (Lond), 455*, 17-32.

Mahler, D. A., Moritz, E. D., & Loke, J. (1982). Ventilatory responses at rest and during exercise in marathon runners. *J Appl Physiol, 52*(2), 388-392.

Mahler, D. A., Shuhart, C. R., Brew, E., & Stukel, T. A. (1991). Ventilatory responses and entrainment of breathing during rowing. *Med Sci Sports Exerc, 23*(2), 186-192.

Mancini, D. M., Henson, D., La Mancha, J., Donchez, L., & Levine, S. (1995). Benefit of selective respiratory muscle training on exercise capacity in patients with chronic congestive heart failure. *Circulation, 91(2)*, 320-329.

Manohar, M. (1986). Blood flow to the respiratory and limb muscles and to abdominal organs during maximal exertion in ponies. *J Physiol, 377*, 25-35.

Markov, G., Orler, R., & Boutellier, U. (1996). Respiratory training, hypoxic ventilatory response and acute mountain sickness. *Respir Physiol, 105*, 179-186.

Markov, G., Spengler, C. M., Knopfli-Lenzin, C., Stuessi, C., & Boutellier, U. (2001). Respiratory muscle training increases cycling endurance without affecting cardiovascular responses to exercise. *Eur J Appl Physiol, 85*(3-4), 233-239.

Martin, B., Heintzelman, M., & Chen, H. (1982). Exercise performance after ventilatory work. *J Appl Physiol, 52(69*, 1581-1585.

Martin, B. J., Robinson, S., Wiegman, D. L., & Aulick, L. H. (1975). Effect of warm-up on metabolic responses to strenuous exercise. *Med Sci Sports, 7*(2), 146-149.

Martin, B. J., Sparks, K. E., Zwillich, C. W., & Weil, J. V. (1979). Low exercise ventilation in endurance athletes. *Med Sci Sports, 11(2)*, 181-185.

Martin, B. J., & Stager, J. M. (1981). Ventilatory endurance in athletes and non-athletes. *Med Sci Sports Exerc, 13(1)*, 21-26.

Martin, B. J., Weil, J. V., Sparks, K. E., McCullough, R. E., & Grover, R. F. (1978). Exercise ventilation correlates positively with ventilatory chemoresponsiveness. *J Appl Physiol, 45*(4), 557-564.

Mateika, J. H., & Duffin, J. (1995). A review of the control of breathing during exercise. *Eur J Appl Physiol & Occup Physiol, 71*(1), 1-27.

McConnell, A. K., Caine, M. P., & Sharpe, G. R. (1997). Inspiratory muscle fatigue following running to volitional fatigue: the influence of baseline strength. *Int J Sports Med, 18*(3), 169-73.

McConnell, A. K., & Davies, C. T. (1992). A comparison of the ventilatory responses to exercise of elderly and younger humans. *J Gerontol, 47*(4), B137-141.

McConnell, A. K., & Semple, E. S. (1989). Day to day variablility of human ventilatory sensitivity to CO_2. *J Physiol, 417*, 122P.

McLoughlin, P., Cope, R., Linton, R. A. F., & Band, D. M. (1993). The effect of inspiration of 100% O_2 on the ventilatory response to incremental exercise in man. *J Physiol, 473*, 57P.

McMahon, M. E., Boutellier, U., Smith, R. M., & Spengler, C. M. (2002). Hyperpnea training attenuates peripheral chemosensitivity and improves cycling endurance. *J Exp Biol, 205*(Pt 24), 3937-3943.

McParland, C., Krishnan, B., Lobo, J., & Gallagher, C. G. (1992). Effect of physical training on breathing pattern during progressive exercise. *Respir Physiol, 90*(3), 311-323.

Metzger, J. M., & Fitts, R. H. (1986). Contractile and biochemical properties of diaphragm: effects of exercise training and fatigue. *J Appl Physiol, 60*(5), 1752-1758.

Miller, J. P., Cunningham, D. J., Lloyd, B. B., & Young, J. M. (1974). The transient respiratory effects in man of sudden changes in alveolar CO_2 in hypoxia and in high oxygen. *Respir Physiol, 20*(1), 17-31.

Miyamura, M., & Ishida, K. (1990). Adaptive changes in hypercapnic ventilatory response during training and detraining. *Eur J Appl Physiol, 60*(5), 353-359.

Miyamura, M., Yamashina, T., & Honda, Y. (1976). Ventilatory responses to CO2 rebreathing at rest and during exercise in untrained subjects and athletes. *Jpn J Physiol, 26*(3), 245-254.

Mizuno, M. (1991). Human respiratory muscles: fibre morphology and capillary supply. *Eur Respir J, 4*(5), 587-601.

Mizuno, M., Juel, C., Bro-Rasmussen, T., Mygind, E., Schibye, B., Rasmussen, B., & Saltin, B. (1990). Limb skeletal muscle adaptation in athletes after training at altitude. *J Appl Physiol, 68*(2), 496-502.

Moffatt, R., Chitwood, L., & Biggerstaff, K. (1994). The influence of verbal encouragement during assessment of maximal oxygen uptake. *J Sports Med Phys Fitness, 34*(1), 45-49.

Mohan, R. M., Amara, C. E., Cunningham, D. A., & Duffin, J. (1999). Measuring central-chemoreflex sensitivity in man: rebreathing and steady-state methods compared. *Respir Physiol, 115*(1), 23-33.

Moore, R. L., & Gollnick, P. D. (1982). Response of Ventilatory Muscles of the Rat to Endurance Training. *Pflugers Arch, 392*, 268-271.

Morgan, D. W., Kohrt, W. M., Bates, B. J., & Skinner, J. S. (1987). Effects of respiratory muscle endurance training on ventilatory and endurance performance of moderately trained cyclists. *Int J Sports Med, 8(2)*, 88-93.

Moxham, J., Wiles, C. M., Newham, D., & Edwards, R. H. (1980). Sternomastoid muscle function and fatigue in man. *Clin Sci, 59*(6), 463-468.

NHLBI, W. (1990). Respiratory Muscle Fatigue: report of the repiratory muscle fatigue workshop group. *Am Rev Respir Dis, 142*, 474-480.

Nye, P. C. (1994). Identification of peripheral chemoreceptor stimuli. *Med Sci Sports Exerc, 26*(3), 311-318.

O'Kroy, J. A., & Coast, J. R. (1993). Effects of flow and resistive training on respiratory muscle endurance and strength. *Respiration, 60*(5), 279-283.

Paterson, D. J., & Nye, P. C. (1991). Effect of oxygen on potassium-excited ventilation in the decerebrate cat. *Respir Physiol, 84*(2), 223-230.

Pianosi, P., & Marchione, T. (1995). Hypoxic response is inversely related to degree of exercise hyperventilation. *Respir Physiol, 101*(1), 71-78.

Pokorski, M., & Lahiri, S. (1983). Aortic and carotid chemoreceptor responses to metabolic acidosis in the cat. *Am J Physiol, 244*(5), R652-R658.

Poortmans, J. R. (Ed.). (1993). *Principles of Exercise Biochemistry* (2nd, revised ed.). Brussels, Switzerland: Krager.

Powers, S. K., Criswell, D., Lawler, J., Martin, D., Ji, L. L., Herb, R. A., & Dudley, G. (1994). Regional training-induced alterations in diaphragmatic oxidative and antioxidant enzymes. *Respir Physiol, 95*(2), 227-237.

Powers, S. K., & Dempsey, J. A. (1996). Adaptive strategies of respiratory muscles in response to endurance exercise. *Med Sci Sports Exerc, 28*(9), 1115-1122.

Powers, S. K., Grinton, S., Lawer, J., Criswell, D., & Dodd, S. (1992). High intensity exercise training-induced metabolic alterations in respiratory muscles. *Respir Physiol, 89*, 169-170.

Powers, S. K., Lawler, J., Criswell, D., Dodd, S., Grinton, S., Bagby, G., & Silverman, H. (1990). Endurance-training-induced cellular adaptations in respiratory muscles. *J Appl Physiol, 68*(5), 2114-2118.

Rausch, S. M., Whipp, B. J., Wasserman, K., & Huszczuk, A. (1991). Role of the carotid bodies in the respiratory compensation for the metabolic acidosis of exercise in humans. *J Physiol, 444*, 567-578.

Rebuck, A. S. (1976). *Measurement of ventilatory response to CO2 by rebreathing. Chest, 70*(1 Suppl), 118-121.

Rebuck, A. S., & Read, J. (1971). Patterns of ventilatory response to carbon dioxide during recovery from serve asthma. *Clin Sci, 52*, 388-392.

Reid, W. D., Cairns, C. L., McRae, D. J., Chung, F., Wiggs, B. R., & Pardy, R. L. (1994). Regional and fibre type glycogen utilization patterns in the hamster diaphragm following swimming. *Respir Med, 88*(6), 421-427.

Ries, A. L., & Moser, K. M. (1986). Comparison of isocapnic hyperventilation and walking exercise training at home in pulmonary rehabilitation. *Chest, 90*(2), 285-289.

Robinson, E. P., & Kjeldgaard, J. M. (1982). Improvement in ventilatory muscle function with running. *J Appl Physiol, 52*(6), 1400-1406.

Roussos, C. (1995). *The Thorax*. (2nd ed.): Marcel Decker, Inc.

Roussos, C., & Macklem, P. (Eds.). (1985). *The Thorax*. New York: Marcel Dekker Inc.

Rowell, L. B., & Sheperd, J. T. (Eds.). (1996). *Exercise: Regulation and Integration of Multiple Systems* (1st ed.). (Vol. 1). New York: Oxford University Press Inc.

Scoggin, C. H., Doekel, R. D., Kryger, M. H., Zwillich, C. W., & Weil, J. V. (1978). Familial aspects of decreased hypoxic drive in endurance athletes. *J Appl Physiol, 44*(3), 464-468.

Severinghaus, J. W. (1976). Proposed standard determination of ventilatory responses to hypoxia and hypercapnia in man. *Chest, 70*(1 Suppl), 129-131.

Sheel, A. W. (2002). Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. *Sports Med, 32*(9), 567-581.

Sliwinski, P., Yan, S., Gauthier, A. P., & Macklem, P. T. (1996). Influence of Global Inspiratory Fatigue on Breathing During Exercise. *J Appl Physiol, 80*(4), 1270-1278.

Sonetti, D. A., Wetter, T. J., Pegelow, D. F., & Dempsey, J. A. (2001). Effects of respiratory muscle training versus placebo on endurance exercise performance. *Respir Physiol, 127*(2-3), 185-199.

Spengler , C. M., Lenzin, C., Stuessi, C., Markov, G., & Boutellier, U. (1998). Decreased perceived respiratory exertion during exercise after respiratory endurance training. *Am J Respir Crit Care Med, 157*, A782.

Spengler, C. M., & Boutellier, U. (2000). Breathless Legs? Consider Training Your Respiration. *News Physiol Sci, 15*, 101-105.

Spengler, C. M., Lenzin, C., Markov, G., Stuessi, C., & Boutellier, U. (1998). Improved cycling endurance after respiratory endurance training without cardiovascular improvement. *FASEB J, 12*(5), A1117.

Spengler, C. M., Roos, M., Laube, S. M., & Boutellier, U. (1999). Decreased exercise blood lactate concentrations after respiratory endurance training in humans. *Eur J Appl Physiol, 79*(4), 299-305.

Stephenson, R., Mohan, R. M., Duffin, J., & Jarsky, T. M. (2000). Circadian rhythms in the chemoreflex control of breathing. *Am J Physiol Regul Integr Comp Physiol, 278*(1), R282-286.

Stockley, R. A. (1977). The estimation of the resting reflex hypoxic drive to respiration in normal man. *Respir Physiol, 31*(2), 217-230.

Stockley, R. A. (1978). The contribution of the reflex hypoxic drive to the hyperpnoea of exercise. *Respir Physiol, 35*(1), 79-87.

Stuessi, C., Spengler, C. M., Knopfli-Lenzin, C., Markov, G., & Boutellier, U. (2001). Respiratory muscle endurance training in humans increases cycling endurance without affecting blood gas concentrations. *Eur J Appl Physiol, 84*(6), 582-586.

Swanson, G. D., & Bellville, J. W. (1975). Step changes in end-tidal CO2: methods and implications. *J Appl Physiol, 39*(3), 377-385.

Syabbalo, N. C., Krishnan, B., Zintel, T., & Gallagher, C. G. (1994). Differential ventilatory control during constant work rate and incremental exercise. *Respir Physiol, 97*, 175-187.

Taylor, R., & Jones, N. (1979). The reduction by training of CO_2 output during exercise. *Euro J Cardiol, 9*, 53-62.

Tzelepis, G. E., Vega, D. L., Cohen, M. E., Fulambarker, A. M., Patel, K. K., & McCool, F. D. (1994). Pressure-flow specificity of inspiratory muscle training. *J Appl Physiol, 77*(2), 795-801.

Ward, S. (1994a). Peripheral and Central Chemoreceptor Control of Ventilation During Excercise in Humans. *Can J Appl Physiol, 19*(3), 305-333.

Ward, S. A. (1994b). Assessment of peripheral chemoreflex contributions to exercise hyperpnea in humans. *Med Sci Sports Exerc, 26*(3), 303-310.

Ward, S. A., & Bellville, J. W. (1983). Peripheral chemoreflex suppression by hyperoxia during moderate exercise in man. In B. J. Whipp & D. M. Wiberg (eds.) (Eds.), *Modeling and Control of Breathing* (pp. 54-61). New York: Elsevier.

Weil, J. V., Byrne-Quinn, E., Sodal, I. E., Kline, J. S., McCullough, R. E., & Filley, G. F. (1972). Augmentation of chemosensitivity during mild exercise in normal man. *J Appl Physiol, 33*(6), 813-819.

Weil, J. V., & Swanson, G. D. (1991). Chemoreceptors and control of breathing. In B. J. Whipp & K. Wasserman (eds.) (Eds.), *Exercise: Pulmonary Physiology and Pathophysiology* (Vol. 52, pp. 371-403). New York: Marcel Dekker.

Wetter, T. J., Harms, C. A., Nelson, W. B., Pegelow, D. F., & Dempsey, J. A. (1999). Influence of respiratory muscle work on VO(2) and leg blood flow during submaximal exercise. *J Appl Physiol, 87*(2), 643-651.

Yerg, J. E., 2nd, Seals, D. R., Hagberg, J. M., & Holloszy, J. O. (1985). Effect of endurance exercise training on ventilatory function in older individuals. *J Appl Physiol, 58*(3), 791-794.