MODERATORS OF RESPIRATORY MUSCLE FUNCTION IN HEALTH, EXERCISE AND DISEASE

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ABSTRACT

This research presents a series of studies investigating the role of the respiratory muscles in both healthy and diseased populations.

To test directly the role of the respiratory muscles in a healthy population various strategies were used. Two studies examined the effects of training these muscles to bring about improvements in inspiratory strength and endurance and observe the effects of these changes on whole body performance.

Following a 10-week intervention of inspiratory resistive loading (IRL) study one demonstrated a 34% increase in maximum inspiratory pressure and a 36% increase in cycling endurance capacity at 75% $\dot{V}O_2$peak (increased time by 1292 ± 607 seconds). During the trial heart rate (HR), ventilation ($\dot{V}_E$), and ratings of perceived exertion (RPE) were significantly reduced at the same time points.

Study two examined the long-term effects (3 months) of non-invasive positive pressure ventilation (NIPPV) use in type II respiratory failure patients (PaO_2 <7.3 kPa & $PaCO_2$ >6.5 kPa). This treatment modality improved daytime blood gas tensions (PaO_2 increased to 8.06 ± 1.24 kPa; $PaCO_2$ decreased to 6.45 ± 0.73 kPa), oxygen saturation (increased to 89.9 ± 3.7%) , bicarbonate retention (decreased to 25.8 ± 3.86) and patient perception of health (as measured by St George's Respiratory Questionnaire) in a group of patients receiving otherwise optimal treatment. In addition, both inspiratory muscle strength and endurance were improved relative to baseline following the three month intervention.

Prevention of loss in body mass, particularly in the lean body compartment, is a potential method of alleviating muscle dysfunction in Chronic Obstructive Pulmonary Disease (COPD). Therapeutic doses of anabolic androgenic steroid (AAS) administration have been shown to increase maximal inspiratory pressure. Study three was designed to investigate the long-term effects of taking AAS (>20 yrs) in a population of bodybuilders to help determine the safety aspect of the drugs with specific application to the respiratory system. Spirometry was within normal range and there were no differences between a group using AAS, a group abstinent from AAS use for three months; bodybuilders who had never used AAS; and sedentary controls. Maximum inspiratory pressure (MIP), a gauge of inspiratory muscle strength, and grip strength were both significantly greater in the group using AAS. MIP was 148 ± 24 cm H_2O in the AAS using group compared to 117 ± 26 cm H_2O in the bodybuilding control group. Although the use of AAS is associated with a variety of potentially hazardous consequences on cardiovascular and hepatic health, we demonstrated no adverse effect on respiratory (muscle) function, lung volumes or indices of flow, or breathing profiles.

Investigating the use of AAS provided an opportunity to examine a subset of bodybuilders self administering recombinant human growth hormone (rhGH). As a potential means of improving body composition and respiratory function in cachectic COPD patients, study four examined the effects of short-term high dose rhGH administration on body composition and respiratory function. Male subjects self-administered 0.019 $mg.kg^{-1}.day^{-1}$ for six days. After this intervention maximum inspiratory and expiratory pressures (MIP & MEP respectively) significantly increased when compared with a control group, (MIP 144 ± 24 cm H_2O ; MEP 179 ±

35 cm H_2O). Increased body mass index and fat free mass were observed with a decrease in body fat when compared with a control group (Body fat pre = 20.0 ± 6.0%, during = 19.0 ± 6.0%, post = 19.1 ± 5.8%.

Differing results from various published studies may be related to the intensity of the intervention. Study five compared two training protocols, the first set at 80% of maximum (SUB), and a second at 100% of maximum (MAX), a third control group performed no training (CON). Both training protocols brought about improvements in inspiratory muscle performance (increases in MIP of +32 ± 19 cm H_2O for MAX and +37 ± 25 cm H_2O for SUB) but only the maximally trained group demonstrated a decrease in exercising heart rate (-6 ± 9 beats.min^{-1}) and RPE (-0.5 ± 1.4).

Study six, examined stroke volume during respiratory manoeuvres against resistance, and was undertaken as a pilot investigation to ascertain possible mechanisms via which improvements in whole body exercise can be produced through respiratory muscle loading. Stroke volume during supine rest (~100ml) was greatly decreased during a maximal inspiration against resistance (~80ml) and very quickly increased (~110ml) on cessation of this manoeuvre. Chronic exposure to these manoeuvres, which are a feature of an IRL intervention, potentially has a profound effect on the heart through the Frank-Starling relationship. These interesting results require further investigation.

CERTIFICATE OF RESEARCH

This is to certify that, except where specific reference is made, the work described in this thesis is the result of the candidate. Neither this thesis, nor any part of it, has been presented, or is currently submitted, in candidature for any degree at any other University.

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Gething, A.D., Baker, J., Davies, B. Respiratory muscle training in healthy populations – A review.

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Gething, A.D., Grace, F., Baker, J., Davies, B. Long term anabolic-androgenic steroid (AAS) administration and respiratory function.

Gething, A.D., Graham, M., Baker, J., Davies, B. Potential for recombinant human growth hormone administration in cachectic COPD patients.

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AAS	Anabolic Androgenic Steroids
ACSM	American College of Sports Medicine
ANOVA	Analysis Of Variance
B_f	Breathing frequency
BPNS	Bilateral Phrenic Nerve Stimulation
CO_2	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease
CSA	Cross Sectional Area
EMG	Electromyographic
FEV_1	Forced Expiratory Volume in 1 second
FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
HR	Heart Rate
IRL	Inspiratory Resistive Loading
MEP	Maximum Expiratory Pressure
MIP	Maximum Inspiratory Pressure
MSV	Maximum Sustainable Ventilation
MSVC	Maximum Sustainable Ventilatory Capacity
MVV	Maximum Voluntary Ventilation
NIPPV	Non-Invasive Positive Pressure Ventilation
O_2	Oxygen
PEF	Peak Expiratory Flow
$P_{ET}CO_2$	End tidal CO_2
rhGH	Recombinant human Growth Hormone
RM	Respiratory Muscles
RMT	Respiratory Muscle Training
RPE	Ratings of Perceived Exertion
RV	Residual Volume
SD	Standard Deviation
SMIP	Sustained Maximum Inspiratory Pressure
T_E	Expiratory Time
T_I	Inspiratory Time
TIRE	Test of Incremental Respiratory Endurance
$Tlim_{75}$	Time to exhaustion at 75% of peak oxygen consumption
TLC	Total Lung Capacity
T_{LIM}	Endurance Time
VC	Vital Capacity
$\dot{V}_E$	Ventilation
VIH	Voluntary Isocapnic Hyperpnoea
$\dot{V}O_2$	Oxygen Consumption
$\dot{V}O_2peak$	Peak oxygen consumption
V_T	Tidal Volume
W	Watts
Wmax	Maximum Power Output

CHAPTER 1 - INTRODUCTION

1-1 General Overview

The provenance of this thesis derived primarily from research conducted at the laboratory of Prof Boutellier (Boutellier *et al.* 1992; Boutellier and Piwko 1992). The demonstration that increasing the endurance of the respiratory muscles could improve whole body exercise performance became the starting point for this thesis. The design of study one, in particular to use 75% $\dot{V}O_2$peak as an outcome measure, followed a visit to Prof Boutellier's laboratory in Zurich, Switzerland where he discussed his work, his training intervention, and theories behind why training the respiratory muscles improves whole body exercise endurance. The potential for a respiratory steal of blood from the exercising limbs was mentioned as a possible mechanism following the demonstration that unloading the work of breathing with a ventilator by 50% increases exercising limb blood flow by 5-7% (Harms *et al.* 1998). To this end pilot studies were conducted at the University of Glamorgan with commercially available positive pressure ventilators to investigate respiratory muscle unloading during exercise. However, commercially available ventilators are not capable of producing pressures great enough to keep up with ventilation during exercise in healthy subjects. The proportional assist ventilators being used by other groups (Gallagher *et al.* 1989; Harms *et al.* 1998) are capable of producing pressures necessary to keep up with exercise but are not commercially available and expensive to construct (Dr Younes, personal communication). However, the use of non-invasive positive pressure ventilators (NIPPV) in diseased populations where the work of breathing is increased is potentially of benefit.

Study two focused on the respiratory muscles in patients with type II respiratory failure where the work of breathing is constantly increased. Rather than increasing

resistive work as study one did, study two investigated resting these muscles to allow the opportunity for them to adapt.

During this study, loss of body mass was a major predictor of outcome for patients and is not addressed by therapy. This cachexia comes from an increased work of breathing, reduced calorie intake because of breathlessness and in some cases the metabolic effects of chronic obstructive pulmonary disease (COPD). Anabolic androgenic steroids (AAS) along with nutritional support have been suggested as a way of alleviating the loss in body mass in chronically ill patients with pulmonary disease (Schols 1995; Ferreira *et al.* 1998). Health care professionals are unlikely to use AAS however as the effectiveness and safety of their use has not been adequately investigated.

Study three recruited long term users (bodybuilders) of AAS (>20 years) to examine the effects of their use with particular focus on the respiratory system.

A subset of the bodybuilders interviewed prior to study three admitted to using growth hormone as a means of increasing body mass ad strength gains. Study four investigated the short term use (6 days self administering) of growth hormone. This study was undertaken as growth hormone has also been suggested for treating patients with advanced COPD (especially those presenting loss of muscle mass) (Villaca *et al.* 2006).

The respiratory training intervention in study one was substantial requiring 30 minute training sessions three times per week for ten weeks. Study five was designed find a training intervention which would minimise the investment of time whilst maintaining the adaptations to the respiratory muscles (and whole body exercise). A reduction from ten weeks to six and from 30 min to 15 min makes investigating respiratory muscle training in the future a lot easier.

To explain the findings of study one and five, study six was designed as a pilot study to investigate stroke volume during an inspiration against resistance. This study outlines a potential mechanism to explain improvements in whole body exercise after respiratory muscle training requires further study.

As the first and last line of defence for oxygen (O_2) transport and the major regulator of acid-base status via carbon dioxide (CO_2) elimination, alveolar ventilation must be provided in large quantities and in a mechanically efficient manner during exercise. Muscular exercise places unique and multifactorial demands on the respiratory system that can result in more than a 20-fold increase in ventilation ($\dot{V}_E$) over resting values (Astrand and Rodahl 1986). These tasks are critically dependent upon both the structural capacities and mechanical properties of the airways, lung, and chest wall and the precision with which their functions are regulated by nervous control mechanisms (Dempsey *et al.* 1995).

The ability of the respiratory musculature to meet these demands is of importance, but it is equally important that the physiologic cost of providing this ventilation is not excessive (Sheel 2002; Padula and Yeaw 2007).

It is generally accepted that the respiratory system may limit functional exercise capacity in disease (Folgering and von Herwaarden 1994), but to what extent the respiratory system limits exercise performance in healthy individuals remains contentious. Inspiratory muscle fatigue occurs following prolonged submaximal exercise (Loke *et al.* 1982) and short-term maximal exercise (Johnson *et al.* 1993; Mador *et al.* 1993). Furthermore, experiments that have deliberately fatigued the inspiratory muscles prior to exercise using either sustained maximal isocapnic

hyperpnoea (Martin *et al.* 1982) or resistive loading (Mador and Acevedo 1991) have observed decreases in time to fatigue during subsequent short-term high intensity exercise. Several authors have noted improvements in exercise capacity in response to partial unloading of the inspiratory muscles using either reduced viscosity gas mixtures i.e. helium (Aaron *et al.* 1985) or proportional assist ventilation (Harms *et al.* 2000), probably due to prevention of inspiratory muscle fatigue. A more "natural" approach to testing for respiratory limitation during exercise is to specifically train the respiratory muscles. If this training can increase exercise performance / capacity then it is more likely that exercise was limited by the respiratory system before the specific training (Spengler and Boutellier 2000).

Respiratory muscle training (RMT) improves respiratory muscle function in patients (Keens *et al.* 1977) and healthy individuals (Leith and Bradley 1976). Although functional exercise capacity can be improved in patients following RMT (Belman *et al.* 1986), the question of whether RMT improves endurance performance in healthy individuals remains controversial. Improvements in whole body exercise capacity (Boutellier *et al.* 1992; Boutellier and Piwko 1992; Spengler *et al.* 1999; Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Holm *et al.* 2004; Guenette *et al.* 2006; Griffiths and McConnell 2007; Leddy *et al.* 2007; Wylegala *et al.* 2007) have been reported by some, whilst others have shown no ergogenic effect of RMT in healthy individuals (Morgan *et al.* 1987; Fairbarn *et al.* 1991; Hanel and Secher 1991; Inbar *et al.* 2000; Williams *et al.* 2002; Wells *et al.* 2005; Downey *et al.* 2007). The explanation for this apparent conflict in research findings is unclear but may result from variations in the intensity, duration and mode of exercise used to evaluate performance in addition to differences in experimental design, fitness level of subjects and intervention type.

In a disease such as chronic obstructive pulmonary disease (COPD), the role of these respiratory muscles are far more pronounced than when compared to that of a healthy population (Levine *et al.* 2001).

In COPD, both the force of contraction generated by the inspiratory muscles and the mechanical load against which they are required to act are abnormal (Gibson 1996). The inspiratory load is increased as a result of the airway obstruction and the force of contraction is reduced as a consequence of: the effect of hyperinflation altering the mechanical advantage of the muscles (both intercostals and diaphragmatic); malnutrition; and in some cases respiratory muscle fatigue (Levine *et al.* 2001).

Inspiratory muscle dysfunction is central to the development of hypercapnia (Begin and Grassino 1991). Patients who experience difficulty in excreting CO_2 as a result of inspiratory muscle fatigue, ventilation perfusion mismatch and possibly alveolar hypoventilation respond by altering the frequency and depth of breathing to maintain adequate alveolar ventilation and there is an adaptive response to the control of breathing, with a reduced ventilatory response to the arterial CO_2 (Calverley 1995).

It has been suggested that in COPD the respiratory muscles are subject to fatigue, and that improvements in lung mechanics and a consequent reduction in workload may facilitate rest and relief of fatigue. Several investigators have shown that the application of non-invasive ventilation may produce reductions in the work of breathing (Belman *et al.* 1990). However, at present no long-term study has yet shown an improvement in measures of respiratory muscle strength and endurance with the use of assisted ventilation.

Furthermore, a common complication of COPD is involuntary weight loss (Yeh *et al.* 2002), and is an independent predictor of outcome, which is infrequently addressed by

therapy. Depending on the population studied, between 20% and 70% of patients with COPD are underweight (Braun 1984; Wilson *et al.* 1989; Schols *et al.* 1993).

Reversal of body weight has been associated with improved outcomes, including increased muscle strength and exercise capacity, as well as increased survival (Efthimiou *et al.* 1988; Rogers *et al.* 1992; Schols 1995). Restoration of body weight may be difficult to achieve and to maintain using nutritional intervention alone (Rogers *et al.* 1992; Schols 1995).

Several studies have shown the merits of using Anabolic Androgenic Steroids (AAS) in the treatment of weight loss in chronically ill patients with pulmonary disease (Schols 1995; Ferreira *et al.* 1998; Yeh *et al.* 2002). In normal eugonadal men and in subjects with COPD, AAS has been shown to produce increases in lean body mass and muscle strength (Schols 1995; Bhasin *et al.* 1996; Graham *et al.* 2006). In addition, therapeutic doses of the AAS Nandrolone have been shown to increase maximal inspiratory pressure (MIP) by 10% in patients with tetraplegia (Spungen *et al.* 1999).

However, before these drugs can be adopted into clinical practice, additional studies are necessary to evaluate the effectiveness (Casaburi 2000) and more importantly, the safety of prolonged AAS administration (Dobs 1999). Information regarding the long-term effects of AAS has both clinical and social relevance. Additionally, there is little available data on the effects of AAS on indices of respiratory function.

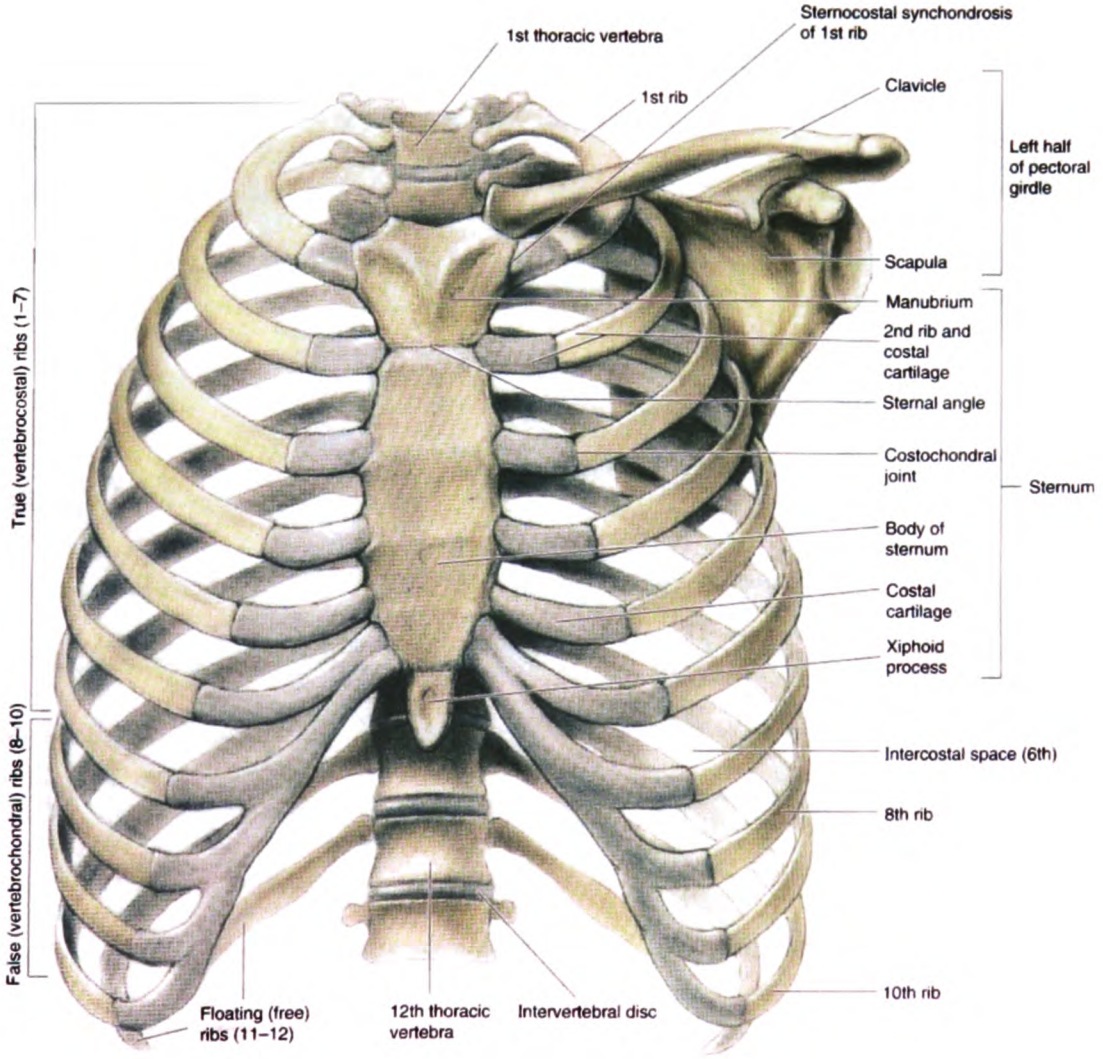
A description of the structure and function of the thorax and respiratory muscles is provided in section 1-2 and 1-3 respectively, whilst section 1-4 reviews the most common procedures for the assessment of the respiratory muscles. Section 1-5

examines the mechanical limitations on respiratory performance. A critical review of the existing respiratory muscle training literature in the context of exercise performance in a healthy population is discussed in section 1-6. Aims and objectives of this investigation can be found in section 1-7.

1-2 Structure and Function of the Thorax

The thoracic skeleton is an osteocartilaginous frame that contains / protects the principal organs of respiration and circulation. It is narrow above, broad below, flattened anteroposteriorly and longer behind (Soames 1995). The thoracic skeleton includes 12 pairs of ribs and costal cartilages; 12 thoracic vertebrae and intervertebral discs; and the sternum (Figure 1-1).

Figure 1-1 The skeleton of the thorax. From Moore and Dalley (1999)



Thoracic variations in dimensions and proportions are partly individual and also linked to age, sex, and race. At birth the transverse diameter is relatively less but adult proportions develop as walking begins. In females capacity is less, absolutely and proportionately, the sternum being shorter, the thoracic inlet more oblique and the suprasternal notch level with the third thoracic vertebra (second in males). The upper ribs are more mobile in females, allowing greater upper thoracic expansion. In tall thin individuals the thorax usually shows corresponding proportions, and a similar correspondence also occurs in the short and broad individuals. Racial variations are also linked to stature and proportions in a like manner (Moore and Dalley 1999).

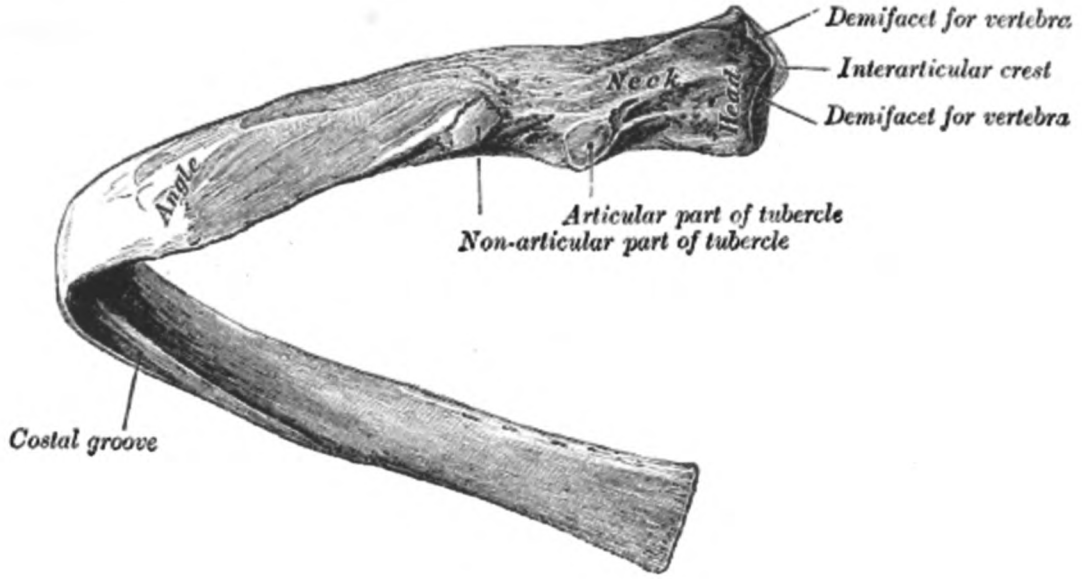
The prime function of the thorax is respiration. The obvious protection afforded is fortuitous but many muscles are attached to it, not all primarily concerned with respiration, although they may assist it. Muscles of the arm, especially those acting on the pectoral girdle and humerus, those of the abdominal wall and spinal column, all have widespread thoracic attachments.

1-2.1 Ribs

The ribs are elastic arches, and 12 pairs connect posteriorly with the vertebral column, forming much of the thoracic skeleton, although the number may be reduced to 11 by absence of the twelfth pair. The superior seven pairs are connected by costal cartilages to the sternum as *true* ribs. The remaining five are so-called *false* ribs, cartilages of the eighth to tenth joining the superjacent costal cartilage; the eleventh and twelfth being free at their anterior ends are sometimes referred to as *floating* ribs.

Between ribs are intercostals spaces, which are deeper in front and between the upper ribs. The ribs increase in length from the first to the seventh, and diminish to the twelfth, whilst their breadth decreases downwards (Soames 1995).

Figure 1-2 Typical rib: The 3rd though 9th ribs have common characteristics. From Soames, (1995).

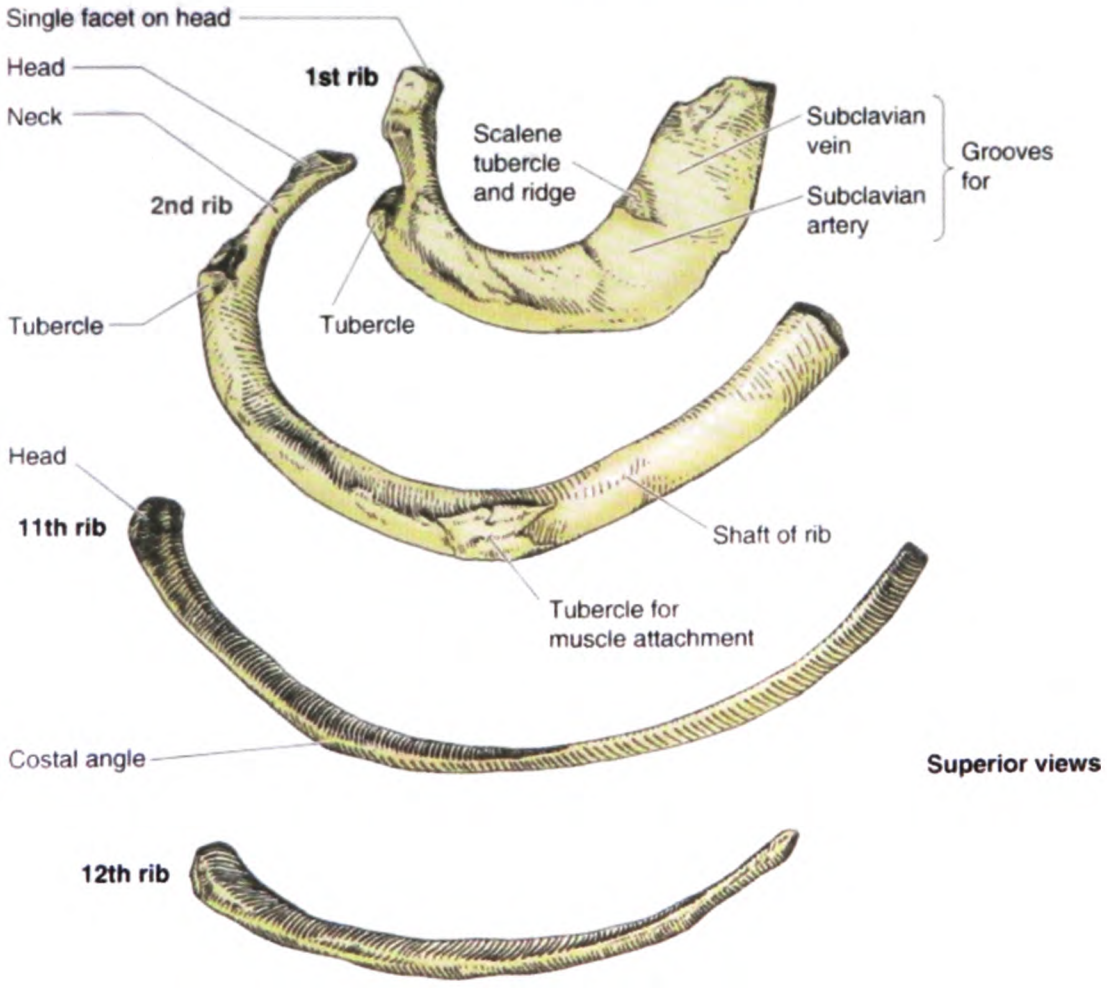


Typical ribs (3rd to 9th) (Figure 1-2) have a:

- **Head** that is wedge-shaped and has two facets, separated by the crest of the head: one facet for articulation with the numerically corresponding vertebra and one facet for the vertebra superior to it.
- **Neck** that connects the head with the body (shaft) at the level of the tubercle.
- **Tubercle** occurring at the junction of the neck and shaft. The tubercle has a smooth articular part for articulating with the corresponding transverse process of the vertebra, and a rough nonarticulating part for attachment of the costotransverse ligament.

- **Shaft** that is thin, flat, and curved – most markedly at the costal angle where the rib turns anterolaterally; the concave internal surface has a **costal groove** that protects the intercostals nerve and vessels.

Figure 1-3 Atypical ribs. These ribs (1st, 2nd, and 10th to 12th) differ from typical ribs. From Moore and Dalley, (1999)



However, the 1st, 2nd, and 10th to 12th ribs are dissimilar (Figure 1-3):

- The first rib is the broadest, shortest and most sharply curved of the seven true ribs; it has a single facet on its head for articulation with T1 vertebra and two

transversely directed grooves crossing its superior surface for the subclavian vessels, which are separated by a scalene tubercle and ridge.

- The second rib is twice the length of the first, with a similar curvature. The nonarticular area of the tubercle is small. The angle is slight and near the tubercle. The shaft is not twisted, but at the tubercle is convex upwards, as in the first rib but less so. The external surface of the shaft is convex and superolaterally marked centrally by a rough, muscular impression. The interior surface, smooth and concave, faces inferomedially with posteriorly a short costal groove.
- The tenth rib has a single facet on its head, which may articulate with the intervertebral disc above, as well as the upper border of the tenth thoracic vertebra near its pedicle.
- The eleventh and twelfth ribs each have one large, articular facet on the head, but no neck or tubercle; their pointed anterior ends are tipped with cartilage. The eleventh has a slight angle and shallow costal groove. The twelfth has neither, being much shorter and sloping cranially at its vertebral end. The internal surface of both ribs face slightly upwards, more so in the twelfth.

Costal cartilages prolong the ribs anteriorly and contribute to the elasticity of the thoracic wall. The cartilages increase in length through the first seven and then gradually decrease. The first seven cartilages (and sometime the 8^{th}) join the sternum; the 8^{th}, 9^{th} and 10^{th} articulate with the cartilages just superior to them. In some people the 10^{th} pair of ribs may be floating. The 11^{th} and 12^{th} cartilages form caps on the anterior ends of these ribs. Intercostals spaces separate the ribs and their costal

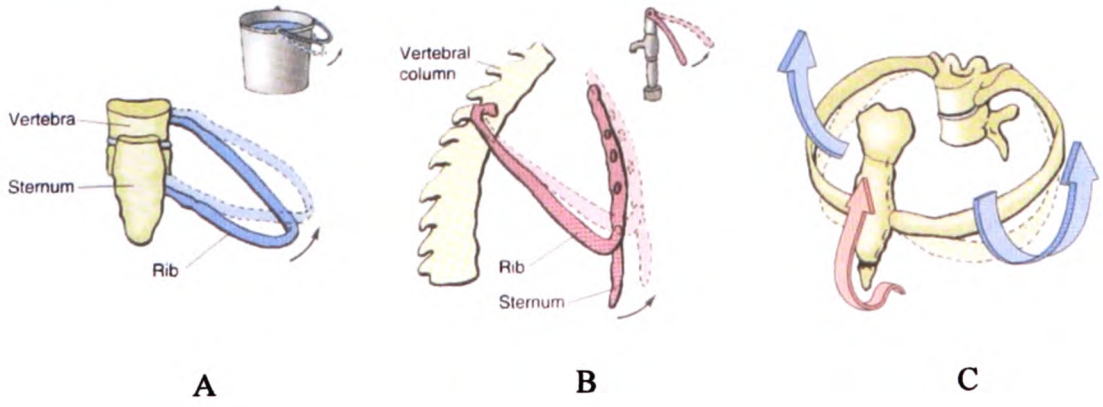
cartilages from one another. These spaces are occupied by intercostals muscles, vessels, and nerves (Moore and Dalley 1999).

1-2.2 Mechanism of the Thorax

Each rib has its own range and direction of movement that contributes to thoracic respiratory excursions. Each acts as a lever, its fulcrum immediately lateral to its costotransverse articulation, which means that when the shaft is elevated, the neck is depressed and vice versa. Since the lever's arms differ in length a significant amount, slight movement at the vertebral end is much magnified at the anterior end (Moore and Dalley 1999).

Anterior costal ends are lower than posterior such that when shafts are raised they move forwards. The midshaft is below the ends so that when the shaft is raised it also spreads laterally. In addition, each rib is part of a curve greater than that of the rib above, therefore costal elevation increases transverse thoracic diameter at higher levels (Figure 1-4).

Figure 1-4 Movements of the thoracic wall. From Moore and Dalley (1999)



The transverse diameter of the thorax increases slightly when the intercostals muscles contract, raising the middle of the ribs – the bucket handle movement (Figure 1-4A). The anteroposterior diameter of the thorax also increases considerably when these muscles contract: movement of the ribs (primarily 2^{nd} through 6^{th}) at the costovertebral joints about an axis passing through the necks of the ribs causes the sternal ends of the ribs to rise – the pump handle movement (Figure 1-4B). Because the ribs slope inferiorly, their elevation also results in anterior-posterior movement of the sternum especially its inferior ends. The combination of all these movements moves the thoracic cage anteriorly, superiorly, and laterally (Figure 1-4C).

1-3 Structure and Function of the Respiratory Muscles

By definition, any skeletal muscle that changes the dimensions of the chest wall is a respiratory muscle and there are many muscles located between the waist and head that can move the chest wall. The muscles of respiration share similar functional and morphological characteristics with other skeletal muscles and are classified as striated skeletal muscles. However, they are unique because in a similar fashion to cardiac muscle, they must continually and rhythmically contract in order to sustain life (Dempsey *et al.* 1995).

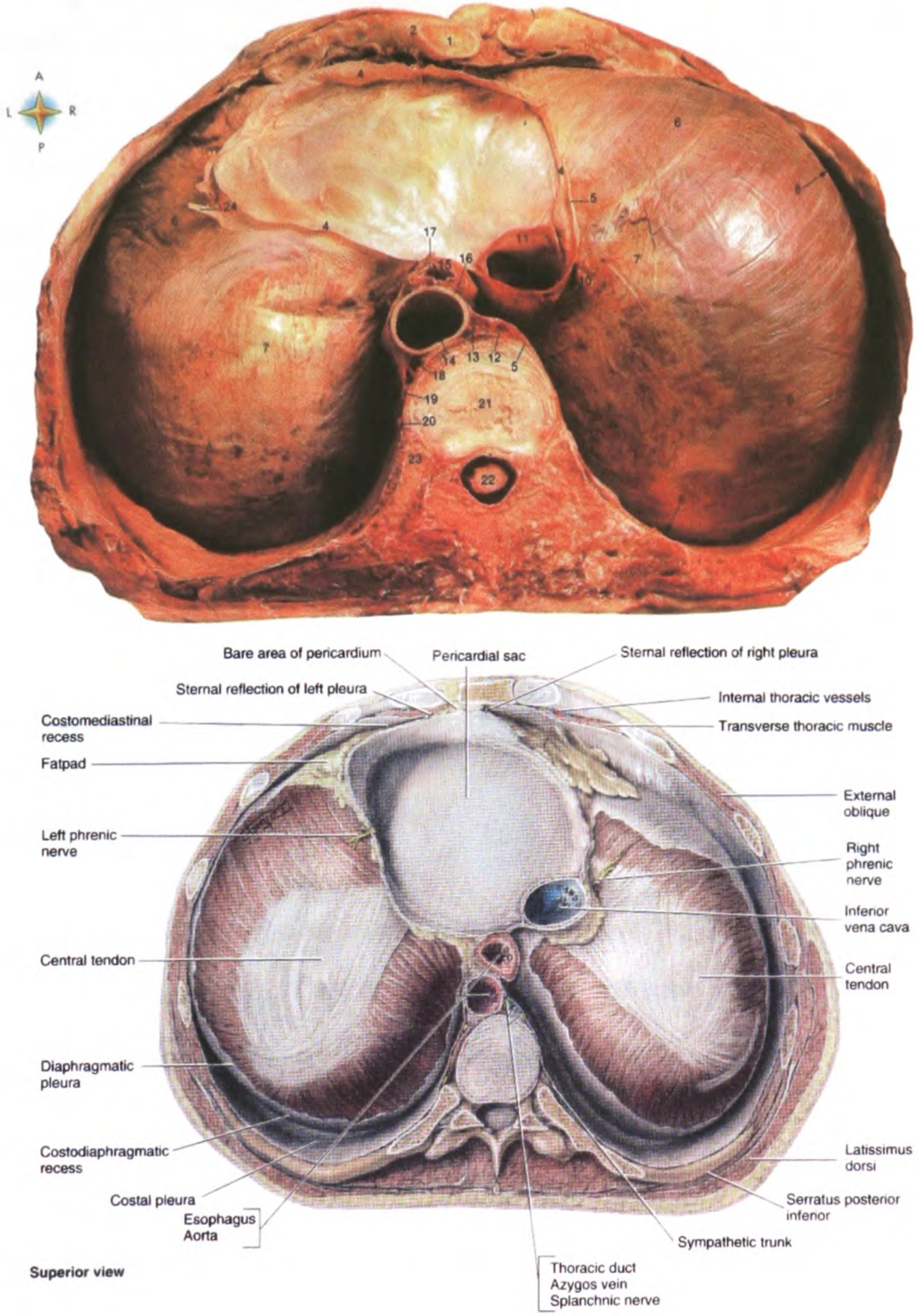
An appreciation of respiratory muscle function implies an understanding of the anatomy and structure of the chest wall muscles and the muscles that displace the chest wall. It is the purpose of this section to briefly review these muscles.

1-3.1 Respiratory Muscles

Diaphragm

Inspiration is an active process that occurs when the inspiratory muscles contract bringing about an expansion of the chest cavity (increased volume) and subatmospheric pleural pressure. The pressure change causes alveolar pressure to also become subatmospheric, which induces airflow into the lung from the atmosphere. The diaphragm is the primary muscle of inspiration; it comprises two muscles, the costal and crural portions, believed to have disparate embryological function (De Troyer *et al.* 1981). The two muscles join to a thin, sheet-like central tendon. The crural diaphragm originates at the lumbar vertebrae with the costal diaphragm attaching to the entire boundary of the thoracic aperture (Figure 1-5).

Figure 1-5 Superior view of Diaphragm. From Thibodeau and Patton (2003)



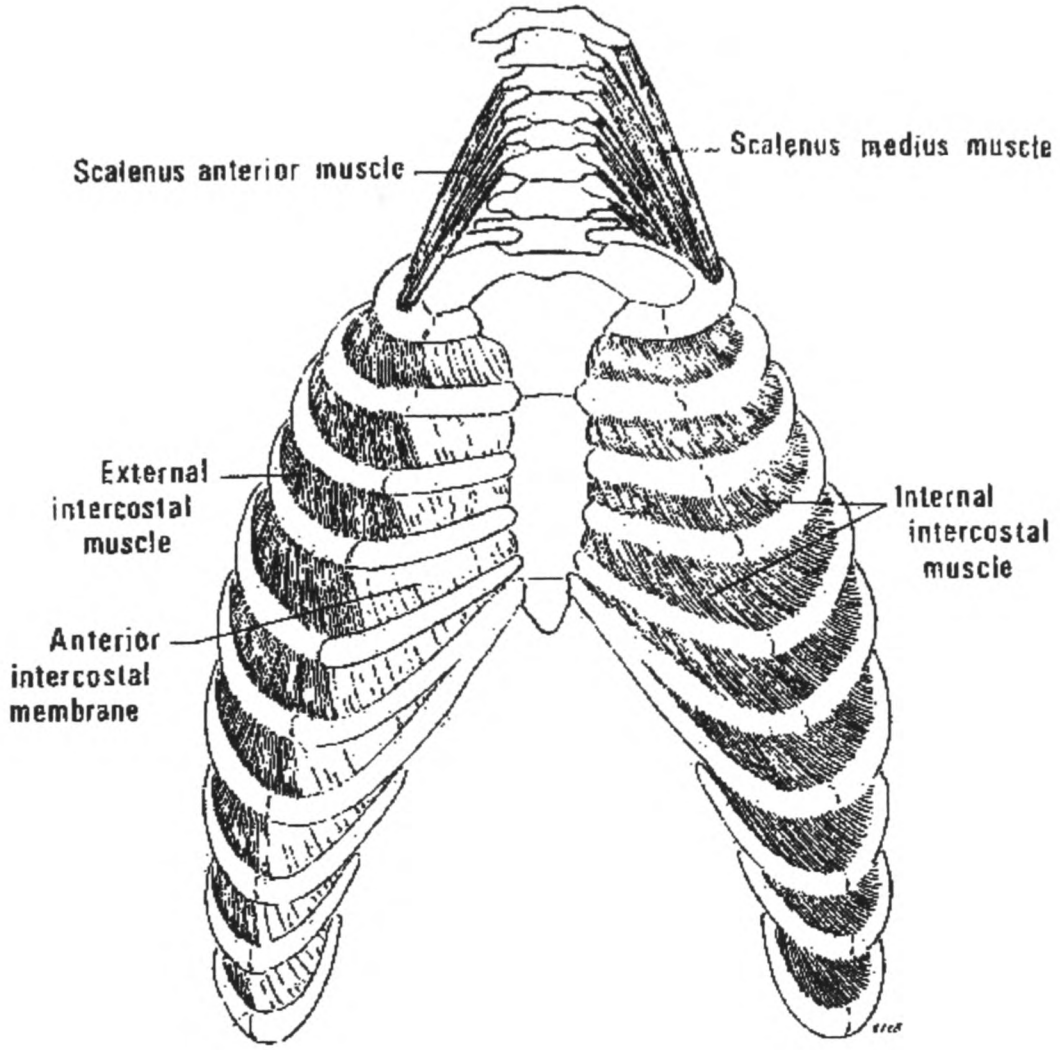
The muscle arises from the cartilage that runs from the end of the 12th rib to the 7th. From its region of attachment, the costal diaphragm ascends steeply such that it is initially in contact with the inner surface of the ribs and intercostals (zone of apposition). During quiet breathing the diaphragm is in contact with the chest wall up to the 10th rib, and it is in contact with the lungs only above that point. The muscle rises to its neutral point at the fourth intercostals space on the right and fifth on the left being opposite the tenth thoracic vertebrae. The phrenic nerves that originate from the 3rd to 5th cervical spinal segments innervate the diaphragm. The aorta, the inferior vena cava, and the oesophagus traverse the diaphragm. Upon contraction of the diaphragm, the initial effect is to draw the central tendon inferiorly from the level of T8 to T9. Further decent is resisted by compression of the abdominal viscera. At this point further contraction leads to the movement of the ribs and sternum, the action of which are described in 1-2.2. During quiet breathing the descent of the diaphragm may be as little as 1.5cm whereas in deep inspiration this may increase to 10 cm.

Intercostals

The intercostals muscles are arranged in two distinct sheets: the internal and external intercostals (Figure 1-6). They are termed external and internal because of their surface relations, the external being superficial to the internal. The external intercostals run obliquely inferiorly and anteriorly beginning lateral to the rib tubercles and extending anteriorly to their costal cartilages. In contrast, the internal intercostals run at right angles to the external intercostal muscles such that their fibres run inferiorly and posteriorly. The intercostal muscles receive afferent and efferent innervation from the anterior primary rami of the intercostal nerves (T1-11). It is

generally accepted that the external intercostal muscles are activated during inspiration, their action being to raise the inferiorly positioned rib.

Figure 1-6 Intercostal and scalene muscles. From Osmond (1995)



Scalenes

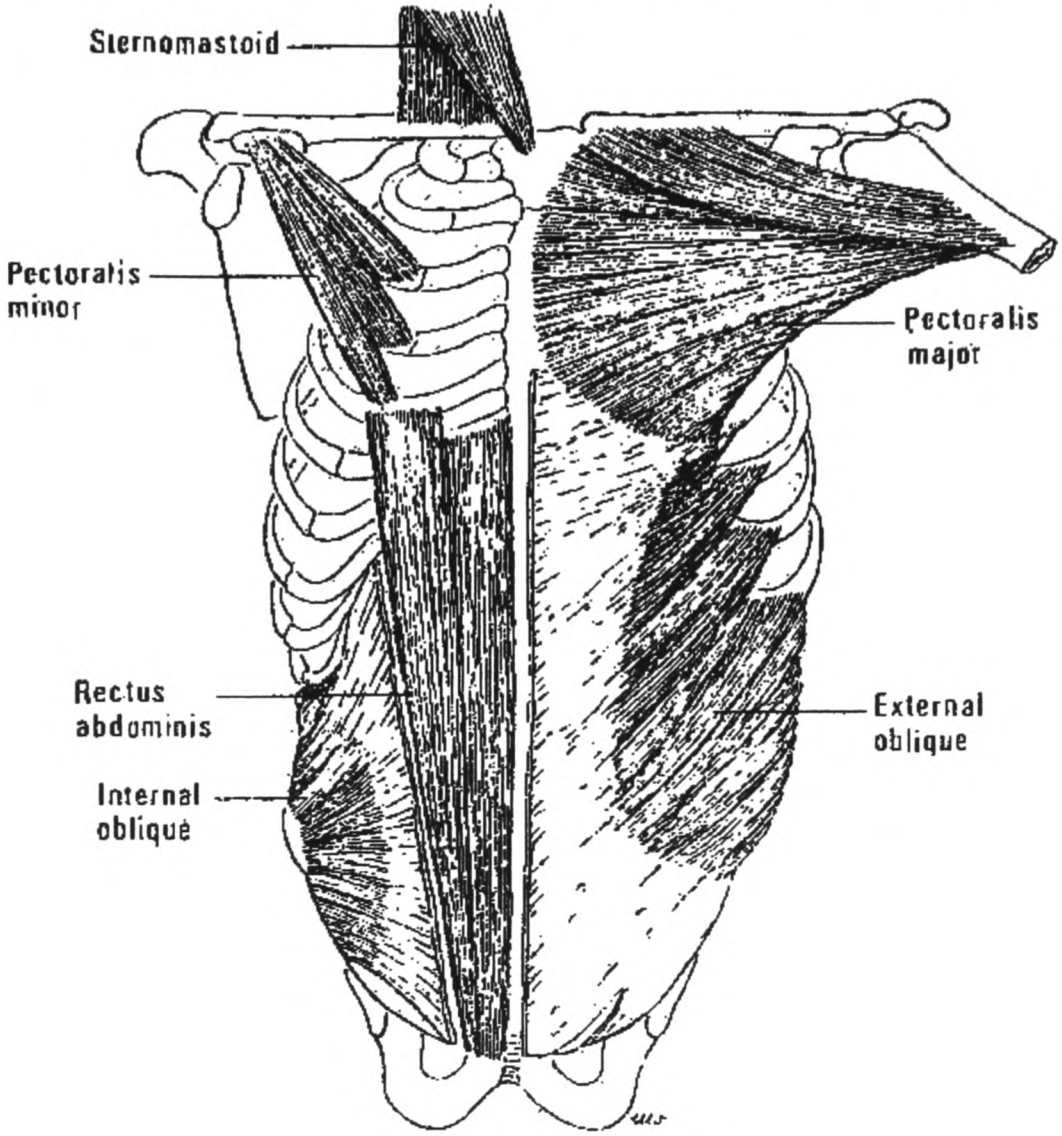
The scalenes comprise three bundles originating from the transverse processes of the lower five cervical vertebrae and inserting into the upper surface of the first two ribs (Figure 1-6). The scalenes are innervated by the ventral rami of the cervical nerves (anterior, C4-6; medius, C3-8). These muscles have traditionally been considered

"accessory" muscles of inspiration (Campbell 1955). However, more recent studies have established that the scalenes are active during inspiration at rest (Raper *et al.* 1966; Hug *et al.* 2006). Hence, these muscles are primary muscles of inspiration, and their contraction is an important determinant of the expansion of the upper rib cage during breathing. In addition, to their inspiratory action the scalenes have prominent postural functions controlling cervical flexion.

Sternocleidomastoids

The sternocleidomastoids descend from the mastoid process to the ventral surface of the manubrium sterni and the medial third of the clavicle (Figure 1-7). The motor innervation of these muscles is from the spinal accessory nerve (C1-5) and additional proprioceptive fibres from the cervical plexus (C2). At rest, the sternocleidomastoids are inactive, being recruited only when ventilation increases substantially, or when the inspiratory muscle pump is abnormally loaded (Campbell 1955; Raper *et al.* 1966).

Figure 1-7 Sternomastoid and abdominal muscles. From Osmond (1995)



Abdominals

The most important muscles of expiration are the abdominal muscles of which there are four that have significant respiratory function and constitute the ventrolateral wall of the abdomen: the rectus abdominus; the external oblique, the internal oblique; and the transverse abdominus (Figure 1-7). The external oblique arises from the external surfaces of the lower eight ribs, above the costal margin, therefore covering the lower ribs and intercostals. The rectus abdominus is the most ventral of these muscles. This

muscle is enclosed in a sheath formed by the aponeuroses of the other three muscles, the most superficial of which is the external oblique. The internal oblique lies deep to the external oblique while the transversus abdominus is the deepest of the muscles of the lateral abdominal wall. These four muscles have important functions as both rotators and flexors of the trunk and during activities such as coughing and speaking (Lim *et al.* 2007). The abdominals have primarily an expiratory function through their effect on the diaphragm and rib cage. Quiet expiration is predominantly the result of passive elastic recoil of the lungs and chest wall. However, with increased demand placed upon the respiratory system e.g. exercise, the abdominals constrict and compress the abdomen. This increases intra-abdominal pressure, helping to elevate the relaxing diaphragm, whilst simultaneously depressing the ribs to which they are attached and compressing the lower thoracic cage (Mier *et al.* 1985).

The abdominals may also reduce the work done by the inspiratory muscles, by stiffening the abdominal wall and making the abdominal contents less compliant. Diaphragmatic contraction results in an increased abdominal pressure that in turn causes outward displacement of the abdominal wall. Through abdominal muscle contraction such displacement is resisted, and due to the reduced abdominal volume, pressure is increased. This serves to increase the displacement of the chest wall during inspiration, and assists in increasing intrathoracic volume (Abraham *et al.* 2002). The interaction between thorax and abdomen in this way is often referred to as the two-compartment model. The activity of the transversus abdominis, which is the most effective in increasing abdominal pressure, is recruited well before activity can be recorded from either the rectus abdominis or the external oblique (De Troyer *et al.* 1990).

1-3.2 Respiratory Muscle Co-ordination

Although the actions of most respiratory muscles on the chest wall and the lung in humans have been qualitatively described, the amount of lung expansion (or deflation) a particular muscle can produce has not been determined (Legrand *et al.* 2003). During normal quiet breathing, approximately 50% of the active inspiratory volume change is caused by diaphragm contraction; the external intercostals and accessory muscles provide the rest (Sheel 2002). When considering the act of breathing, the action of the various muscles described in the preceding section should be considered as a whole. The action of the diaphragm can be viewed as working antagonistically against the abdomen, whereas the other inspiratory muscles act against the upper rib cage. Both muscle groups act in the same direction on the lower rib cage and the lung. If the diaphragm alone were to contract and hence move caudally, abdominal pressure would be increased, and unless simultaneous contraction of the abdominal muscles occurs, the abdomen would be displaced outwards (Abraham *et al.* 2002). The zone of apposition effectively makes the relevant part of the thorax the abdominal container. As abdominal pressure increases within the zone of apposition the effect is to displace the rib cage outwards. As thoracic volume increases then pleural pressure decreases. Due to this decrease in pleural pressure and without any activation of other inspiratory muscles the effect would be to displace the upper rib cage inwards. The diaphragm therefore has an effect of causing an inspiratory effect on the lower rib cage and an expiratory effect on the upper rib cage. In humans, the inspiratory effect is of greater magnitude (De Troyer *et al.* 1981).

If the extra-diaphragmatic inspiratory muscles alone were to contract, the upper rib cage would be displaced outwards, with the effect of reducing pleural pressure. If the

diaphragm were to remain relaxed, the decreased pleural pressure would have the effect of displacing the abdominals wall inwards. It can therefore be seen that during inspiration, in order to achieve the required decrease in pleural pressure, contraction of the diaphragm and the other inspiratory muscles must occur concurrently (Ratnovsky and Elad 2005).

Through monitoring the changes in abdominal and pleural pressures it is possible to assess the relative contributions of the diaphragm and extra-diaphragmatic muscles. Through this technique it has been shown that with increasing ventilation caused by exercise, the inspiratory rib cage muscles are recruited to a proportionately greater extent than the diaphragm (De Troyer *et al.* 1981).

Breathing pattern during exercise is a highly coordinated effort of the respiratory muscles to produce efficient gas exchange. The pattern of respiratory muscle contractions during exercise produces changes in tidal volume (V_T), end-inspiratory and end-expiratory lung volumes, inspiratory and expiratory flow rates and respiratory timing. The increase in $\dot{V}_E$ during exercise is caused by increases in both V_T and breathing frequency (B_f). In normal young individuals engaged in low-intensity exercise, both increases in V_T and B_f contribute to the rise in $\dot{V}_E$. V_T usually plateaus at 50-60% of vital capacity (Dejours 1966). During higher intensity exercise, additional increases in $\dot{V}_E$ are brought about by increases in B_f whilst V_T changes little. V_T typically increases 3- to 5-fold from rest to maximal exercise and B_f increases 1- to 3-fold, although these values are often higher for highly trained athletes. The rise in V_T is caused by an increase in end inspiratory lung volume and decrease in end expiratory lung volume. Increases in B_f are caused by a fall in both inspiratory time (T_I) and expiratory time (T_E) (Dempsey *et al.* 1995).

During rest, $\dot{V}_E$ ranges from 6 to 8 L.min^{-1} in healthy individuals and reaches values of 120 to 130 L.min^{-1} in untrained individuals during strenuous aerobic exercise. In highly trained endurance athletes $\dot{V}_E$ can exceed 200 L.min^{-1}. The type of exercise is an important determinant of the B_f, V_T and $\dot{V}_E$ responses; at equivalent rates of oxygen consumption ($\dot{V}O_2$), B_f will be higher and V_T lower for treadmill running than for cycle exercise (Henke *et al.* 1988; Rassler and Kohl 2000).

1-3.3 Respiratory Muscle Morphology

Only the diaphragm, parasternal intercostals, internal and external intercostals, scalenes, and sternocleidomastoids muscles have been morphologically examined in humans. The dominant procedure used to characterise human respiratory muscles has been histochemical staining for myofibrillar adenosine triphosphatase (ATPase) activity (Dempsey *et al.* 1995).

The Brooke and Kaiser (1970) myosin ATPase-based scheme of fibre classification can be used to separate skeletal muscle fibres into three main categories: 1) type I – slow twitch oxidative; 2) type IIa – fast-twitch oxidative glycolytic; and 3) type IIb – fast-twitch glycolytic. It should be recognised that discreet divisions between these fibre types may not exist but rather, a continuum in morphology may actually be found (Edwards and Faulkner 1995). These three categories have since been superseded by slow 1, fast 2A, fast 2X, and fast 2B, identified by the isoform of myosin heavy chain present. Slow fibres are resistant to fatigue due to their high oxidative metabolism whereas 2X and 2B fibres are easily fatigued and fast 2A fibres exhibit intermediate resistance (Polla *et al.* 2004).

Although most mammalian skeletal muscles contain a mixture of all three fibre types, the ratio of type I to type II fibres in a muscle is commonly linked with the muscle's activity pattern (Ariano *et al.* 1973). In general, most human respiratory muscle fibres contain a high percentage of type I and type IIa fibres and relatively few type IIb fibres. For example, the sum of type I and type IIa fibres in the costal diaphragm, internal intercostals, and parasternal intercostals ranges from between 77% and 99% of the total fibre pool (Mizuno 1991). The structural and functional characteristics of respiratory muscle fibres are not fixed, however, and can be modified in response to severe physiological conditions such as training, hypoxia, age related changes, and changes associated with respiratory diseases (Polla *et al.* 2004).

The number of capillaries surrounding each muscle fibre is surprisingly similar across most human skeletal muscles. However, a factor in untrained humans that distinguishes key respiratory muscles from less active locomotor muscles is the fibre cross sectional area (CSA) / capillary ratio (Dempsey *et al.* 1995). This ratio is significantly lower in the costal diaphragm compared to the vastus lateralis. The physiological significance of this observation is that this ratio provides an index of diffusion distance between capillaries and muscle fibres i.e. the lower the ratio, the shorter the diffusion distance (Mizuno 1991). In humans, this ratio is closely linked to the oxidative potential of muscle fibres and to the endurance capacity of the muscle (Saltin and Rowell 1980).

Numerous animal studies have demonstrated that the activity of oxidative (e.g. TCA cycle) enzymes in healthy sedentary rats is 35-65% greater in the costal diaphragm compared to mixed fibre locomotor muscle (i.e. plantaris) (Grinton *et al.* 1992; Powers *et al.* 1994). However, when we consider that the diaphragm is chronically active with a resting breathing rate of 70-100 breaths.min^{-1} and that a caged rat has

little opportunity to exercise the plantaris muscle, then this finding is not unexpected. Bioenergetic enzyme activity studies in humans are few, and those that do exist are measurements from patients admitted for surgery or from hospitalisation (Mizuno and Secher 1989).

In summary, the structural and morphological properties of the respiratory muscles are well suited to the unique functional requirements of respiration. These observations are consistent with the notion that the human respiratory muscles possess a high oxidative capacity and are resistant to fatigue. Despite this conclusion, evidence exists (Boutellier *et al.* 1992) for respiratory limitation during whole body exercise and section 1-6 will examine this in more detail.

1-4 Assessment of Respiratory Muscle Function

Muscles have two functions: to shorten and develop force. In the respiratory system, force is usually estimated as pressure and shortening as lung volume change or displacement of chest wall structures (Green *et al.* 2002).

Routine measurements of respiratory function i.e. volumes, flows, and indices of gas exchange, are non-specific in relation to diagnosis, but can supply indirect information about respiratory muscle performance in a clinical setting at least (Gibson *et al.* 2002). Indeed, the most frequently noted abnormality of lung volumes in patients with respiratory muscle weakness is a reduction in vital capacity (VC). However, VC has poor specificity for the diagnosis of respiratory muscle weakness and is generally less sensitive to changes than are maximum pressures (Black and Hyatt 1971).

The maximum expiratory and maximum inspiratory flow-volume curves characteristically show a reduction in those flows that are most effort dependent, i.e. maximum expiratory flow at large lung volumes (including peak expiratory flow) and maximum inspiratory flow at all lung volumes (Gibson *et al.* 2002).

When discussing respiratory muscle function then more often than not it is the strength and / or endurance capacity of these muscles that we refer to. The ability to assess these muscles is limited because of their inaccessibility. The most common methods of assessing the respiratory muscles, with a view to detecting a change in both strength and / or endurance will be the subject of this section.

1-4.1 Respiratory Muscle Strength

To test respiratory muscle properties, pressures can be measured either during voluntary manoeuvres or during involuntary contractions, notably in response to phrenic nerve stimulation (Green *et al.* 2002). In the former, the combined action of several inspiratory or expiratory muscle groups is tested, whilst in the latter, the pressure produced is specific to the contracting muscle. Table 1-1 summarises the methods of measuring respiratory muscle strength.

Figure 1-8 Typical pressure tracing during inspiratory and expiratory manoeuvres. From Green *et al.* (2002).

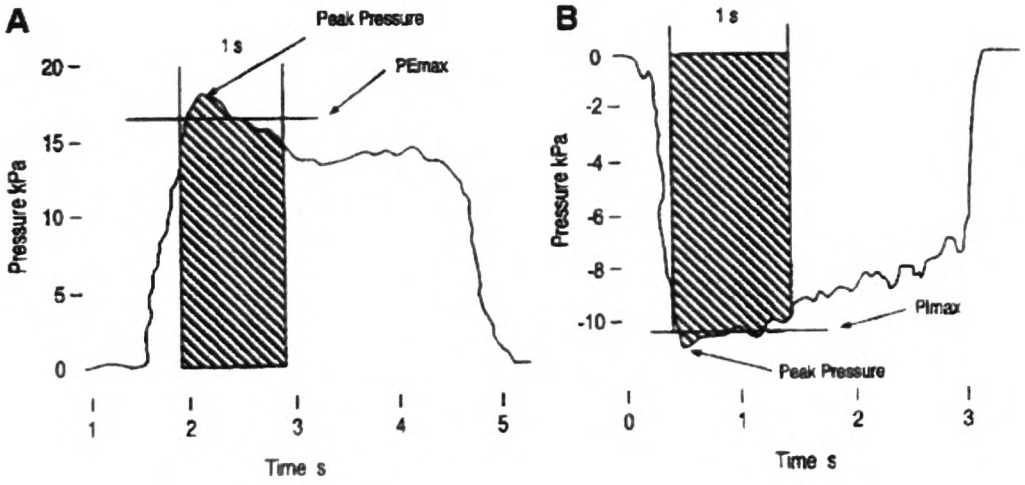
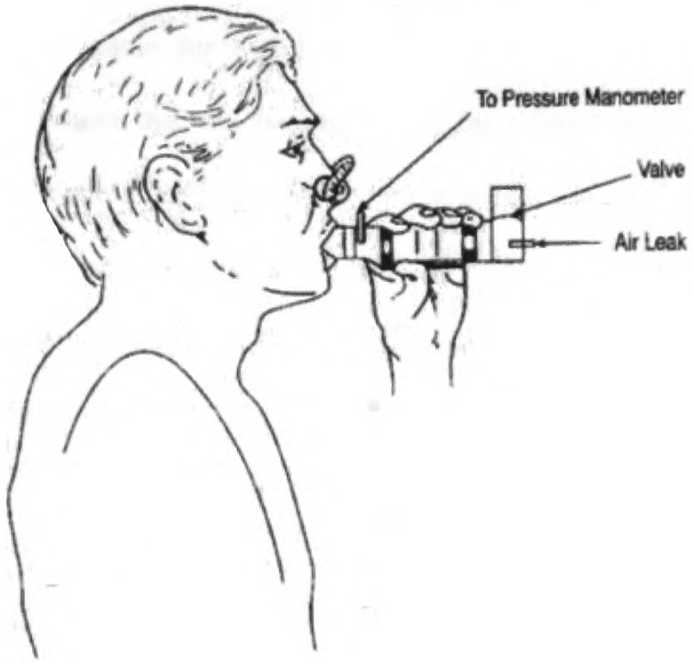


Table 1-1 Measures of Respiratory Muscle Strength

Name of test	Brief description	Comment
Maximal Static Inspiratory Pressure (MIP)	A volitional test: subjects perform a maximal inspiratory (Mueller) manoeuvre into a pressure manometer.	MIP varies with lung volume so should always be measured at RV. Careful instruction and motivation are essential. Subjects often need coaching to prevent air leaks around the mouthpiece.
Maximum static Expiratory Pressure (MEP)	A volitional test where subjects perform a maximal expiratory (Valsalva) manoeuvre into a pressure manometer.	MEP also varies with lung volume so should be read at TLC. Both MIP and MEP are taken as the best of three readings (Fig 1-8; 1-9).
Sniff tests	A short, sharp voluntary inspiratory manoeuvre performed through one or both unoccluded nostrils.	Requires less practice then MIP (Miller *et al.* 1985). However, nasal resistance could be affected by several factors i.e. allergic reactions.
Cough tests	A voluntary expiratory manoeuvre consisting of four phases: inspiratory, compressive, expulsive, relaxation (Bouros *et al.* 1995). Measured at the mouth and used to evaluate the expiratory muscles (Arora and Gall 1981).	Even though coughs, like sniffs, may be more natural manoeuvres than maximal static efforts, their superiority over conventional measurements has not been firmly established (Green *et al.* 2002).
Involuntary test of muscle strength via phrenic nerve stimulation	Phrenic nerve is stimulated either with needle, implanted wire, or transcutaneous electrical and magnetic stimulation. Force development by the diaphragm can be measured (Aubier *et al.* 1981; Hubmayr *et al.* 1989).	Motivation is not an issue with this technique. It is invasive, often using an oesophageal balloon for the measurement of transdiaphragmatic pressure (Johnson *et al.* 1993; Mador *et al.* 1993).The influence of factors such as height, race, fitness etc., is unknown.

Figure 1-9 **Measurement of maximum inspiratory and expiratory pressures. From Green *et al.* (2002).**



1-4.2 Respiratory Muscle Endurance

Muscle endurance is the ability to sustain a specific muscular task over time. It is a highly integrated and complex quality of a muscle or a group of muscles that is related to its resistance to fatigue (Clanton *et al.* 2002). The wide varieties of techniques that have been developed to measure endurance of the respiratory muscles differ largely in the type of task that is being performed. For each specific task, an endurance curve can be generated of task intensity vs. the endurance time (T_{LIM}) for which the task can be sustained prior to task failure (an event defined as the inability to continue performing the required task). At high levels of intensity, a task can be performed for only a few repetitions, but as the intensity decreases then the longer is the time to task failure. Eventually a level is reached where the task can be maintained

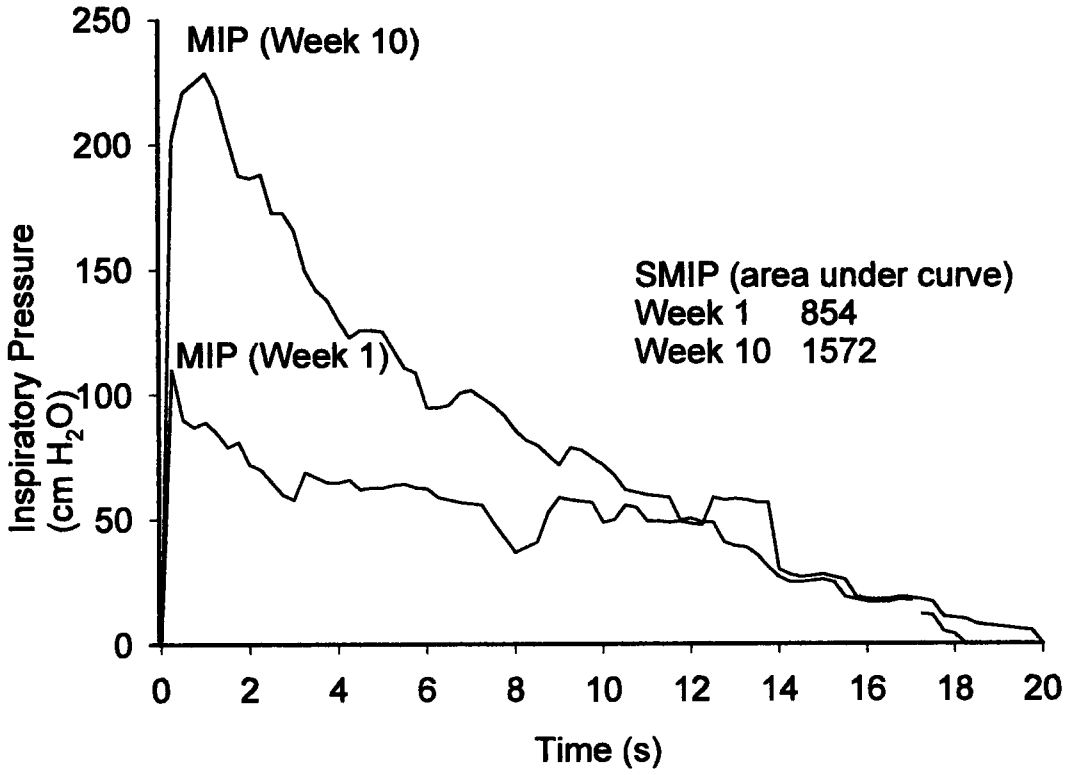
indefinitely (hours), and this is referred to as the maximum sustainable task or load. An additional estimate of endurance involves performing incremental increases in task intensity for a given time period until a peak intensity is reached, which is the maximum that can be maintained for a finite period of time. This intensity is not sustainable but may also be used to reflect endurance properties and is well tolerated by subjects (Mancini *et al.* 1994; Mancini *et al.* 1995).

Although respiratory muscle strength and endurance appear to be closely linked in many conditions, there are numerous examples in which endurance would not be accurately predicted from estimates of maximum pressures or maximum ventilatory capacity (Clanton *et al.* 2002). For example, in normal subjects following respiratory muscle training (RMT) protocols, larger relative effects are seen on endurance measures than for strength (Leith and Bradley 1976). Therefore, measurements of endurance capacity can be useful in some clinical and investigative settings. Table 1-2 summarises these tests.

Name of test	Brief description	Comment
Maximum Voluntary Ventilation (MVV)	Measures the capacity of the subject to ventilate the lungs as fast and as deeply as possible.	Reflects the combined global work capacity of the respiratory musculature against the inherent impedance of the lungs and chest wall. Measurements are difficult to interpret but useful for overall assessment of the respiratory muscles during exercise or high levels of metabolic activity.
Maximum sustainable ventilation (MSV)	Measured as a percentage of MVV. Starting at 20% MVV, the target ventilation is increased by 10% every 3 min until the subject can no longer maintain the target ventilation (Mancini *et al.* 1995).	Advantage: Closely resembles the task performed during exercise. Disadvantage: difficult to estimate the relative contribution of lung and chest wall mechanics to the measure. Measurement are highly susceptible to changes in flow resistance (Roussos and Campbell 1986). Load on the Respiratory muscles is not uniform across subjects or even in the same subject over time (Clanton *et al.* 2002).
Maximum sustainable threshold loading	Nickerson and Keens (1982) developed this method in which endurance times are measured in response to gradually decreasing threshold pressures, starting near MIP. The first pressure that can be sustained for over 10 min is considered the sustainable inspiratory pressure.	The test is non-invasive and tolerated well. Subject do adjust their breathing pattern as they breathe against any kind of mechanical load, and they will learn to do this over time (Belman *et al.* 1986). Approximately 2 h is required to establish sustainable inspiratory pressure using the Nickerson and Keens (1982) method.
Maximum incremental threshold loading	First described in 1987 (Martyn *et al.* 1987) his method was designed to resemble a Bruce protocol. Subjects inspire from a threshold valve ~30-40% of MIP. A unit of weight is added increasing the pressure by 5-10% of MIP, until the load cannot be tolerated for 2 min. The maximum pressure that can be tolerated for the full 2 min is considered the peak pressure (Morrison *et al.* 1989)	This technique has generally been applied to the measure of inspiratory muscle endurance. Limited normal values have been described using this technique, however it is not clear to what extent the test reflects respiratory muscle strength rather than endurance (Clanton *et al.* 2002).

Name of test	Brief description	Comment
Maximal sustainable isoflow	Subjects inspire with MIP against a device that provides a constant inspiratory flow rate to lungs (Clanton and Ameredes 1988). Airflow is humidified and end tidal carbon dioxide levels ($P_{ET}CO_2$) are maintained. Subjects inspire maximally for 10 min. Airway opening pressures decline Exponentially during this period until a sustainable pressure is obtained.	Using curve fitting techniques, it has been shown that sustainable pressures in normal subjects can be calculated within 5% with only 5 min of testing. Normal values have not been described over a wide range of subjects. The test is non-invasive and is tolerated well naïve subjects. Cannot be certain of The relative contributions of the rib cage or the diaphragm during contractions. Equipment to conduct this test is not commercially available and would be expensive to construct.
Repeated maximal inspiratory pressures	McKenzie and Gandevia (1986) developed a technique that uses 18 repeated MIP manoeuvres lasting 10s each, with 5s of rest between contractions.	In normal young subjects, the average inspiratory mouth pressure attained in the last contraction is 87 ± 3% of MIP (McKenzie and Gandevia 1986). This technique provides a measurement that is entirely independent of chest wall mechanics, as well as mechanical work of breathing, making it potentially useful for understanding endurance properties of the respiratory muscles without interference from chest wall or lung mechanics (Clanton 2002).
Test of Incremental Respiratory Endurance (TIRE)	Chatham *et al.* (1995) extended the procedures of Gandevia *et al.* (1983) to include a series of repeated inspiratory efforts at 80% of maximum sustained inspiratory pressure (Fig 1-10). Rest periods start at 60s and decrease every 6 manoeuvres to 45s, 30s, 15s, 10s, and 5s. The test is terminated when the subject can no longer maintain the target load.	This method generates a number of parameters that can be used to appraise endurance performance, including the number of inspirations to task failure or accumulated area generated during the test (reported pressure-time units). Little data exists regarding normal values for this test, but its usefulness as both a method of measuring endurance performance and of training the respiratory muscles remains to be investigated in the present study.

Fignre 1-10 TIRE Template



In the test of incremental respiratory endurance (TIRE) subjects inspire maximally against resistance from residual volume (RV) through the full lung volume to total lung capacity (TLC). MIP is the peak pressure reached and SMIP is the area under the curve as recorded by the connected computer. Figure 1-10 illustrates the use of TIRE as a measure of respiratory endurance and its capacity to be used as a training intervention. The figure shows the training template for one subject at week 1 and following a nine week training intervention (Gething *et al.* 2004).

1-5 The Respiratory Muscles as a Limiting Factor During Exercise

Bye *et al.* (1983) discussed several possibilities for how respiratory factors may limit exercise. Limitations may arise in terms of gas exchange, respiratory mechanics, energetics of the respiratory muscles, or because of the development of respiratory muscle fatigue. This section will focus on how respiratory muscle fatigue develops and the consequences of this happening.

1-5.1 Evidence of Respiratory Muscle Fatigue Post Exercise

Fatigue is defined as a loss of the capacity to generate skeletal muscle force and / or velocity that is accompanied by recovery during rest (NHLBIWorkshop 1990). Therefore, a single measurement of force is inadequate to detect fatigue. The muscle force generating or shortening capability must be demonstrated to fall during serial measurements over time (Supinski *et al.* 2002). Roussos and Macklem (1977) demonstrated that the diaphragm exhibits fatigue in a fashion similar to that which would be expected of any other skeletal muscle. Furthermore, several studies have presented evidence that respiratory muscles may fatigue during exercise (whole body). Shephard (1967) observed that the maximal voluntary ventilation (MVV) performed after 20 min of exercise at a workload of 80% of $\dot{V}O_2max$ was significantly less than that after 5 min of exercise, implying that respiratory muscle fatigue may occur. Warren *et al.* (1989) noted a 17% fall in the 12 s MVV in ultramarathon runners, but only after 24 h of running. Further evidence for respiratory muscle fatigue during exercise was presented by Mahler and Loke (1981) and Loke *et*

al. (1982), who demonstrated a decrease in vital capacity as well as decreases in maximal inspiratory and expiratory pressure development after a marathon. More recently, fatigue of the respiratory system, as measured by breathing endurance, was equivalently reduced after exhaustive cycling at either 65, 75, 85, or 95% $\dot{V}O_2$peak (Perret *et al.* 2000). In contrast, several other studies have not demonstrated alterations in volitional measurements of ventilatory performance after exercise (Anholm *et al.* 1989; Nava *et al.* 1992). A more objective measure, bilateral phrenic nerve stimulation (BPNS) has also been utilised to examine the existence of diaphragmatic muscle fatigue following exercise. Applying this technique to endurance exercise to exhaustion at a fixed work rate has revealed some interesting results. Johnson *et al.* (1993) tested relatively fit subjects who were able to maintain 85% and 95% of $\dot{V}O_2$max for 30 and 14 min respectively, while Mador *et al.* (1993) tested relatively unfit subjects who were only able to maintain 80% $\dot{V}O_2$max for 8 min. A similar average reduction in the diaphragmatic pressure produced was observed between studies at the lower exercise loads despite the marked difference in exercise duration, while subjects exercising at the higher intensity averaged a 60% greater reduction in the pressure produced (Johnson *et al.* 1993; Mador *et al.* 1993). These studies were the first to demonstrate objectively that diaphragm muscle is susceptible to exercise-induced fatigue, especially when exercise intensity progressed beyond 85% of $\dot{V}O_2$max and was of the endurance type.

Although, these studies concentrate mainly on fatigue of the diaphragm, the main inspiratory muscle, potential fatigue of the accessory muscles should also be considered, as well as the expiratory muscles, since these muscles also come into play during heavy exercise. For instance, Johnson *et al.* (1993) demonstrated that the relative contribution of the diaphragm to total respiratory output is progressively

reduced with exercise duration, indicating an increasing activity of extra diaphragmatic muscles. As far as the expiratory muscles are concerned, Fuller *et al.* (1996) demonstrated that the ability to voluntary maximally activate abdominal expiratory muscles and to generate maximum expiratory pressures is impaired after exhaustive exercise. Moreover, after only 2 min of maximal isocapnic ventilation, the force-generating capacity of abdominal muscles is reduced for >90 min (Kyroussis *et al.* 1996), which indicates that the reduced ability to maximally activate expiratory muscles after exhaustive exercise is likely to be caused by muscular fatigue. Since respiratory muscles other than the diaphragm become increasingly active during exercise, it is also likely that fatigue of those muscles can contribute to exercise limitation.

1-5.2 Consequence of Respiratory Muscle Fatigue

It is unlikely that expiratory muscle fatigue would translate into reduced exercise performance. The expiratory gastric pressures generated during dynamic exercise are less than those observed during voluntary expiratory manoeuvres (Dempsey *et al.* 1990). Moreover, the expiratory flow rate becomes maximal and independent of effort at levels of gastric pressure that are well below the maximum attainable values (Johnson *et al.* 1992); any additional effort would therefore be wasted. Thus, although expiration during heavy exercise is an active process, the intensity of the expiratory efforts is probably not sufficient to impair expiratory airflow, overall pulmonary ventilation, or exercise performance.

Potentially, inspiratory muscle fatigue might limit exercise performance via alveolar hypoventilation or by an increased sensation of dyspnoea. Alveolar hypoventilation

may occur as a result of the inspiratory muscles not being able to generate the required pressures or when an altered breathing pattern, such as the tachypneic pattern sometimes associated with inspiratory muscle fatigue, occurs (Romer 2001). It is unlikely that alveolar hypoventilation contributes to exercise limitation because in the previously cited studies documenting inspiratory muscle fatigue, ventilation was generally appropriate for the given metabolic demand. However, as previously noted, diaphragm pressure production tends to plateau (or occasionally fall slightly) beyond the initial few minutes (~6-10 min) of strenuous exercise (>85% $\dot{V}O_2max$), even though ventilation and total inspiratory muscle pressure continue to rise throughout exercise (Johnson *et al.* 1993; Babcock *et al.* 1995; Babcock *et al.* 1996). Therefore, the diaphragm contributes less to total inspiratory muscle work as exercise continues. Whether this is the result of the observed diaphragm fatigue or simply a normal muscle recruitment strategy by the inspiratory system remains to be determined (Johnson *et al.* 1996). Nevertheless, this increasing use of accessory respiratory muscles as exercise continues might be a mechanically inefficient means of producing ventilation because of chest wall distortion, which might in turn translate into a higher cost of breathing and command a higher blood flow to the chest wall muscles (Ward and Macklem 1995).

The consequence of respiratory muscle fatigue has been investigated after subjects have voluntarily fatigued their respiratory muscles. Martin *et al.* (1982) had their subjects breathe at 60% of MVV for 150 min. After this huge ventilatory work, the subjects running time at high speed was significantly reduced from 7.6 to 6.5 min. Also, respiratory muscle fatigue induced by breathing with a threshold inspiratory load of ~80% of the MIP compromised endurance time of a subsequent cycling test

(Mador and Acevedo 1991). At the same time, minute ventilation and breathing frequency was increased during exercise (Mador and Acevedo 1991).

However, studies of similar design have not shown these significant effects of fatiguing respiratory efforts (Dodd *et al.* 1989; Nava *et al.* 1992). This discrepancy has not been adequately explained, but may be related to the level of fatigue induced in the respective studies or by the rate of recovery from the fatigue. The level of fatigue induced by the voluntary tasks used in the studies mentioned above is likely to be much greater than that which follows exercise. Therefore, it is still unresolved whether the diaphragmatic fatigue measured after exhaustive exercise tests are large enough to impair endurance performance.

1-5.3 Unloading the Work of Breathing During Exercise

An alternative method to investigate the role of respiratory muscle work, and hence development of fatigue, on exercise performance is to unload the work of breathing during exercise. This has been achieved in the past through either assisting the subjects breathing with a ventilator or by having subjects breathe reduced viscosity gas mixtures (helium / oxygen).

Aaron *et al.* (1985) examined the influence of helium breathing (79% He, 21% O_2) on rowers exercising at 80 and 90-95% $\dot{V}O_2$max to volitional exertion. These authors concluded that respiratory muscle fatigue and / or the respiratory load played a significant role in limiting human performance at exercise intensities >90-95% $\dot{V}O_2$max.

More recent studies have used pressure-assist to unload the respiratory muscles (Gallagher and Younes 1989; Marciniuk *et al.* 1994; Krishnan *et al.* 1996; Harms *et*

al. 2000). Several of these studies have shown no benefit of respiratory muscle unloading upon constant-load exercise at ~70-80% $\dot{V}O_2$max despite significant reductions (~20-40% less than control) in respiratory loads during exercise (Gallagher and Younes 1989; Marciniuk *et al.* 1994; Krishnan *et al.* 1996). In contrast, Harms *et al.* (2000) recently reported an ~14% improvement in time to exhaustion with respiratory muscle unloading and ~15% decrease with respiratory muscle loading compared to control conditions in seven male cyclists exercising at a constant work rate of 90% $\dot{V}O_2$max.

Collectively, these results suggest no effect on exercise time if subjects were studied at <80% Wmax and significant increases in exercise time at intensities >90% $\dot{V}O_2$max, independent of the method used (Aaron *et al.* 1985; Harms *et al.* 2000). However, these two methods may have effects other than unloading the work of breathing. For example, helium can increase the flow-volume loop in responsive individuals, increasing the breathing reserve and potential ventilatory capacity. Alternatively, it may alter the perception of load rather than actually preventing respiratory muscle fatigue. A further concern is that helium may act by unloading the expiratory apparatus, which often provides a small but significant resistance to breathing, rather than by having its major influence on the lungs and upper airways (Johnson *et al.* 1996). In addition, a ventilator may also alter respiratory sensations and normal breathing mechanics, and change the distribution of blood flow.

A more "natural" approach to testing for respiratory limitation during exercise is to specifically train the respiratory muscles. If this training can increase exercise performance / capacity then it is more likely that exercise was limited by the respiratory system before the specific training and is the focus of section 1-6.

1-6 Respiratory Muscle Training

Investigators studying the effects of training on respiratory muscles have used either whole-body endurance exercise training or specific respiratory muscle training as a means of providing a training stimulus.

Most of the literature considering the effects of whole body endurance exercise has focused upon rodents (Powers and Criswell 1996). Endurance training results in an increased oxidative capacity of the costal diaphragm through increased activity of various enzymes (citrate synthase, succinate dehydrogenase) (Powers *et al.* 1992). Furthermore, endurance training appears to decrease the cross-sectional area of the costal diaphragmatic fibres with no change in the capillary / fibre ratio. This suggests that an increase in the perfusion of the diaphragm was elicited by the training (Sharpe 1999). Although such data is of interest, extrapolation of the findings to the human condition should be made with caution.

Studies examining the effects of whole-body exercise on human respiratory muscle strength and endurance have involved healthy subjects as well as patients with obstructive disease (Dempsey *et al.* 1995). The literature suggests that whole-body endurance exercise training results in improved ventilatory muscle performance as evidenced by increases in the maximal sustainable ventilation and maximum voluntary ventilation (Clanton *et al.* 1987). Furthermore, studies comparing ventilatory performance of highly trained subjects with that of untrained subjects reveal that endurance training improves the ability of respiratory muscles to maintain a high-power output (Coast *et al.* 1990).

It appears that exercise hyperpnoea (through whole-body endurance exercise) provides a strong enough training stimulus to the respiratory muscles to elicit a

general training effect (Powers *et al.* 1997). Although whole-body exercise may enhance respiratory muscle performance, it should be noted that this training effect is not necessarily optimal.

1-6.1 Modes of Training

Respiratory muscle training interventions can be very broadly classified in one of two modes of training: 1) voluntary isocapnic hyperpnoea (VIH) to improve respiratory muscle endurance; 2) inspiratory resistive loading (IRL) to improve respiratory muscle strength (Sheel 2002).

Voluntary Isocapnic Hyperpnoea (VIH)

VIH is accomplished by having subjects maintain high target levels of ventilation for varying periods of up to 30 min. Training sessions are generally conducted 3-5 times / week at ~70-90% MVV and the training effect is evaluated by monitoring the change in the time to volitional exhaustion during either sustained or incremental ventilation. In order to prevent lowering of $PaCO_2$, subjects may simply re-breathe through a dead space or employ more elaborate apparatus that supply supplemental oxygen to avoid hypoxaemia, while at the same time keeping $PaCO_2$ constant (Leith and Bradley 1976). Both healthy subjects and patients with airway disease appear to show improvement in respiratory muscle endurance with this type of training (Leith and Bradley 1976; Morgan *et al.* 1987; Belman and Gaesser 1988; Fairbarn *et al.* 1991; Boutellier *et al.* 1992; Boutellier and Piwko 1992; O'Kroy and Coast 1993).

Inspiratory Resistive Loading (IRL)

During IRL training, loads are applied to inspiratory muscles three to five times per week for a duration of up to 30 min with some regimes. The training effect is evaluated as the increase in time that a fixed resistive load can be tolerated or the increase in the maximum resistance that can be produced i.e. MIP.

The load to which the inspiratory muscles are exposed can be brought about in a number of ways. Flow resistive loading requires individuals to inspire via a variable diameter orifice whereby, for a given flow rate, the smaller the orifice the greater the resistive load. Although flow resistive loading appears to improve respiratory muscle function (Aldrich and Karpel 1985; Clanton *et al.* 1985), the findings should be interpreted with caution. As indicated above, inspiratory pressure, and therefore training load, varies with flow rate and not just orifice size (Pardy *et al.* 1988). Therefore, it is important that breathing pattern is monitored during this mode of training if a quantifiable training stimulus is to be provided.

The *test of incremental respiratory endurance* (TIRE) is a flow resistive device which does exactly that (Chatham *et al.* 1995). The TIRE system utilises an electronic manometer attached via a serial interface to a computer and dedicated software (See Section 1-4.2). Initially, the subject performs several sustained maximal inspiratory efforts to provide a baseline pressure-time profile. The subject is then presented with a pressure-time profile typically set at 80% of the maximal effort. The inspiratory manoeuvre is then repeated with an ever-decreasing rest period with 6 efforts and 60, 45, 30, 15, 10, and finally 5 s rests. The exercise is terminated when the subject either completes the full range of breathing exercises or falls beneath the reference pressure-

time profile. This technique can be applied to a wide range of subject populations because the respiratory work is fixed in direct relation to individual capacity.

Pressure threshold loading requires individuals to produce a negative pressure sufficient to overcome a threshold load and thereby initiate inspiration. Threshold loading permits variable loading at a quantifiable intensity by providing near flow independent resistance to inspiration (Romer 2001). This has been achieved in a number of ways: weighted plunger (Nickerson and Keens 1982; Clanton *et al.* 1985); and a spring-loaded poppet valve (Caine and McConnell 2000). Threshold training can be utilised effectively without regulating breathing pattern or gas exchange.

Elastic loading utilises a procedure requiring strapping of the rib cage or abdomen. This method typically reduces V_T and T_I such that B_f increases with $\dot{V}_E$ often being maintained (Daubenspeck 1995). Whilst elastic loading appears attractive due to its simplicity of use, it is generally difficult to standardise and quantify the additional work imposed on breathing. To date, this loading device has not been used to specifically train the inspiratory muscles.

1-6.2 Specific Respiratory Muscle Training

Perhaps the first investigators to conclusively show that the respiratory muscles are responsive to specific training were Leith and Bradley (1976). The authors found that both the strength and the endurance properties of the inspiratory muscles could be significantly increased after a period of 5 weeks of specific training. A purely strength training regime was performed by one subject group, and consisted of maximum static inspiratory and expiratory efforts. These were held for 3-5 s at 20% intervals

over vital capacity (VC) for 30 min daily (IRL). An endurance-training program was performed by a separate group, which comprised voluntary isocapnic hyperpnoea (VIH) for around 30-45 min daily. The strength-training group improved maximal inspiratory pressures (MIP) by 55% whereas their ventilatory endurance remained unaltered. The endurance-training group increased their 15 s MVV by 14%, along with increasing the fraction of MVV, which could be sustained for 15 min from 81% to 96%. Endurance training had no effect on MIP (Leith and Bradley 1976).

Although a learning effect may account for some of the changes outlined above, it appears that the inspiratory muscles exhibit training-induced increases in functionality, and further that these changes are large and occur swiftly. It is interesting that no cross-over effect was observed between strength improvements and endurance capacity or vice versa. Presumably, quite different morphological and / or neurological changes must occur in order to elicit improvements in strength and endurance.

In a follow-up study, Bradley and Leith (1978) examined the oxygen cost of maximum sustainable ventilation prior to and following programmes of endurance and strength training similar to those outlined above. Following endurance training it was again found that maximum sustainable ventilation had increased by around 19% and this extra ventilatory capacity incurred an additional oxygen cost of 67%. Only small changes were observed in ventilatory endurance and oxygen cost following strength training. These results support the notion that ventilatory endurance is increased by muscular adaptations, which allow a greater metabolic rate to be achieved.

More recently, the specificity of respiratory muscle training adaptations was also demonstrated by O'Kroy and Coast (1993) and Wells *et al.* (2005). The authors found

that inspiratory muscle strength training (IRL) resulted in significant increases in MIP but not in endurance. Conversely, those subjects trained specifically for endurance showed significant increases in maximum sustainable voluntary ventilation with no increase in MIP.

It is quite clear that the respiratory muscles show adaptive responses to training, seen both as improvements in strength and endurance. The question of whether these improvements alone, independent of any other type of physical training, have any influence on human whole body exercise performance has been the focus of numerous studies (Sheel 2002) (Table 1-3).

A review of the literature reveals that the effects of respiratory muscle training, whether it is VIH or IRL, on exercise performance are controversial. The reason for much of the controversy surrounds the fact that studies have used different RMT regimens, different laboratory tests of exercise performance / capacity, and differences in the training status of individuals.

Of the 23 out of 27 studies listed in Table 1-3 that measured performance 18 showed statistically significant improvements. In the majority of these studies, performance was determined using a fixed work rate i.e. submaximal percentage of $\dot{V}O_2$peak or maximal power output (Wmax). The reported performance improvements ranged from 24 to 50%. While these improvements are seemingly large, it is important to note that the variation common to these type of trials is large (Jeukendrup *et al.* 1996). In addition, the only study that used a true placebo group, a 16% improvement was seen in the placebo group compared with a 26% improvement in the respiratory muscle trained individuals (Sonetti *et al.* 2001). In those studies that demonstrated an improvement in performance, the fixed work-rate ranged from 64% to 85% of $\dot{V}O_2$peak or Wmax (Boutellier *et al.* 1992; Boutellier and Piwko 1992; Spengler *et al.*

1999; Markov *et al.* 2001; Stuessi *et al.* 2001; McMahon *et al.* 2002; Guenette *et al.* 2006; Leddy *et al.* 2007). In the remaining studies which did not show improvements, the work rate was near maximal i.e. 85% to 95% of $\dot{V}O_2$peak or Wmax (Morgan *et al.* 1987; Fairbarn *et al.* 1991; Sonetti *et al.* 2001; Williams *et al.* 2002; Downey *et al.* 2007; Verges *et al.* 2007). This remains a curious observation, as at intensities above 85% $\dot{V}O_2$peak diaphragm fatigue has been observed (see Section 1-6.1) and there may be an inter-relationship between respiratory muscle training, diaphragm fatigue, recruitment of accessory respiratory muscles and exercise intensity (Sheel 2002).

From the limited work completed to date it would appear that respiratory muscle training can improve time to volitional exhaustion at fixed-rate submaximal workloads (although this is not a completely consistent finding) and not at near-maximal exercise intensities. Controversy surrounds the value of fixed work-rate laboratory tests for two reasons. Firstly, these tests are not true measures of athletic performance, as they do not mimic competitive situations. Athletes often change velocity for strategic or environmental reasons i.e. hill, wind etc. Secondly, those studies which evaluated performance using fixed work-rate type performance tests are reported to have poor reliability (McLellan *et al.* 1995). The coefficient of variation for this type of trial vary from 17 to 40% (Jeukendrup *et al.* 1996) and Hinckson and Hopkins (2005) demonstrated that a longer trial results in greater variation.

Boutellier (1998) suggests two reasons for using submaximal constant workloads instead of high intensity exercise. First, the literature e.g. Dempsey (1986) provides ample evidence that respiration does not limit $\dot{V}O_2$max. Second, short lasting, maximal exercise hardly applies to daily life situations in contrast to long lasting, more moderate intensities of exercise (endurance exercise). In addition, subjects set their own pace in time trials, usually increasing the pace toward the end (Schabort *et*

al. 1998). A more even pace may be optimal for longer endurance (Foster *et al.* 1994) and the simple fact that so many studies have used fixed work-rate tests to exhaustion as an outcome measure in the past suggest that a new study conducted within this interesting area should also incorporate this type of test into the study design in order to compare the new results to the old.

Table 1-3 Summary of reported changes in exercise performance / capacity following specific respiratory muscle training

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
Leith & Bradley (1976)	Untrained: n = 4 E1, strength	E1: static inspiration and expiration at 20% intervals over vital capacity, 30 min/day, 5 d/week, 5wk	E1: no change breathing endurance	Exercise not performed
	n = 4 E2, endurance	E2: ventilate to exhaustion, 3-5 × 5 d/wk, 5wk	E2: +19% breathing endurance*	Exercise not performed
	n = 4 C	C: no RM training	C: no change breathing endurance	Exercise not performed
Morgan *et al.* (1987)	Trained: n = 4 E	E: 85% MVV increasing duration, 5 d/wk, 3wk	E: +14% MVV*, +1575% endurance breathing time*	E: -6% (NS) cycling time to exhaustion at 95% $\dot{V}O_2peak$
	n = 5 C	C: no RM training	C: 0% MVV (NS), 0% endurance breathing time (NS)	C: -8% (NS) cycling time to exhaustion at 95% $\dot{V}O_2peak$
Hanel and Secher (1991)	Untrained: n = 10 E	E: 50% MIP, 10 min 2/d, 27.5d	E: +10% MIP*	E: +8% (NS) Cooper's 12 min run test
	n = 10 C	C: 0% MIP, 10 min, 2/d, 27.5d	C: 4% MIP (NS)	C: +6% (NS) Cooper's 12 min run test
Fairbarn *et al.* (1991)	Trained: n = 5 E	E: >MSVC 3 sessions of 8 min, 3-4 d/wk, 4 wk	E: +12% breathing endurance*	E: +25% (NS) cycling to exhaustion at 90% Wmax
	n = 5 C	C: no RM training	C: -4% breathing endurance (NS)	C: +4% (NS) cycling to exhaustion at 90% Wmax

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
Boutellier and Piwko (1992)	Untrained: n = 4	58-63% MVV 20-30 min, 5d/wk, 4wk	+268% breathing endurance*	+50% (*) increase in cycling to exhaustion at 64% $\dot{V}O_2$peak
Boutellier *et al.* (1992)	Trained: n = 8	55-68% MVV 30 min, 5d/wk, 4wk	+555% breathing endurance*	+38% (*) cycling to exhaustion at 77% $\dot{V}O_2$peak
Suzuki *et al.* (1993)	Untrained: n = 6 E	E: 30% MIP, 15 min, 2/d, 4/wk	E: MIP +30%*, MVV +12%*	Performance not measured. No change in $\dot{V}_E$ or respiratory sensation at any stage of exercise during incremental treadmill test.
	n = 6 C	C: no RM training	C: MIP 0% (NS), MVV +4.8% (NS)	
Spengler *et al.* (1999)	Trained, n = 20	60-85% MVV, 30 min, 5 d/wk, 4wk	+532% breathing endurance*	+28% (*) cycling to exhaustion at 85% Wmax.
Inbar *et al.* (2000)	Trained, n = 10 E	E: 30-80% MIP, 30 min, 6 d/wk, 10wks	E: +25% MIP* and +27% breathing endurance*	No change in $\dot{V}O_2$peak, or $\dot{V}_{E\ MAX}$. Performance not measured
	n = 10 C	C: Same training, no resistance	C: +1% MIP (NS) and +1% breathing endurance (NS)	
Stuessi *et al.* (2001)	Untrained, n = 13 E	E: 40 (15 wk) sessions of 30 min 65-70% MVV	E: +632% breathing endurance*	E: +24% (*) cycling to exhaustion at 70% Wmax.
	n = 13 C	C: no RM training	C: -2% breathing endurance (NS)	C: -4% (NS)

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
Sonetti *et al.* (2001)	Trained, n = 9 E	E: 5 wk, 5 d/wk, 30 min/d, 50-60% MVV and 4-5 min 50% MIP.	E: +8% MIP (*), no change in 15 s MVV, no change breathing Endurance	E: +1.8% (*) 8km time trial (cycling), +26% (*) cycling to exhaustion at 80-85% Wmax
	n = 8 C	C: 5wk, 5 d/wk, 30 min/d, Placebo.	C: +3.7% MIP (NS), no change In 15 s MVV, no change in Breathing endurance	C: -0.3% (NS) 8 km time trial, +16% (*) 80-85% Wmax.
Volianitis *et al.* (2001)	Trained, n = 7 E,	E: 50% MIP, 30 breaths, 2 × day, 7d/wk, 11 wk.	E: +45% MIP*	E: +4% (*) 6 min all out Rowing.
	n = 7 C	C: 15% MIP, 60 breaths, 1 × day, 11 wk	C: +5% MIP (NS)	C: +2% (NS)
Markov *et al.* (2001)	Untrained, n = 13 E	E: 60% MVV, 30 min, 40 sessions over 15 wk	E: +770% breathing endurance*	E: +24% (*) cycling to exhaustion at 70% Wmax.
	n = 9 C1	C1: 30 min cycling / running 40 sessions over 15 wk	C1: +45% breathing endurance*	C1: +41% (*) cycling to exhaustion at 70% Wmax.
	n = 15 C2	C2: no RM training	C2: -25% (NS)	C2: -0.05% (NS).
Williams *et al.* (2002)	Trained, n = 7	50-65% MIP, 25 min, 4-5 sessions/wk, 4 wk	+31% MIP*, +128% breathing endurance time*	No effect on $\dot{V}O_2$peak, or endurance run time (85%).
Romer *et al.* (2002)	Trained, n = 8 E	E: 50% MIP, 30 breaths, 2 × day, 7d/wk, 6 wk.	E: +28% MIP*	E: +3.8% (*) 20 km cycling time trial, +4.6% (*) 40 km.
	n = 8 C	C: 15% MIP, 60 breaths, 1 × day, 7d/wk, 6 wk.	C: -1% MIP(NS)	C: No change in 20 km or 40 km cycling time trial

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
Romer *et al.* (2002)	Trained,	E: 50% MIP, 30 breaths,	E: +31% MIP*	E: -7% (*) recovery time in Repetitive sprint (15).
	n = 12 C	C: 15% MIP, 60 breaths, 1 × day, 7d/wk, 6 wk.	C: +1% MIP (NS)	C: -0.7* (NS) recovery time.
Romer *et al.* (2002)	Trained, n = 8 E	E: 50% MIP, 30 breaths, 2 × day, 7 d/wk, 6 wk.	E: +28% MIP*	E: Attenuation of post exercise respiratory fatigue (reduction in MIP).
	n = 8 C	C: 15% MIP, 60 breaths, 1 × day, 7 d/wk, 6 wk.	C: -1% MIP (NS)	C: No effect on level of RM fatigue.
McMahon *et al.* (2002)	Trained, n = 10 E	E: 60% MVV, 30 min, 20 sessions, 4 wks	E: +11% MVV*, and +260%* breathing endurance	E: +3.3min* cycling endurance at 85% Wmax and reduced chemosensitivity
	n = 10 C	C: no RM training.	C: -1.5% MVV (NS), and +4% (NS) breathing endurance	E: -1.5min (NS) cycling endurance, and no change in chemosensitivity
Holm *et al.* (2004)	Trained, n = 10 E	E: 45 min, 20 sessions, 4 wks	E: + 12% MSVC*	E: + 4.7%* cycling trial
	n = 6 C	C: no RM training	C: NS	C & P: 0.46% (NS) cycling trial
	n = 4 P	P: 5 min, 20 sessions, 4 wks	P: NS	

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
McConnell and Sharp (2005)	Non-endurance trained, n = 6 E	E: 50% MIP, 30 breaths, 1 x day, 7 d/wk, 6 wk.	E: +26% MIP*	E: Blood lactate decreased -1.17 mmol.L^{-1} cycling at maximum lactate steady state
	n = 6 P	P: 15% MIP, 60 breaths 1 x day, 7 d/wk, 6 wk.	P: No change	P: +0.37 mmol.L^{-1}.
Wells *et al.* (2005)	Trained swimmers, n = 17 E,	E: combined inspiratory and expiratory training,	E: FIV_1, FEV_1 increase*	E: No change in critical swim speed.
	n = 17 P	10 sessions / wk, 12 wks. P: sham training	P: No change.	P: No change.
Guenette *et al.* (2006)	Untrained, n = 7 males, n = 8 females	50% MIP, 2 x 30, 5 day/wk, 5 weeks.	+37 ± 18% MIP*	Males: +17%* cycling time to exhaustion (80% $\dot{V}O_2$peak). Females: +23%* cycling time to exhaustion (80% $\dot{V}O_2$peak).
Verges *et al.* (2007)	Untrained, n = 13 E,	E: 30min isocapnic hyperpnoea 1/day, 5 weeks	Increased fatigue resistance	E: No change in cycling test at 85% Max power output.
	n = 8 C.	30 min sham training	No change	C: No change.
Griffiths and McConnell (2007)	Trained, n = 10, E1	E1: 50% MIP, 30 breaths, 2 x day, 5 d/wk, 6 wks.	E1: +26%* MIP	E1: +2.7%* mean power output During 6 min all out effort.
	n = 7, E2	E2: 50% MEP, 30 breaths, 2 x day, 5 d/wk, 6 wks.	E2: +31%* MEP	E2: No change.

Study	Participants	Intervention	Change in Respiratory Muscle Function	Change in Endurance Capacity / Performance
Wylegala *et al.* (2007)	Untrained,	30 min/day, 5 d/wk, 4 weeks.		
	n = 10 E1	E1: Isocapnic hyperpnoea	E1: Respiratory endurance +217%*	E1: +66%* underwater endurance; +33%* end. swim
	n = 10 E2	E2: Resistance 50 cm H_2O	E2: Respiratory endurance + 31%* MIP +12%*; MEP +15%*.	E2: +26%* und. endurance; +38% endurance swim.
	n = 10 P	P: 10 s breath hold, 1/min	P: No change	P: No change
Leddy *et al.* (2007)	Trained,			
	n = 15, E	E: Isocapnic hyperpnoea, 30 min/d, 4 weeks.	E: +10%* MVV	E: +50%* Run to exhaustion at 80% $\dot{V}O_2$peak; -4%* 4mile time
	n = 7, P	P: sham training.	P: No changes.	P: No changes.
Downey *et al.* (2007)	Trained,	E: 50% MIP, 2 x 30, 5 d/wk,	E: +24.5% MIP*,	E: Time to exhaustion
	n = 7 E,	4 weeks.	+8-12% diaphragm thickness*	85% $\dot{V}O_2$peak: no change
	n = 5 C.	C: 15% MIP, 2 x 30, 5 d/wk, 4 weeks	C: No change MIP	C: No change.

Studies are listed in chronological order.

* = Statistically significant; C = control; E = experimental; P = placebo; MIP = maximum inspiratory pressure; MVV = maximum voluntary ventilation; NS = not statistically significant; RM = respiratory muscles; $\dot{V}_E$ = minute ventilation; $\dot{V}O_2$peak = peak oxygen consumption; Wmax = maximum power output.

Although, fixed work-rate trials to volitional exhaustion have been included when discussing athletic performance, it may be pertinent to make the distinction between time trials (exercise performance) and fixed work-rate trials (exercise capacity).

Indeed, the number of studies that have actually examined performance (time trial) in this way are few (Sonetti *et al.* 2001; Volianitis *et al.* 2001; Romer *et al.* 2002; Holm *et al.* 2004). During an 8 km laboratory time trial, cycling performance was increased significantly (+1.8%) in well trained athletes who performed five weeks of respiratory muscle training (Sonetti *et al.* 2001). The authors proposed that the effect of RMT does not exceed that observed in a placebo group which highlights the need for adequate familiarisation and placebo groups. However, Holm *et al.* (2004) reported improvements in a cycling time trial (+4.7%; improvements in 9 out of 10 subjects) that were not evident in either the control or placebo groups.

Romer *et al.* (2002) assessed performance using 20 km and 40 km cycling time trials and noted a significant reduction in the trained group which was not apparent in the placebo group.

The lack of a consistent effect of RMT on exercise performance may be due to effects such as familiarisation to the outcome measure and / or the placebo effect. Further study is required to fully discern the effects of RMT, familiarisation and placebo effects on exercise performance (Sheel 2002).

In a different study, following 11 wks of IRL training, female rowers improved rowing performance (as measured by distance covered in 6 min), by +3.5 ± 1.2% (Volianitis *et al.* 2001). The control group in this study performed sham training (15% MIP vs. 50% MIP in the training group) and also improved performance (+1.6% ± 1.0%). The +1.9% improvement in the RM-trained group over and above the improvement in the sham-trained group suggests that the RM training did indeed have

an effect on the rower's performance and may be relevant within the context of competitive athletics. These results have since been repeated with a +2.7% improvement in mean power output during a 6 minute all out rowing performance (Griffiths and McConnell 2007).

The most likely explanation for the observed differences between these four studies is the intervention used. Both Romer *et al.* (2002), Volianitis *et al.* (2001) and Griffiths and McConnell (2007) used an IRL intervention (6 wk, 11 wk and 6 wk respectively), whilst Sonetti *et al.* (2001) used a combination of both VIH and IRL (5 wk) which has been suggested a less than optimal intervention (Romer *et al.* 2002). Indeed, Wells *et al.* (2005) presented data that a combined respiratory training intervention had no effect on critical swim speed. The duration of the RMT intervention has also been cited as a possible explanation for these findings (Sheel 2002).

In summary, when exercise capacity is evaluated using submaximal fixed work-rate tests, significant improvements are seen in RMT participants. Smaller, but significant improvements have also been reported in placebo-trained individuals. When performance is measured using time-trial type measures, performance is increased to a much lesser extent with RMT than during fixed work-rate tests. Further research is needed to establish in which conditions RMT exhibits an effect on whole body exercise performance; to determine a minimal dose of RMT; and to unravel a mechanism which would explain any improvements from the many that researchers in the field have thus far suggested or even to suggest my own.

1-7 Aims & Objectives

The primary aim of this investigation was to examine the role of the respiratory muscles in health and disease. To this end, a number of objectives were planned which are briefly discussed in this section.

There is considerable interest in the use of specific RMT to improve exercise performance (See Section 1-6.2). Controversy exists with regard to the effectiveness of just such an intervention on whole body exercise capacity. The explanation for the apparent conflict in research findings is unclear but may stem from variations in the intensity, duration and mode of exercise used to evaluate performance in addition to differences in experimental design and intervention (type of RMT). Furthermore, significant improvements have also been reported in placebo-trained individuals. In view of these discrepancies, an aim of this study was to determine the functional significance of changes in respiratory muscle function for prolonged endurance performance whilst taking into account both familiarisation and placebo effects. Previous studies have included either a control group who receive no intervention, or a placebo group who perform sham training, but none have included both so that the magnitude of the placebo effect can be separated from the effect of familiarisation.

VIH and some types of IRL (TIRE for instance) require a large commitment of time (~30 min / session) on an almost daily basis. However, the minimum amount of training required to induce a physiological adaptation in the respiratory muscles (as measured using MIP) has not been investigated. Although an exhaustive investigation into various regime intensities and durations would be a project of epic proportions, a further aim of the current study is to compare different intensities of training.

Establishing the intensity and duration of training necessary to improve RM performance will help with the prescription of IRL in the future.

Respiratory muscle training has been applied in a variety of populations including chronic obstructive pulmonary disease (COPD) (Padula and Yeaw 2007). Recent data suggest that the increased load experienced by the respiratory muscles in such diverse diseases as COPD, kyphoscoliosis, and obesity can eventually result in respiratory failure (Lumb 2000). Therefore, an additional load from RMT is not likely to be beneficial. However, there is growing interest in the use of non-invasive ventilation as a means of facilitating rest and relief of respiratory muscle fatigue (Wijkstra *et al.* 2003). The effect that this unloading intervention will have on respiratory muscle strength and endurance capabilities is an aim of this investigation. Exploring the interactions between respiratory muscle load, rest, and performance in type II respiratory failure may also provide some rationale for conducting RMT in this patient group.

Anabolic androgenic steroids (AAS) have also been suggested as deserving of attention in the clinical arena for the potential benefit of preventing body mass loss, an independent indicator of outcome in COPD. The greater load on respiratory muscles experienced by COPD patients may also result in an increase in respiratory muscle strength with AAS administration (Casaburi 2000). However, before these claims can be examined in a clinical setting the effectiveness and safety of their prolonged use in healthy population needs to be determined (Dobs 1999). This investigation will examine the long-term effects of AAS use (>20 yrs) in a group of body-builders. Although, the side effects are well known, we are interested in the

specific effects on the respiratory system i.e. static and dynamic flow rates, and respiratory muscle performance with a view to examining whether AAS use will acerbate the condition of a COPD patient for example.

Examining the effect of 20 years AAS use on bodybuilders provided the opportunity to examine a subset of weightlifters taking growth hormone as an ergogenic aid. Growth hormone has been suggested as a means of improving body composition, muscle wasting, and functional capacity in cachectic patients with COPD (Nagaya *et al.* 2005). Before suggesting the use of growth hormone in a clinical setting, its effect on its users (the respiratory system) should be investigated. This study will examine the effect of short term growth hormone administration on respiratory function.

In summary, primary aims of this study were:

- To determine the functional significance of IRL training on cycling endurance capacity, whilst accounting for both familiarisation and placebo effects.
- To establish the relationship between the intensity of IRL (as a percentage of MIP) and duration of an IRL training intervention on the respiratory muscles.
- To investigate a possible mechanism behind improvements following IRL such as diaphragm thickness and stroke volume.
- To investigate the effect of an inspiration against resistance on stroke volume.
- To examine and evaluate the effect of non-invasive ventilation on respiratory muscle performance in a group of patients suffering with type II respiratory failure.
- To determine the long term effects of AAS use on respiratory system including respiratory muscle performance.
- To determine the effects of growth hormone on respiratory function.

CHAPTER 2 - GENERAL METHODS

2-1 Pre – test Preparation

2-1.1 Subject Preparation

Written informed consent was obtained from all participants prior to testing and the ethics committee of the University of Glamorgan approved all procedures. Participants completed a general health questionnaire and were removed from studies if they reported a respiratory tract infection within the two weeks prior to data collection because of the potential effects upon respiratory muscle function (Mier-Jedrzejowicz *et al.* 1988). Participants were thoroughly familiarised with all test procedures prior to formal testing. Participants were instructed not to engage in strenuous activity on the day before a test or to exercise on a test day. Participants presented to the laboratory following a 12-hour (hr) overnight fast, having consumed 500ml of water 2-hr prior to arrival to ensure that they were euhydrated as confirmed during a pilot study. Participants also provided a 48-hr dietary recall on the initial visit to the laboratory, and were given a copy of this diet to follow for the 48-hr prior to the next laboratory visit, and every time thereafter, in an attempt to standardise dietary status. On a test day, participants were instructed to avoid drinking alcohol or caffeinated beverages; and taking any substances that are known to affect or may be suspected to affect human physiological functions. All participants were non-smokers.

2-1.2 Testing Environment

Environmental conditions were conducted at the same temperature (21 ± 2 °C) and relative humidity (<70%). Each testing session was scheduled at a similar time of day

(all am) (± 1 hr) so that the effect of diurnal biological function was minimised (Atkinson and Reilly 1996).

2-2 Apparatus & Procedures

The following sections provide detailed information of the general methods used through out this study. Details of more specialised apparatus and procedures are contained within the relevant chapters.

2-2.1 Anthropometry

Age, Stature, Body Mass and Body Fat

Subjects' date of birth was expressed as year, month, and day and then was converted to a decimal age. Freestanding stature was measured to the nearest 0.1 cm with a stadiometer (Seca 220, Birmingham, UK). When recording the measurement, subjects were required to stand barefoot with the heels together and arms hanging naturally by the sides. Buttocks and scapulae were in contact with the back of the stadiometer. The subjects were instructed to look straight ahead and inhale upon measurement. The measurement was taken as the maximum distance from the floor to the vertex of the head. The vertex was defined as the highest point on the skull when the head was held in the Frankfort plane i.e. with the line joining the orbitale to the tragion at right angles to the long axis of the body. Body mass in minimal clothing was recorded on a calibrated lever balance scales (Seca 220, Birmingham, UK).

Body Fat

Harpenden skinfold callipers (John Bull, British Indicators Ltd., Bedfordshire, UK) with a constant spring pressure of $10g.mm^{-2}$ were calibrated for accuracy with a Vernier scale and were used to measure skin-fold thickness at the biceps, triceps,

subscapular and suprailiac sites on the right side of the subjects body. Three repeated measurements were obtained from each skin-fold site. Body fat was estimated from the sum of four skin-folds using the procedures of Durnin and Womersley (1974). The coefficient of variation (CV) of repeated skin-fold measurements has been estimated at 5% (Durnin and Womersley 1974) and has been demonstrated to correlate highly with hydrostatic weighing (Durnin and Womersley 1973). Body density was determined using the equations of Siri (1956).

2-2.2 Lung Function

Flow-volume profiles were measured using an online spirometer (Spirosense Spirometry System, Burdick Inc, Milton, USA). This device utilises a Silverman-Lilly or pressure differential pneumotachograph to measure flow in terms of the proportional pressure drop across a known resistance consisting of a very fine mesh screen. The software incorporated a calibration routine which measured inspiratory and expiratory volumes over 3 repeated full pumping cycles (3L). Calibration was repeated until the agreement between values for inspiratory and expiratory factors were <3%. These calibration factors were then used during subsequent calculations performed by the software.

With both the resistance (R) and pressure (P) known, it is possible to calculate flow:

$$\text{FLOW} = \Delta P / \text{R}$$

The flow is then integrated with respect to time to give volume and the two are then displayed in real time on the display of the computer to give a flow-volume curve.

The accuracy and precision of this spirometer met the minimal recommendations for diagnostic spirometry recommended by the American Thoracic Society (ATS 1995).

The spirometer was calibrated prior to each testing session using syringes of known volumes (Hans Rudolph Inc, Kansas, USA). Dedicated software was used to manage pulmonary function data from the on-line spirometer (Burdick Spirosense Spirometry Software, V2.25).

All measurements were made via a mouthpiece attached to the spirometer whilst subjects were seated in an upright position with the nose occluded. The appropriate technique was explained and demonstrated prior to testing. For flow-volume manoeuvres, participants were instructed to inhale completely from FRC and begin the forced expiratory manoeuvre with minimal hesitation. From the position of maximal expiration, participants were directed to make a maximal forced effort to a position of maximal inspiration. The following variables were derived from the flow-volume profiles: forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), peak expiratory flow (PEF) and peak inspiratory flow (PIF).

All flow-volume measurements were performed according to specific criteria (ATS 1995): at least three reproducible trials were performed (i.e. the FVC and FEV_1 measurement values of the two best trials did not differ from each other by more than 0.2 L); the expiration time in the maximum expiratory effort was not shorter than 6 s; an expiratory plateau existed (i.e. over the last 2 s of expiration the volume did not change by more than 40 ml). All volume and flow measurements were expressed as BTPS. The largest FVC and the largest FEV_1 were recorded after examining the data from all of the acceptable curves, even if they did not come from the same curve. Other measures, such as the peak expiratory flows, were obtained from the single curve that met the acceptability criteria and gave the largest sum of FVC plus FEV_1. All measured values were compared with selected reference values based on European Respiratory Society (ERS) prediction equations (Quanjer *et al.* 1993).

2-2.3 Respiratory Muscle Function

Diaphragm thickness

Diaphragm thickness, when assessed, was measured at maximum inspiratiration and expiration using B-mode ultrasonography. A 7.5 MHz linear probe (PLE 705S Toshiba Medical System, UK) was held perpendicular to the chest wall in the 10^{th} intercostal space on the right side between the anterior and mid-axillary lines. The diaphragm was identified by two clear echodense lines and measured from the middle of the pleural to the middle of the peritoneal line (Figure 3-1). The mean of three measurements made at the zone of apposition at maximal inspiration and expiration were recorded.

Mouth Pressure Meter

A hand-held mouth pressure meter (Micro Medical Ltd, Rochester, Kent, UK) was used for the determination of maximum static inspiratory and expiratory mouth pressures (MIP and MEP, respectively). The pressure meter incorporated a 1 mm orifice to prevent the subject from producing artificially high inspiratory pressures with the muscles of the buccal cavity when the glottis is closed (Black and Hyatt 1969). The leak was small enough that it did not greatly affect lung volume during maximal efforts. Subjects breathed via a flanged mouthpiece and a nose clip was attached to the subject's nose to prevent leakage. The device was capable of measuring differential pressure between ± 300 cm H_2O at a rate of 16 Hz. An integral microprocessor was used to determine and display the maximum pressure averaged over one second for both inspiratory and expiratory efforts.

Since MIP and MEP measurements are dependent on lung volume, each effort was initiated from residual volume (RV) or total lung capacity (TLC), respectively. All manoeuvres were performed whilst standing and subjects were instructed to make contractions rapidly and maximally i.e. Mueller or Valsalva manoeuvre. Subjects were provided with strong verbal encouragement and visual feedback from the pressure device output. The procedure was repeated three times, with the best value recorded (Green *et al.* 2002).

Inspiratory Muscle Endurance

The test of incremental respiratory endurance (TIRE), as described by Chatham et al (1995), was used to provide an index of inspiratory muscle endurance. Computer generated targets are presented to the subject, set at 80% of maximum, across the functional range of volume and the frequency at which the templates are presented increases as the test progresses. The device is a hand-held pressure meter with a one-way valve and controlled air leak to generate mouth pressure when the subject inspires (Figure 2-1). The handset consists of a pressure transducer, electronics to process the signal and send it to a computer via its infrared transmitter. The base station, which also acts as the battery charger, receives the information and sends it to the PC via the serial port. On the PC there is a custom written software program in MS Windows format that processes, displays the test in real time and databases the information.

Clanton and Ameredes (1988) reported a significant familiarisation effect between the first and fourth trials when conducting breathings trials of a similar type. To determine if this was the case in this instance 5 subjects were required to conduct the TIRE test once per week for five weeks (Table 2-1).

Table 2-1 Measurement of TIRE

Week	MIP (cm H_2O)	Pav (cm H_2O)	SMIP (PTU)	ΣSMIP x 10^3 (PTU)
1	127 ± 40	116 ± 24	897 ± 231	23.9 ± 1.1
2	132 ± 26	120 ± 22	946 ± 324	24.2 ± 2.0
3	130 ± 22	119 ± 18	920 ± 290	22.8 ± 1.7
4	134 ± 29	117 ± 21	936 ± 314	24.5 ± 2.2
5	137 ± 31	121 ± 26	916 ± 312	23.4 ± 2.1

Mean ± SD are shown. MIP = Maximum Inspiratory Pressure; Pav = Average pressure; SMIP = Sustained Maximum Inspiratory Pressure; ΣSMIP = Sum of SMIP.

There were no statistically significant differences between measures of MIP, SMIP, and sum of SMIP made on week one compared with week two through five. This is most likely because during a single TIRE test a subject will likely perform inspiratory manoeuvres from RV to TLC >39 times. Although MIP and SMIP are determined from the first three manoeuvres, an experienced operator will ensure that efforts are maximal each time. Therefore, familiarisation does not appear to be a great problem with TIRE. However, participants were expected to complete three TIRE tests prior to baseline testing thereby ensuring familiarisation was minimised. In addition, baseline testing took place a minimum of four days after familiarisation to ensure respiratory fatigue was not a factor (Loke *et al.* 1982).

Figure 2-1 TIRE device

2-2.4 *Variation in TIRE*

At present very little data exists regarding the TIRE test (Chatham *et al.* 1995) in terms of the variation of the test. Therefore, 10 male volunteers (age 20-24) were recruited to perform TIRE once a week for 10 weeks (Table 2-2).

Table 2-2 Variation in TIRE

Week	MIP (cm H_2O)	ΣSMIP x 10^3 (PTU)
1	120.4 ± 10	26.9 ± 4.9
2	110.3 ± 11	15.8 ± 6.6
3	141.7 ± 10	36.1 ± 2.4
4	112.0 ± 17	32.5 ± 5.2
5	100.9 ± 18	19.0 ± 9.1
6	151.1 ± 16	24.9 ± 8.4
7	103.8 ± 20	17.6 ± 5.2
8	124.9 ± 11	19.1 ± 7.3
9	158.5 ± 19	25.3 ± 14.2
10	123.2 ± 20	21.8 ± 13.5

Mean ± SD are shown. MIP = Maximum Inspiratory Pressure; ΣSMIP = Sum of SMIP. n = 10.

Participants were familiarised with the test by performing TIRE three times before taking a baseline measurement. These data suggest that this familiarisation strategy is effective in negating the demonstrable learning effect for TIRE and is to be used in later studies within this study.

The above data were collected to determine the variation apparent when using TIRE. The coefficient of variation (CV) is a measure of dispersion of a distribution; it is

defined as the ratio of the standard deviation (SD) to the mean (CV = SD / mean). It is often reported as a percentage by multiplying the above calculation by 100. The total CV for measuring TIRE based on the above data (where mean = 124.7 ± 19.8) is 15.9%.

The analytical variation is the variation present in a measurement due to the instrument used for that measure. In this case the instrument is the TIRE device (Figure 2-1). In order to measure the analytical variation the TIRE device was connected to a vacuum cleaner (Fig 2-2) reported by the manufacturer to produce a suction of 28.4 cm.H_2O (Dustbuster, 4.8V, Black & Decker, Maryland, USA). Any variation in the measurement of pressure produced by the vacuum should be a result of the instrument (TIRE) and used to calculate the analytic coefficient of variation.

The MIP of the vacuum was consistently found to be exactly 28 cm.H_2O^{-1} after 10 "manoeuvres" held for 10 s each. This resulted in a CV_A of 1 (1/1). Biological variation is the variation due to the changes in the subject and can be calculated as the total CV (CV_T) minus the analytical variation (CV_A). This would give a biological variation for TIRE as 15.9 – 1 = 14.9%.

The critical difference (CD) was assessed using the equation of Fraser and Fogarty (1989):

$$CD = 2.77 \sqrt{CV_A^2 + CV_B^2}$$

where 2.77 probability of $P = 0.05$.

Using this equation, the critical difference for MIP based on the present data is 41.4%.

The standardised difference (Altman and Bland 1997) can be calculated using the expected proportion for the specified outcome in each group ($p1$ and $p2$), where:

$p1$ = proportionate increase in MIP to achieve a physiological effect (20% from Green *et al.* (2002));

$p2$ = CD in MIP based on present data (41.4%).

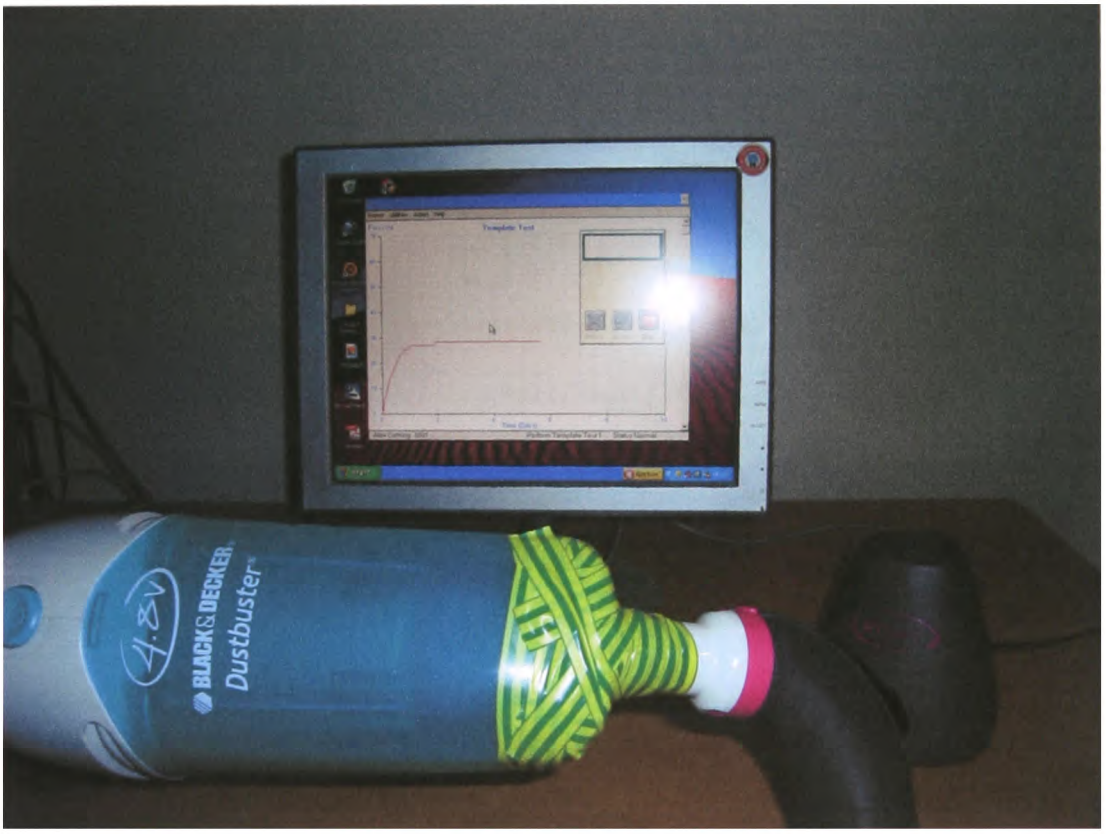
The standardised difference = $\frac{p1 - p2}{\sqrt{P}}$

where P = $(p1 + p2) / 2$

These data provide a standardised difference of 1.2. The power was set at 0.80 and a 5% significance level between groups was required to provide a high probability to detect any differences between variables.

Using the nomogram of Altman (1980), the sample size was 22 (11 in each group).

Figure 2-2 Measuring the analytical variation of TIRE



2-2.5 Ventilation & Pulmonary Gas Exchange

An online, computerised, metabolic system (Medgraphics CPX/D System, Minnesota, USA) was used to measure ventilation and gas exchange. Flow was measured by a bi-directional differential pressure pneumotach (Range ±18 $L.s^{-1}$) and a reported accuracy of ± 3% or 50 ml, whichever is greater. Volumetric calibration of the flow meter was performed prior to each laboratory test. Before performing the volumetric calibration the zero offset reading of the flow meter was measured. A 3-litre syringe (Hans Rudolph Inc, Kansas, USA) was then used to perform the volumetric calibration. The software incorporated a calibration routine which measured inspiratory and expiratory volumes over 3 repeated full pumping cycles. Calibration was repeated until the agreement between values for inspiratory and expiratory factors were <3%. These calibration factors were then used during subsequent calculations performed by the software.

Expired air was analysed for the concentrations of O_2 and CO_2 by sampling through Zirconium and infra-red analysers respectively. The O_2 and CO_2 analysers were calibrated before each test over the expected physiological range using certified zero and balance N_2 gas mixtures (British Oxygen Company, London, UK). Analysers were warmed up for at least an hour before testing to eliminate electrical drift. All respiratory gases were sampled from the manifold of a mouthpiece via a sample tube, which was a fine polythene catheter approximately 2m in length. A delay existed between the time that the gas concentration existed and the time that this concentration was detected and recorded by the online system. To allow the flow signals to be matched accurately to the gas analysers from the online systems the delay times were calculated automatically during calibration.

In order to assess the accuracy of gas exchange and ventilatory measurements made using the online system, these were compared with measurements made using the Douglas bag technique. Measurements were made during a range of constant power output cycle ergometer exercise. In order to achieve steady-state conditions subjects cycled for a period of 4 minutes prior to measurements being made. A Douglas bag sample, connected in series with the online system, was then collected for a known duration enabling comparison between the two methods at the same time.

Such calibrations were made periodically in order to verify the validity of the laboratory system. Examples of the results are illustrated in Table 2-3.

Table 2-3 Ventilation and gas exchange variables during constant power exercise measured by Douglas bag and laboratory breath-by-breath system

Cycling Power	Method	$\dot{V}_E$	$\dot{V}O_2$	$\dot{V}CO_2$
0W	Bag	15.4	0.50	0.40
	Online	16.7	0.50	0.49
60W	Bag	37.2	1.55	1.38
	Online	38.7	1.61	1.50
90W	Bag	56.3	2.23	2.16
	Online	54.6	2.18	2.14
120W	Bag	65.1	2.43	2.39
	Online	64.0	2.48	2.44

$\dot{V}_E$ = Ventilation; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide excretion.

There were no statistically significant differences between measures of $\dot{V}_E$, $\dot{V}O_2$ or $\dot{V}CO_2$ made using the laboratory based online system and the Douglas bag technique. The online system therefore offers an excellent means of measuring true breath by breath gas exchange and has the flexibility to be used in a wide range of ergometry tests.

2-2.6 Cycle Ergometry

Exercise tests were conducted using a friction-loaded ergometer (Monark 824E, Varberg, Sweden). The cycle ergometer was modified with a racing saddle, with the height individually adjusted so that the knee remained slightly flexed after completion of the power stroke (final knee angle approximately 170-175°, where full knee extension is defined as 180°; crank position at bottom dead-centre). The subjects' feet are firmly held in contact with the pedals by toe-clips. The handlebar can also be adjusted to an angle comfortable to the subject. Saddle height and position of handlebar remained constant from test-retest for each subject. The flywheel of the bike is braked by means of a brake lacing which runs around the brake surface of the flywheel. A schematic diagram of the ergometer is shown in Figure 2-3. The design of the ergometer is such that a point on the circumference of the flywheel travels 6m for every single revolution of the pedals. The power can then be calculated as the product of the pedal cadence and the mass applied to the basket (Monark instruction manual, onark, Vansbro, Sweden). This makes the calculation of work and power relatively simple compared with having to either determine the theoretical brake torque or taking direct measurements of the brake torque (Gordon *et al.* 2006). The brake power is changed either by using another pedalling speed or by increasing the tension of the brake lacing against the flywheel by means of weights. Brake resistance and brake

power for the cycle ergometer is shown in Table 2-4. For instance, if a subject were to place 2kg on the weight basket, then the brake force is 3 Kg (the weight basket has a mass of 1 kg). If the subject then cycled with a pedalling frequency of 60 revolutions per minute (rpm), then the brake power would be 180 Watts (W).

Figure 2-3 Flywheel arrangement for a rope / belt braked ergometer from Gordon *et al.* (2006)

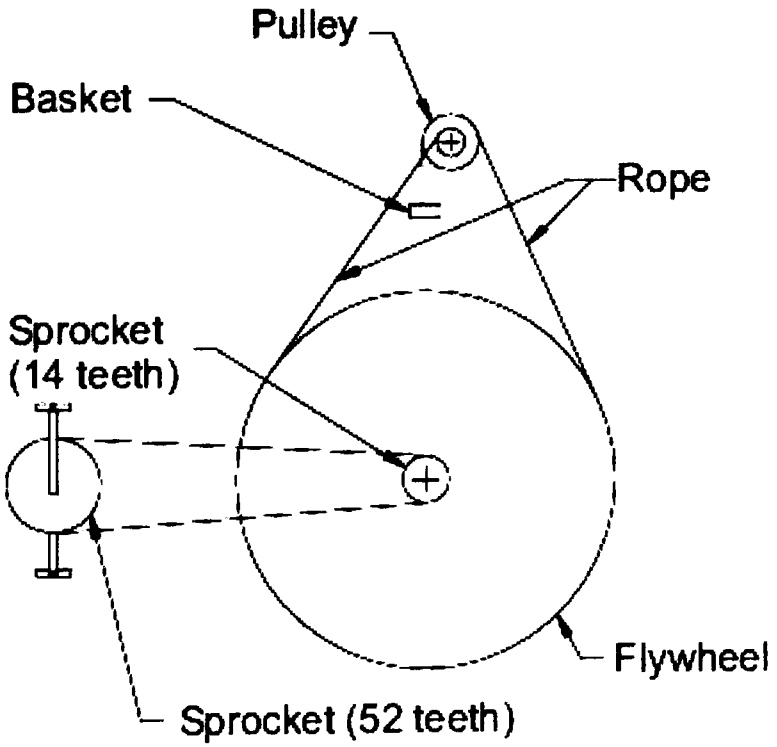


Table 2-4 Relationship between cycling cadence, brake resistance, and power

	Kp (Mass + 1kg cradle)						
Rpm	1	2	3	4	5	6	7
30	30	60	90	120	150	180	210
40	40	80	120	160	200	240	280
50	50	100	150	200	250	300	350
60	60	120	180	240	300	360	420
70	70	140	210	280	350	420	490
80	80	160	240	320	400	480	560
90	90	180	270	360	450	540	630
100	100	200	300	400	500	600	700
110	110	220	330	440	550	660	770

2-2.7 Perceived Exertion

The overall perceived exertion rating integrates various information, including the many signals elicited from the peripheral working muscles and joints, from the central cardiovascular and respiratory functions, and from the central nervous system. All these signals, perceptions, and experiences are integrated into a configuration of perceived exertion (Borg 1982). The 15-grade scale for ratings of perceived exertion, the RPE scale (Borg 1970), was used to measure perceived exertion during exercise.

The instructions to subjects emphasised that the perceptual ratings should reflect sensations of exertion / effort, strain, and / or discomfort. Subjects were asked to recall known sensations to anchor the bottom and top ratings on the scale for each exercise mode.

The rating scale was A4 mounted and situated in full view of the subject at all times throughout testing. Subjects were encouraged to use verbal expressions on the scale to

aid in selecting the appropriate rating. However, subjects were encouraged to rate naively without making judgements or evaluating correctness. Subjects were familiarised with the scale on at least one previous occasion in order to improve the reliability of data collection (Eston and Williams 1988).

2-2.8 Cardiovascular measurements

Blood Pressure

Systemic arterial blood pressure (BP-mm/Hg) was measured in the brachial artery non-invasively using the auscultatory method and using a mercury sphygmomanometer (Accoson Freestyle, Cardiokinetics, UK) and stethoscope (Littmann, 3M, USA). Systolic pressure was recorded at the first appearance of clear repetitive tapping sounds (Korotkoff, Phase 1) and diastolic pressure was recorded at the disappearance of repetitive sounds (Korotkoff, Phase 5).

Stroke Volume

Stroke volume was derived echocardiographically using a HP Sonos 5500 Ultrasound System (Hewlett Packard, Andover, MA) with a 2.5Hz transducer. A 2-D sector scan of the left ventricle outflow tract was gained from a parasternal long axis view. Placement of the cursor line at the level of the valve allowed an M-mode trace of the aortic tract and delineation of the opening and closure of the aortic valve leaflets. Placement of the ultrasound transducer in the suprasternal notch allowed a 2-D sector scan of the ascending aorta (Figure 7-2). The sample volume was oriented parallel to flow in the aorta to facilitate pulsed-wave Doppler echocardiographic representation of the aortic flow waveform. From the M-mode trace, the diameter (D) of the annulus of the aortic valve was measured. The cross-sectional area (CSA) of the open valve

was then determined using the following equation: πD^2 / 4. from the Doppler trace, the outline of the waveform was digitised to calculate flow velocity integral (FVI, cm). The FVI is calculated by the computer of the echo machine as the area under the curve from the continuous wave Doppler of aortic outflow (Figure 7-1). The FVI and CSA measurements permitted the calculation of stroke volume according to the following equation: SV = CSA x FVI.

2-2.9 Blood sampling

Venous blood

Venous blood was sampled from an anticubital vein following an overnight fast and 30 minutes supine rest (Pronk 1993) using the standard Venepuncture method. Blood samples were appropriately centrifuged and immediately stored at -70°C until analysis.

Blood gas tensions

An earlobe arterialised capillary blood sample was taken as described by Spiro and Dowdeswell (1976). This involves spreading an earlobe (usually the patients left, with a right-handed operator) with nicotinate paste for at least 10 minutes to induce capillary vasodilation. A stab incision was made in the inferolateral aspect of the pinna from which blood usually flows freely but may require a small amount of manual massage. The arterialised blood was collected in a drop on the inferior aspect of the earlobe. It was drawn into a thin glass capillary tube by surface tension under the control of a gloved finger over the open end of the tube and then aspirated into the blood gas analyser (ABL500, Radiometer, Copenhagen).

CHAPTER 3 – RESEARCH STUDIES

STUDY ONE – THE EFFECTS OF INSPIRATORY RESISTIVE LOADING (IRL) ON CYCLING ENDURANCE CAPACITY

3-1 Abstract

Respiratory muscle training (RMT) has been demonstrated to improve both respiratory muscle strength and endurance. The effect these improvements have on whole body exercise performance remains controversial. Fifteen apparently healthy subjects (ten males and five females) were randomly allocated to one of three groups. One group underwent inspiratory resistive loading (IRL) set at 80% of maximum inspiratory pressure with ever decreasing work-rest ratios until task failure, for 3-d per week (wk) over a ten week period (IRL group). A second placebo group performed the same training procedure but with a minimal resistance (PLA group). The remaining five control subjects performed no IRL (CON group) during the 10 week study period. Cycling endurance capacity at 75% $\dot{V}O_2$peak was measured before and after the intervention. Following the 10 week IRL intervention, a significant increase relative to pre-test was observed for respiratory muscle strength (maximum inspiratory pressure) by 34% (pre = 134 ± 35 cm H_2O; post = 180 ± 39 cm H_2O), and respiratory muscle endurance (sum of sustained maximum inspiratory pressure) by 38% (pre = 987 ± 144; post = 1303 ± 212). An increase in diaphragm thickness was also observed (+22%). These improvements translated into a 36% increase in the cycling time to exhaustion at 75% $\dot{V}O_2$peak (+1292 ± 607 seconds). During cycling trials heart rate, ventilation, and ratings of perceived exertion were attenuated in the IRL group. No changes were observed for the placebo or control group either in the time to exhaustion or cardiorespiratory response to the same intensity of exercise. Ten weeks of IRL attenuated heart rate, ventilatory and perceptual response to constant workload exercise, and improved cycling endurance capacity. Familiarisation was not a factor and the placebo effect was minimal.

3-2 Introduction

Ever since it has been possible to specifically train the respiratory muscles (RM) for improvements in strength and / or endurance (Leith and Bradley 1976), the effect of doing so on whole-body exercise performance has drawn the attention of many studies (Sheel *et al.* 2002). Despite this attention, results remain equivocal with some studies reporting improvements in exercise capacity (Boutellier *et al.* 1992; Boutellier and Piwko 1992; Spengler *et al.* 1999; Markov *et al.* 2001; Stuessi *et al.* 2001; Volianitis *et al.* 2001; McMahon *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Holm *et al.* 2004; Guenette *et al.* 2006; Griffiths and McConnell 2007; Leddy *et al.* 2007; Wylegala *et al.* 2007) and others reporting no significant effect (Morgan *et al.* 1987; Fairbarn *et al.* 1991; Hanel and Secher 1991; Kohl *et al.* 1997; Inbar *et al.* 2000; Williams *et al.* 2002; Wells *et al.* 2005; Downey *et al.* 2007; Verges *et al.* 2007). Within the literature, the different methods of respiratory training, and performance outcomes used have made it difficult to discern a pattern as to when respiratory muscle training (RMT) will result in an improvement in performance. The majority of RMT studies have employed one of two modes of training: 1) voluntary isocapnic hyperpnoea (VIH) to improve respiratory muscle endurance; or 2) inspiratory resistive loading to improve respiratory muscle strength (Sheel *et al.* 2002). IRL can be achieved with either pressure-threshold or flow resistive devices. A pressure threshold device requires subjects to produce a negative pressure sufficient to overcome a threshold load. Threshold loading in this manner allows variable loading at a quantifiable intensity by providing near flow-independent resistance to inspiration and has been used successfully in several recent studies (Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Guenette *et al.* 2006; Griffiths and McConnell 2007), but has received criticism by a group evaluating the device (Hart *et*

al. 2001). In contrast, inspiratory flow-resistive loading requires subjects to inspire via a variable diameter orifice whereby, for a given flow rate, the smaller the orifice the greater the resistive load. An inherent limitation of most forms of flow-resistive loading is that inspiratory pressure varies with flow rate and not just orifice size. A new device, which is flow-resistive in nature, dictates flow rate and hence inspiratory pressure through the computer to which it is linked. The test of incremental respiratory endurance (TIRE) requires subjects to perform repeated sustained maximum inspiratory pressures, through the full lung volume, at 80% of the individual's sustainable pressure. Evidence suggests that this protocol induces diaphragmatic fatigue (Chatwin *et al.* 2001), and that an improvement in both respiratory muscle strength and endurance will be the net result of performing this protocol three times per wk for 10 wks (Chatham *et al.* 1999). The device differs from other types in that it requires the manoeuvre to be performed through volume, from residual volume to total lung capacity, which is not the case with other types of trainers. For example, the pressure-threshold devices used by some (Caine and McConnell 1998; Inbar *et al.* 2000; Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; McConnell and Sharpe 2005; Guenette *et al.* 2006; Griffiths and McConnell 2007) will close at higher lung volumes where the required threshold pressure cannot be maintained.

In addition, the majority of the studies that have examined RMT have lacked an effective placebo (Boutellier *et al.* 1992; Boutellier and Piwko 1992; McMahon *et al.* 2002; Williams *et al.* 2002; Guenette *et al.* 2006; Griffiths and McConnell 2007) or a control group (Spengler *et al.* 1999; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Leddy *et al.* 2007) or have not used repeated trials for constant workload performance outcomes in order to account for familiarisation (Sonetti *et al.* 2001).

Because of this, despite a positive outcome, the extent to which the improvement is due to an actual training effect, as opposed to familiarisation or a placebo effect casts doubt on the results. More recent studies however, have included a placebo group whilst assessing RMT and performance (Sonetti *et al.* 2001; Volianitis *et al.* 2001; Romer *et al.* 2002; Holm *et al.* 2004; Leddy *et al.* 2007; Wylegala *et al.* 2007). All six of these studies demonstrated an improvement in performance following an RMT intervention, whereas Sonetti *et al.* (2001) reported an improvement in the placebo group equal to that of the training group, Volianitis *et al.* (2001) demonstrated an improvement in both the trained and placebo group, but in this instance the improvement in the trained group was significantly greater than the improvement in the placebo. Finally, Romer *et al.* (2002), Wylegala *et al.* (2007), and Leddy *et al.* (2007) showed no improvement in the placebo group. The extent to which the placebo effect is having on exercise performance with this type of intervention is difficult to ascertain because all studies lack a control group i.e. a group who receive no training (Sheel 2002).

The following study was designed to assess the effect of a 10-wk intervention with the TIRE device on respiratory muscle performance and whole body exercise endurance capacity. Familiarisation (by using repeat trials) and the placebo effect (by using both a placebo and control group) were also considered.

3-3 Methods

3-3.1 Participants

Fifteen apparently healthy subjects who participated regularly in exercise were recruited. Informed written consent was obtained from each subject and the ethics committee of the University of Glamorgan approved all procedures. The physical characteristics of the subjects were as follows: (mean ± SD), age 22.7 ± 2.3 yr, stature 1.75 ± 0.09 m, body mass 75.8 ± 9.6 kg, peak oxygen consumption ($\dot{V}O_2$peak) 3.18 ± 0.69 L.min^{-1}, maximum power output (Wmax) 287 ± 64 W.

3-3.2 Procedure

See Methods (section 2-1.1) for details of subject preparation prior to testing.

Each subject visited the laboratory four times pre-intervention and three times post. The first visit comprised a spirometric assessment, maximum inspiratory and expiratory mouth pressures, and a submaximal cycling test to establish their oxygen consumption vs. workload relationship. On the same day, an incremental test to exhaustion was used to establish $\dot{V}O_2$peak. A minimum of 7 days later, subjects completed a cycling trial to exhaustion at a resistance prescribed to elicit 75% $\dot{V}O_2$peak ($Tlim_{75}$). Subjects repeated this trial, at the same resistance, on the two remaining visits to the laboratory, again separated by 7 days, in an attempt to account for familiarisation and the variation innate in such trials (Jeukendrup *et al.* 1996). Subjects then received a 10-wk intervention of inspiratory resistive loading (IRL), respiratory training placebo (PLA), or no IRL (CON) to which they were randomly allocated. The effects of the interventions were evaluated using the same battery of

tests after the respective interventions over a three-wk period as only two $Tlim_{75}$ trials were performed.

3-3.3 Lung Function

Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1 / FVC%, and peak expiratory flow (PEF) were determined using a computerised spirometry system (Spirosense Spirometry System, Burdick Inc, Milton, USA). Each test was repeated until three acceptable results were obtained. The greatest value from each set of repeated measurements for each test was used for subsequent analysis (Section 2-2.2).

Mouth pressures (MIP and MEP) were measured with a portable hand-held mouth pressure meter (Micro Medical Ltd, Rochester, Kent, UK). Subjects were strongly urged to make maximum inspiratory (Mueller manoeuvre) and expiratory (Valsalva manoeuvre) efforts at or near residual volume (RV) and total lung capacity (TLC), respectively. The maximum of three manoeuvres was recorded. All lung function tests were conducted prior to whole body exercise testing (2-2.3).

3-3.4 Exercise testing

All exercise tests were conducted on the same cycle ergometer (Monark 824 E, Varberg, Sweden).

The relationship between workload or power output and oxygen consumption was calculated by selecting four submaximal workloads that would elicit a heart rate (HR) ranging from 120 to 180 $beats.min^{-1}$, and measuring oxygen consumption using a

computerised O_2 breath-by-breath analysis system (Medgraphics CPX/D, St Paul, Minnesota).

$\dot{V}O_2peak$: After an adequate rest period, subjects performed an incremental test to exhaustion starting at a power output of 60 Watts (W) and increasing by 24W per minute until the criteria for $\dot{V}O_2$peak was reached i.e. rating of perceived exertion (RPE) of 20, volitional exhaustion, respiratory exchange ratio (RER) >1.1, HR $\geq$ HR_{MAX} (220 – age). Following an additional rest period, $\dot{V}O_2$peak was confirmed using a square wave test. Here subjects cycled at the maximum power output achieved during the incremental test for 5-minutes, again at 60 rpm. $\dot{V}O_2$peak was taken as the highest values from the two tests.

For each subject, 75% $\dot{V}O_2$peak was ascertained from the regression of workload vs. oxygen consumption to determine the resistance required to elicit this oxygen consumption. $Tlim_{75}$ trials were performed one wk apart in order to avoid any physiological adaptation. Subjects were verbally encouraged to perform at their best during all tests. Each test continued until volitional fatigue, defined as failure to maintain cadence $\geq$ 60 rpm. During the trial, measurements were made of oxygen consumption ($\dot{V}O_2$), ventilation ($\dot{V}_E$), HR, RER, and RPE.

Subjects performed the trial three times at baseline with data from the first trial excluded to allow for familiarisation. The best time of the second and third trials was taken as $Tlim_{75}$ in an attempt to account for some of the day-to-day variation common to this type of trial. Post-intervention, the subjects performed just two trials, as they should now have been familiarised with the test. Again $Tlim_{75}$ was taken as the better of the two trials. The time between subjects completing their last IRL session and re-evaluation of exercise capacity was greater that 24 hr to provide adequate time for respiratory muscle fatigue to subside (Laghi *et al.* 1995).

3-3.5 Inspiratory Resistive Loading

Both IRL and PLA training was conducted 3d a wk for 10 wk for a total of 30 sessions in all and was supervised throughout the training period by the same investigator (AG) to ensure 100% adherence. All subjects continued with their regular exercise training programs and were required to keep a training diary that included their IRL or PLA training throughout the study. The control group also kept a training diary for the duration of the study but received no additional IRL. Within their diary, subjects recorded both the frequency and duration of training during the 10-wk intervention period.

The IRL device is flow-resistive in nature, with subjects having to breathe through a 2mm leak, present to prevent glottal pressure. A maximum flow was set during the inspiratory effort proportional to the pressure achieved. The measured resistance (pressure / flow) was approximately 270 cm $H_2O.L^{-1}.s^{-1}$. Placebo subjects used the same breathing device with a different mouthpiece that had a greatly reduced flow resistance (a leak of 30mm, resistance approximately 10 cm $H_2O.L^{-1}.s^{-1}$).

IRL was undertaken using the test of incremental respiratory endurance (TIRE) system, which has been previously described (Chatham *et al.* 1999). Briefly, during the TIRE, respiratory work is fixed in direct relation to individual capacity. This is achieved by measuring three SMIPs (sustained maximal inspiratory pressure) i.e. subjects inspire from residual volume to total lung capacity, and the highest of the three is selected by the operator and reduced by the computer to an on-screen template, re-drawn at 80% of this best effort (Figure 1-10). If at any point during the TIRE, the subject fails to achieve at least 90% of this reduced template the test is over. The TIRE has six levels with each level consisting of six inspiratory efforts. Initially, level A presents template at 60-second intervals over its six inspiratory

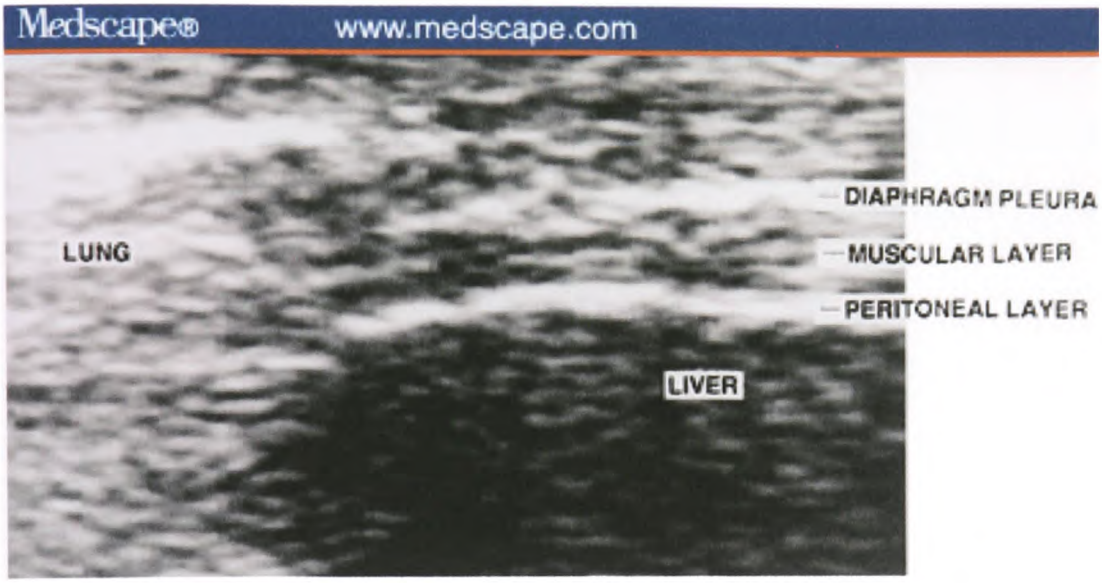
efforts but at levels B through F this rest period is reduced to 45, 30, 15, 10 and finally 5 seconds.

At each TIRE completion, a grid of performance is established which assigns a point of failure (D4, F1, etc) plus summated "work" expressed as total pressure / time units (sum of SMIPs). At each training session SMIPs are reassessed so that the work performed during the TIRE on that day is based on the new maximal effort (see Methods 2-2.4).

3-3.6 Diaphragm Thickness

Diaphragm thickness for three of the control group and four of the IRL group were assessed on maximum inspiration and expiration by B-mode ultrasonography. A 7.5 MHz linear probe (PLE 705S Toshiba Medical System, UK) was held perpendicular to the chest wall in the 10^{th} intercostal space on the right side between the anterior and mid-axillary lines. The diaphragm was identified by two clear echodense lines and was measured from the middle of the pleural to the middle of the peritoneal line (Figure 3-1). The mean of three measurements made at the zone of apposition at maximal inspiration and expiration was recorded. The rationale for making this measure follows work where diaphragm thickness has been correlated with MIP (McCool *et al.* 1997).

Figure 3-1 Ultrasound image of the diaphragm in the zone of apposition in a healthy individual. The diaphragm is identified as the structure bounded by the pleura and peritoneum



3-3.7 Statistical Analysis

Shapiro-Wilks tests were applied to each dependent variable to confirm distribution normality. Mixed between-within analyses of variance (ANOVA) were used to test for between group effects due to treatment (IRL, PLA, CON) and within group effects due to intervention (Pre- and post-treatment). To determine where statistically significant differences existed between pairs of mean values Student's paired sample *t*-tests were used with a Bonferroni correction factor. Specifically, this analysis assessed the changes in mean values over time in each of the two types of performance tests, using peak power and $\dot{V}O_2$peak for the incremental tests, and time to exhaustion in the $Tlim_{75}$ trials. Changes in cardiorespiratory responses and RPE measured at fixed time points during $Tlim_{75}$ were examined in the same manner. To examine the effect of familiarisation on the first three trials, we used Student's paired

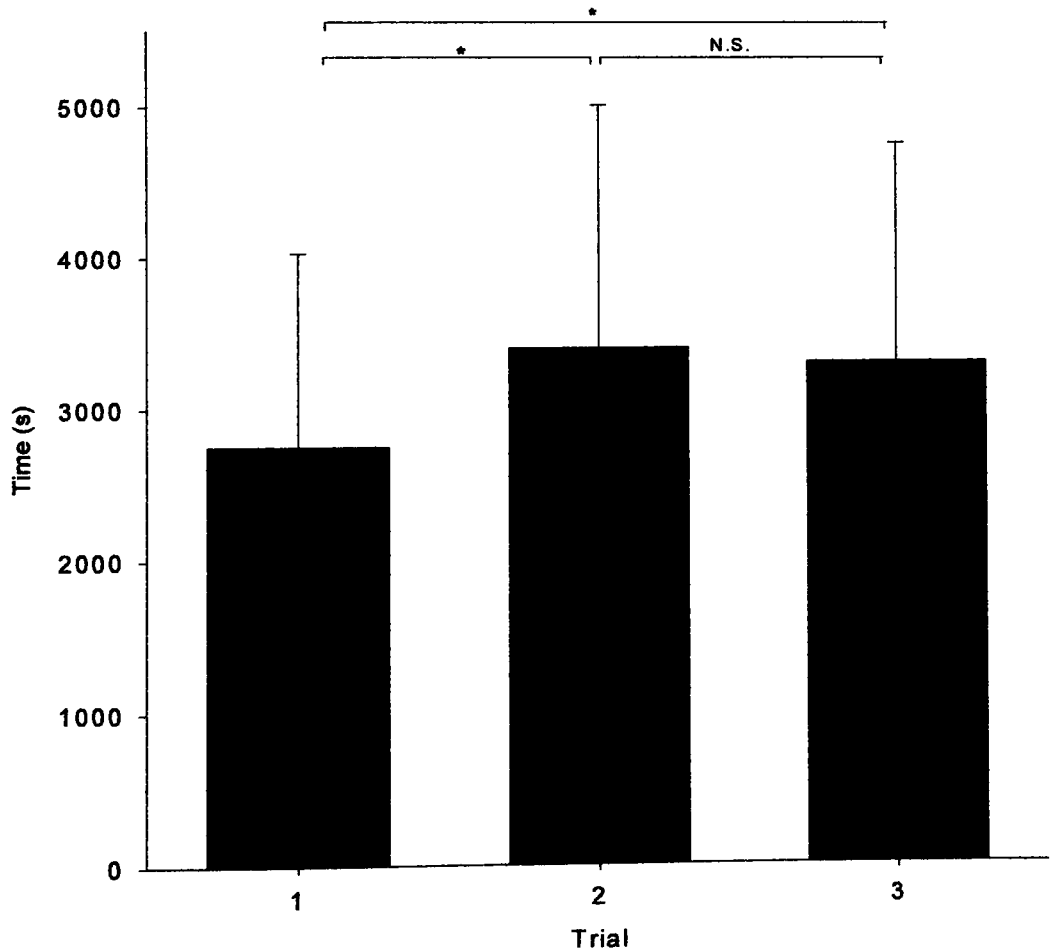
samples *t*-test with a Bonferroni correction. Significance for all two tailed tests was established at an alpha level of $P < 0.05$ and data are expressed as mean ± 1 standard deviation (SD).

3-4 Results

3-4.1 Familiarisation to cycling time to exhaustion

To account for familiarisation in the present study, repeated trials were used. The cycling time to exhaustion at 75% $\dot{V}O_2$peak for all subjects (regardless of group) pre-intervention is illustrated in Figure 3-2.

Figure 3-2 Changes in cycling time to exhaustion at 75% $\dot{V}O_2$peak as a result of familiarisation (n = 15)



* Significantly different from first test ($P < 0.05$). NS not significantly different.

The times for both the second and the third trial are both significantly greater than the time of the initial trial ($P < 0.05$). Importantly, there was no significant difference

between the times of the second and the third trials ($P > 0.05$). The results for subjects' first trial were not included in statistical analyses.

3-4.2 Adherence to training

For both groups that were expected to perform respiratory training (IRL, PLA), there was 100% adherence with all 10 subjects completing 30 sessions during the 10-wk period. In addition, supervision of the training, with appropriate encouragement ensured that subjects were fully motivated, producing a maximal training template, at commencement of each training session.

3-4.3 Whole-body training

All subjects continued their regular exercise training programs during the 10-wk intervention period and were required to keep a training diary of all physical activity, reporting both frequency (sessions / wk) and duration (time training / wk) of training. During this period, training did not differ between / within the three groups in terms of the frequency and duration of training, however, no record of the intensity of training was recorded (Table 3-1).

Table 3-1 Frequency and Duration of Training during 10-wk intervention

Parameter	IRL n = 5	PLA n = 5	CON n = 5
Week One			
Frequency (sessions)	4.4 ± 1.1	4.8 ± 1.1	3.8 ± 1.5
Duration (min)	198 ± 91	200 ± 119	181 ± 68
Week Five			
Frequency (sessions)	4.4 ± 1.1	4.2 ± 1.6	4.0 ± 1.2
Duration (min)	196 ± 82	196 ± 132	177 ± 59
Week Ten			
Frequency (sessions)	4.2 ± 0.8	4.0 ± 1.0	4.0 ± 1.2
Duration (min)	197 ± 59	198 ± 120	179 ± 78

Mean ± SD is shown. No significant differences were found. IRL = inspiratory resistive loading; PLA = placebo; CON = control

3-4.4 Lung Function

Lung function, as assessed by FEV_1, FVC, FEV_1/FVC ratio, and PEF measured at baseline as shown in Table 3-2. All values are within normal limits and values post interventions were unremarkable.

Table 3-2 Luug Function at baseline

Parameter	IRL n = 5	PLA n = 5	CON n = 5
FEV_1 (l)	3.7 ± 0.7	3.7 ± 1.0	3.9 ± 0.8
	(103 ± 12)	(93 ± 8)	(94 ± 10)
FVC (l)	4.4 ± 0.6	4.6 ± 1.1	4.9 ± 0.9
	(106 ± 10)	(99 ± 4)	(98 ± 9)
FEV_1/FVC ratio	0.84 ± 0.06	0.80 ± 0.06	0.80 ± 0.08
	(97 ± 8)	(95 ± 10)	(96 ± 10)
PEF ($l.s^{-1}$)	10.5 ± 1.8	8.7 ± 1.6	8.2 ± 2.4
	(114 ± 12)	(98 ± 10)	(97 ± 8)

Mean ± SD is shown. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory flow. Figures in brackets are % of predicted (Quanjer *et al.* 1993).

3-4.5 Respiratory muscle function

Following intervention, MIP (an indicator of inspiratory muscle strength), sustained MIP (SMIP), sum of SMIP (indicator of inspiratory muscle endurance), and the duration of inspiration were all significantly improved compared to baseline in the IRL group (Table 3-3). However, MEP was not improved following the intervention. The control group, who performed one TIRE at baseline and again after the 10-wk period did not improve in any of the above parameters.

Table 3-3 Effect of IRL on respiratory muscle function

Parameter	IRL n = 5		PLA n = 5		CON n = 5	
	Pre	Post	Pre	Post	Pre	Post
MIP (cm H_2O)	134 ± 35	180 ± 39*	136 ± 26	140.2 ± 29	127 ± 40	128 ± 39
MEP (cm H_2O)	138 ±26	144 ± 26	126 ± 40	126 ± 28	117 ± 21	126 ± 32
SMIP (PTU)	987 ± 144	1303 ± 212*	~	~	897 ± 231	915 ± 219
ΣSMIP × 10^3 (PTU)	29.6 ± 3.1	40.7 ± 5.8*	~	~	23.9 ± 1.1	24.9 ± 2.9
Time (s)	13.6 ± 3.0	17.4 ± 1.6*	~	~	15.8 ± 3.6	15.8 ± 4.4

Mean ± SD is shown. * Significantly different change from baseline (P<0.05).
MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; SMIP, sustained maximum inspiratory pressure; ΣSMIP, sum of SMIP; Time, duration of inspiration. ~ these parameters were not measured in the placebo group because performing the TIRE with normal resistance would unblind the study.

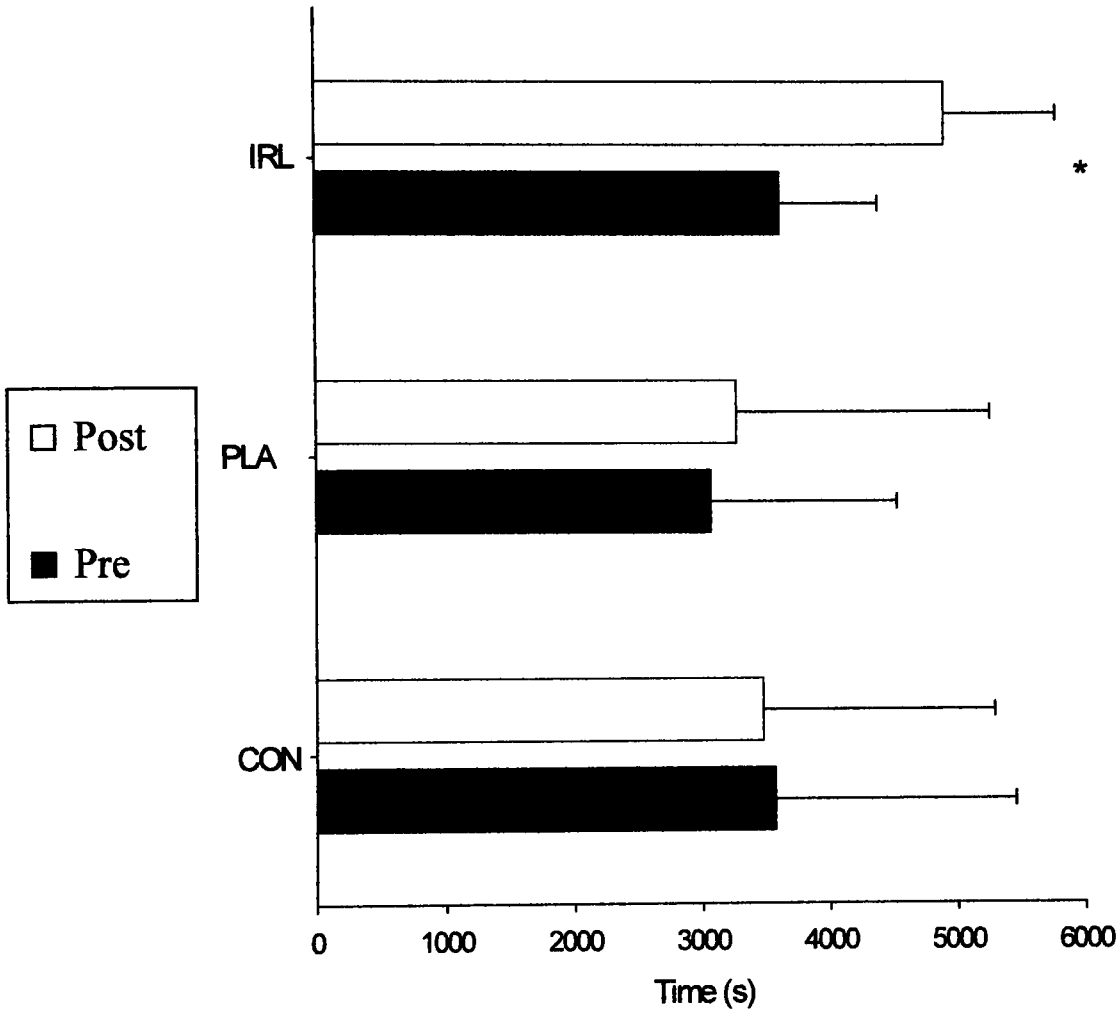
3-4.6 Incremental Cycle Test

Maximum power output (Wmax) for the subjects did not differ between the inspiratory resistive loading, placebo and control groups. Similarly, these values remained unchanged after the intervention for the three groups. Peak oxygen uptake ($\dot{V}O_2$peak) remained unchanged in all three groups.

3-4.7 Cycling endurance ($Tlim_{75}$)

Prior to the intervention, cycling time to exhaustion did not differ between the three groups (IRL, PLA, CON). After the intervention $Tlim_{75}$ improved by 36% in the IRL group ($P < 0.05$), a change that was not apparent in either the placebo or control group (Figure 3-3).

Figure 3-3 Effects of 10 wks of inspiratory resistive loading (IRL), placebo (PLA), or no respiratory training (CON) on $Tlim_{75}$



Values are mean ± SD. * Significantly different from pre-test ($P < 0.05$). All data are the best of the two trials. Shaded bars, pre-; unshaded, post-intervention.

Of the subjects in the IRL group, all five improved their individual cycling time to exhaustion by an average value of 1292 ± 607 s, whereas in the placebo group an average increase of 202 ± 526 s was not significant. Of the controls, an overall decrease in the cycling time to exhaustion of –96 ± 157 s was also not significant.

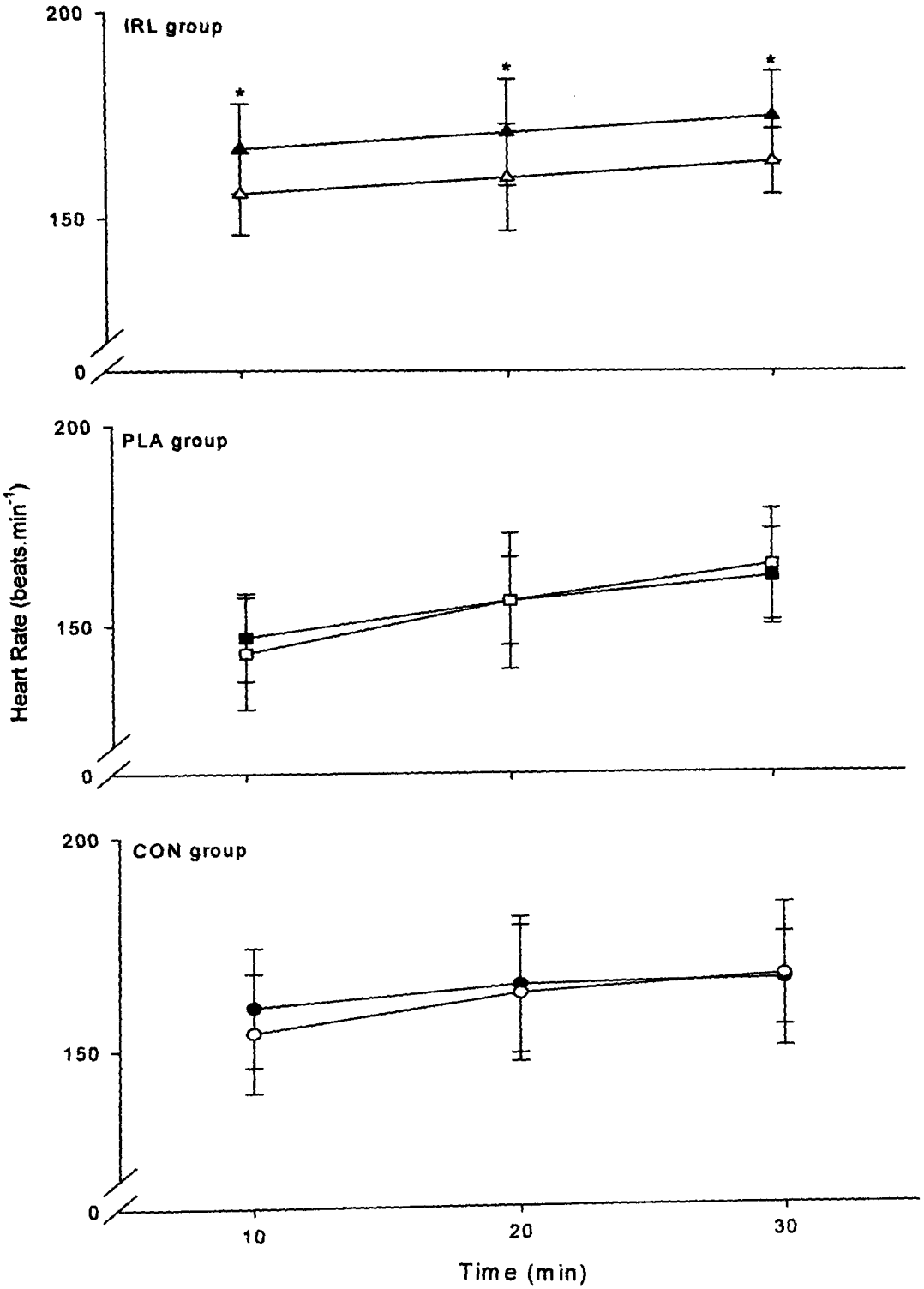
Heart rate, ventilation, $\dot{V}O_2$, and RER were measured throughout the time to exhaustion trial. We compared these parameters (pre- and post) at 10, 20, and 30 minutes into exercise). After the IRL intervention heart rate is significantly decreased

at 10, 20 and 30 minutes (Figure 3-4), and ventilation is significantly decreased at 20 and 30 minutes (Figure 3-5) ($P < 0.05$).

$\dot{V}O_2$, respiratory frequency and RER were not significantly altered following the IRL intervention (Table 3-4). There was no change in any of the measured parameters during exercise for either placebo or control groups.

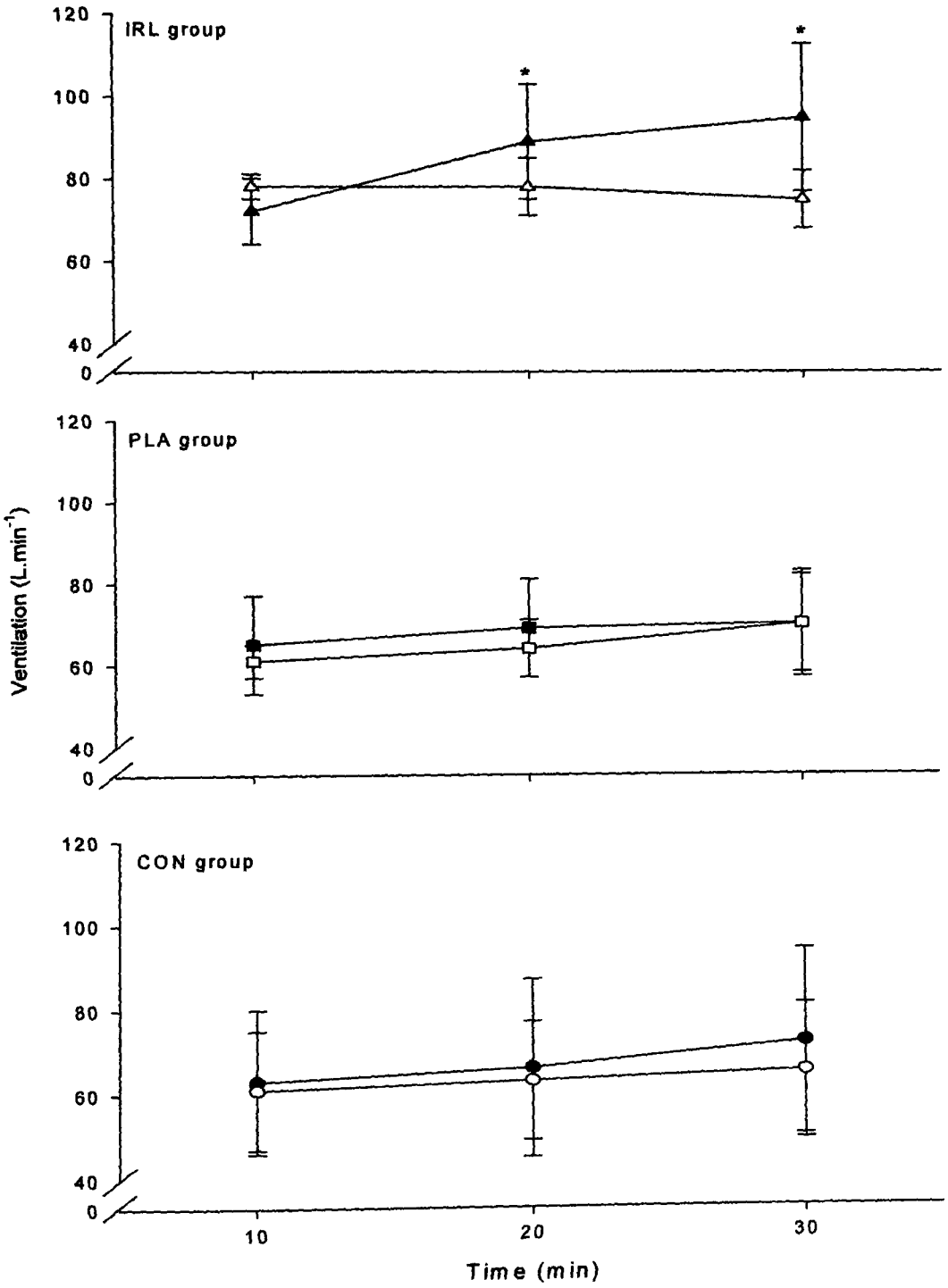
All subjects expressed their perceived exertion at set time intervals throughout the trial using the Borg scale (Figure 3-6). Substantial effects were observed for the IRL group during the trial with ratings consistently lower for all IRL subjects, but reaching significance only at the end of exercise (17.2 ± 1.1 and 15.4 ± 0.9, $P < 0.05$) or when averaged across the whole trial (14.2 ± 1.3 pre-IRL and 12.9 ± 1.4 post-IRL, $P < 0.05$). There was no observed difference in the RPE for either the PLA or CON groups (Figure 3-6).

Figure 3-4 **Effects of 10-wks of IRL, PLA, or CON on heart rate during $Tlim_{75}$**



Values are mean ± SD. * Significantly different from pre-test ($P < 0.05$). Shaded symbols, pre-; unshaded, post-intervention.

Figure 3-5 Effects of 10-wks of IRL, PLA, or CON on minute ventilation during $Tlim_{75}$



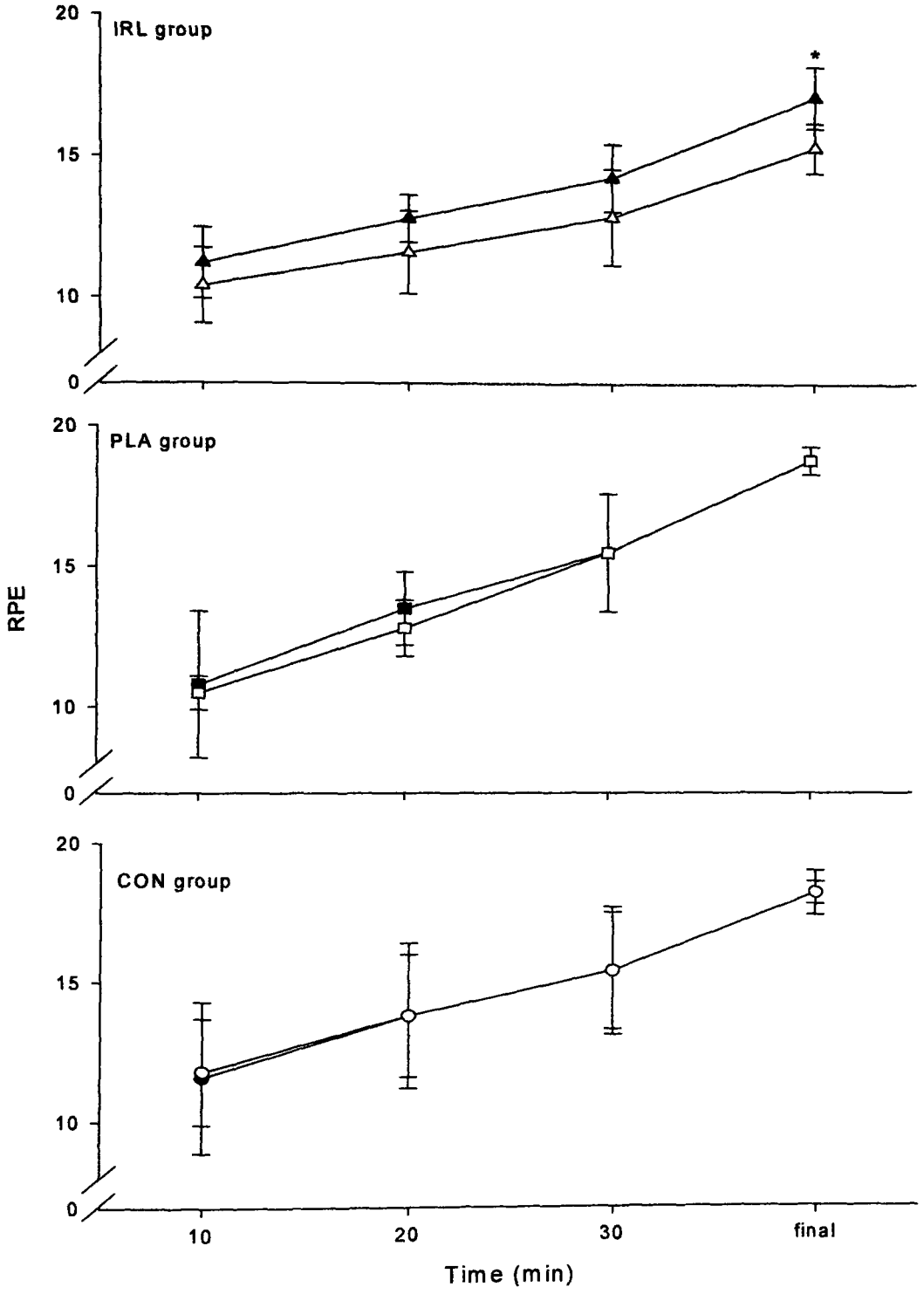
Values are mean ± SD. * Significantly different from pre-test ($P < 0.05$). Shaded symbols, pre-; unshaded, post-intervention.

Table 3-4 B_f, $\dot{V}O_2$, and RER during the cycling endurance trial at 75% $\dot{V}O_{2PEAK}$

Parameter	Time (min)	IRL (n = 5)		PLA (n = 5)		CON (n = 5)	
		Pre	Post	Pre	Post	Pre	Post
	10	34.2 ± 4.0	34.6 ± 2.2	34.2 ± 4.2	33.3 ± 4.9	31.6 ± 5.4	32.8 ± 6.3
B_f (breaths.min^{-1})	20	38.0 ± 4.4	37.2 ± 3.1	37.8 ± 5.2	36.5 ± 2.1	33.2 ± 6.1	32.8 ± 4.7
	30	44.2 ± 10.6	39.4 ± 3.3	44.0 ± 8.0	45.3 ± 10.9	40.6 ± 7.8	35.6 ± 7.2
	10	2.6 ± 0.3	2.7 ± 0.2	1.9 ± 0.4	1.9 ± 0.2	2.2 ± 0.7	2.2 ± 0.4
$\dot{V}O_2$ (L.min^{-1})	20	2.6 ± 0.3	2.7 ± 0.3	1.9 ± 0.4	2.0 ± 0.3	2.2 ± 0.8	2.2 ± 0.5
	30	2.6 ± 0.5	2.8 ± 0.2	1.8 ± 0.5	2.0 ± 0.4	2.2 ± 0.8	2.2 ± 0.4
	10	1.04 ± 0.09	0.99 ± 0.03	1.08 ± 0.04	1.03 ± 0.13	1.09 ± 0.05	1.06 ± 0.09
RER	20	1.03 ± 0.10	0.96 ± 0.03	1.07 ± 0.05	1.02 ± 0.17	1.09 ± 0.03	1.04 ± 0.06
	30	1.03 ± 0.10	0.96 ± 0.05	1.06 ± 0.07	1.00 ± 0.12	1.08 ± 0.03	1.02 ± 0.09

Mean ± SD are shown. B_f = respiratory frequency; $\dot{V}O_2$ = oxygen consumption; RER = respiratory exchange ratio.

Figure 3-6 Effects of 10-wks of IRL, PLA, or CON on ratings of perceived exertion (RPE) during $Tlim_{75}$

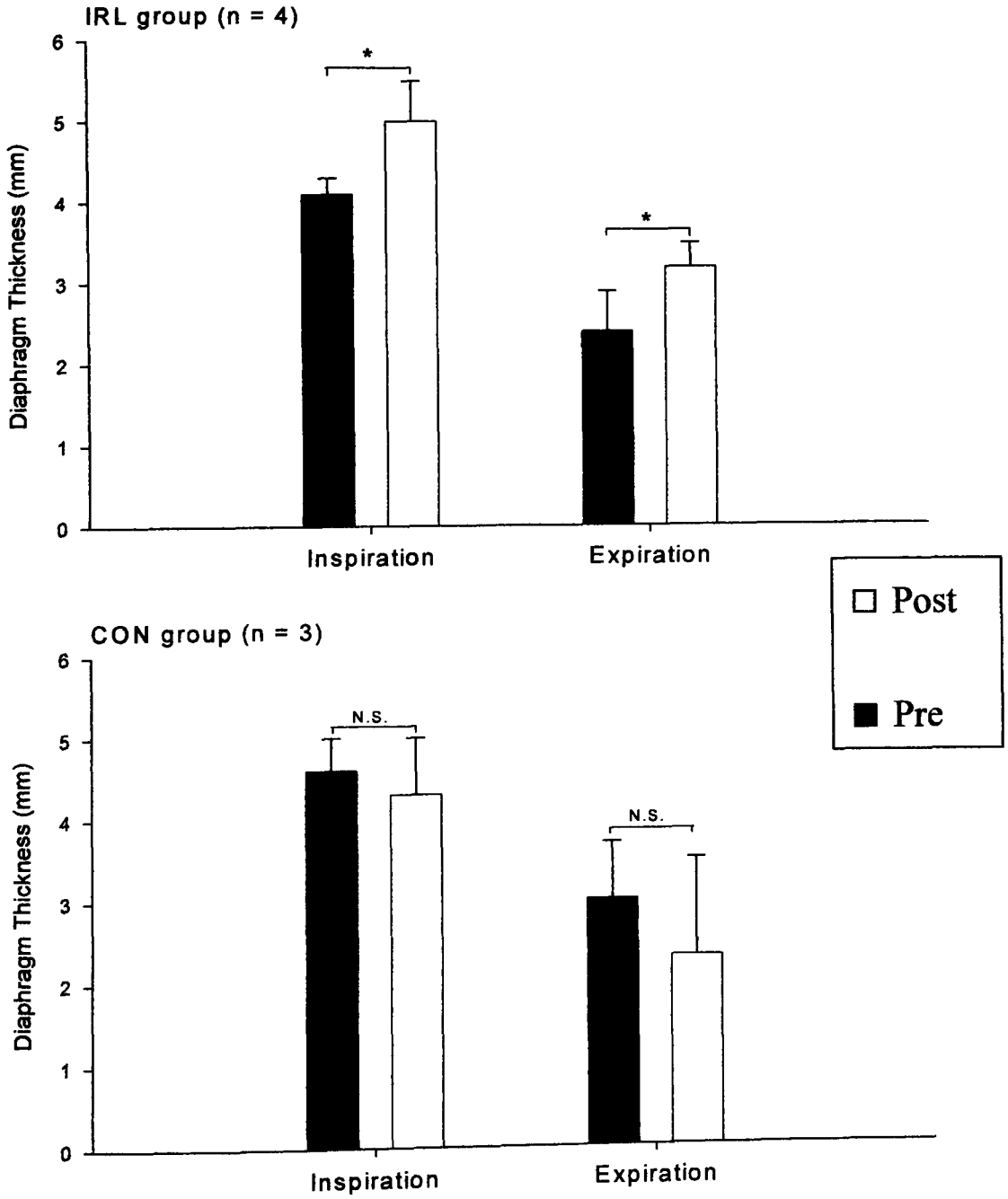


Values are mean ± SD. * Significantly different from pre-test ($P < 0.05$). Shaded symbols, pre-; unshaded, post-intervention.

3-4.8 Diaphragm thickness

A significant increase in diaphragm thickness at both maximal inspiration and expiration was observed in the IRL group (Figure 3-7). This effect was not observed in the control subjects.

Figure 3-7 Effect of 10-wks of IRL vs. CON on diaphragm thickness



Values are mean ± SD. * Significantly different from pre-test ($P < 0.05$). Pre- black bars; post, clear bars.

3-5 Discussion

3-5.1 Main findings

The aim of the present study was to assess the effect of a 10-wk flow-resistive IRL intervention on the cycling time to exhaustion at an intensity prescribed to elicit 75% of peak oxygen consumption. A placebo and control group facilitated the effect of familiarisation and placebo. The main finding was that inspiratory muscle training attenuated the heart rate, ventilatory and perceptual response to constant workload sub-maximal exercise, and improved the cycling time to exhaustion. Familiarisation was not likely a factor following the decision to drop the initial cycling time to exhaustion (Section 3-4.1; Figure 3-2). No changes were apparent in the placebo group.

3-5.2 Placebo effects

The placebo intervention was designed to fulfil the criteria for a true placebo, as outlined by Ojaunen (1994) i.e. to be both inert, and to generate expectations, involvement, subjective utility, and be meaningful to the subjects. The majority of the studies into IRL have used the placebo employed in the present study, which employs a minimal resistance (Caine and McConnell 1998; Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Holm *et al.* 2004; Wells *et al.* 2005) but have been recently criticised for failing to activate the important placebo factors mentioned above (Sonetti *et al.* 2001). However, we feel that the assessment of the placebo effect in the present study, with all subjects exposed to the same respiratory protocol and biofeedback, was comprehensive.

It is possible that our placebo group underwent neuromuscular development as they performed the same protocol at 80% of their 'maximal' inspiration. However, we found no evidence for this in the measurements of respiratory muscle strength, or in terms of exercise performance. The level of subject involvement in this study was the same as for the actual IRL group, and thus enables us to document the effect that this attention has on an open-ended cycling trial to exhaustion.

3-5.3 Respiratory muscle function

Inspiratory muscle strength, as measured by MIP, was improved in the IRL group but not in the PLA or CON group, whereas MEP remained unchanged in all three groups. The significant 34% increase in MIP demonstrates the specificity of the training and is consistent with previous improvements reported following an IRL intervention (Inbar *et al.* 2000; Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Williams *et al.* 2002; Guenette *et al.* 2006; Downey *et al.* 2007; Griffiths and McConnell 2007), although Sonetti *et al.* (2001) reported only an 8% improvement albeit following a shorter intervention (5 wk) compared with the present study.

Previously, respiratory muscle training performed at different lung volumes has demonstrated greatest effect within the lung volume in which the training was prescribed (Tzelepis *et al.* 1994). The method of training utilised in this study required subjects to inspire at 80% of their maximum through the full range of volume i.e. from residual volume to total lung capacity. Therefore, an effect would be expected throughout the whole lung volume. This is reflected by the increase in the sustained maximal inspiratory pressure, determined by measuring pressure generation over a full inspiratory effort. A demonstrable increase throughout the inspiration in terms of

the pressure generated was apparent for all of the subjects who received the IRL intervention.

The inability to adequately fix workload or volume at which training occurred has confounded earlier studies (Sheel 2002). Our methodology ensured accurate prescription of the workload with reassessment of SMIP and resetting of the workload before each training session. In addition, biofeedback via an on-screen training template was provided so that subjects could consistently perform IRL at 80% of the maximal inspiratory effort. Previously, this method has been shown to induce diaphragmatic fatigue (Chatwin *et al.* 2001) suggesting that the type of training used in the present study provides an adequate stimulus to induce an adaptation. Indeed, the increased diaphragm thickness on inspiration and expiration reported here would suggest that this is the case. However, although measurement of diaphragm thickness was not performed for a minimum of 48 hrs after the last IRL session, the possibility that diaphragm thickness was increased as a result of intracellular fluid changes exists. That inspiratory muscle strength was significantly increased in the same subjects may suggest that this was not the case.

That respiratory muscle function can be improved following a specific inspiratory muscle training intervention is well established (Sheel 2002) but translation into an improvement in whole-body exercise performance is more controversial (Fairbarn *et al.* 1991; Hanel and Secher 1991; Inbar *et al.* 2000; Sonetti *et al.* 2001; Williams *et al.* 2002; Downey *et al.* 2007; Verges *et al.* 2007). This controversy remains with the different methods of respiratory training (IRL or VIH) and outcome measures (maximal exercise, endurance exercise, or performance) being the obvious confounding variables. When discussing respiratory muscle training, the majority of authors have produced the widespread impression that resistive methods of respiratory

training are synonymous with the hyperpnoea types. It is our opinion however, that because the respiratory muscles can be trained specifically for strength or endurance (Leith and Bradley 1976) then these types of training are worthy of separate investigation. Sonetti *et al.* (2001) overcame this problem by combining the two types of training, but as pointed out by Romer *et al.* (2002) the concurrent strength and endurance training might have inhibited strength development and would explain the small 8% increase in maximum inspiratory pressure reported. Investigating and reviewing the results of IRL and VIH training separately may well provide insights into the mechanism behind respiratory muscle training which is yet to be adequately explained.

3-5.4 Diaphragm thickness

Our data demonstrates a significant change in diaphragm thickness following a 10-wk IRL intervention as measured by using B-mode ultrasonography (4.1 ± 0.2 mm to 5.0 ± 0.2 mm). Previously, McCool *et al.* (1997) have reported a correlation between diaphragm strength and thickness, so this finding is not unexpected. Diaphragm dimensions and IRL deserve more in depth examination as these measures may provide more insight into the mechanism behind IRL-induced improvements in endurance capacity. An increase of 12% in diaphragm thickness following an IRL intervention has been reported previously and coincided with an increase in respiratory muscle strength of 41% and exercise capacity (Enright *et al.* 2006). However, Downey *et al.* (2007) reported increases in diaphragm thickness of 8-12% in seven healthy subjects following an IRL intervention set at 50% MIP but reported no improvements in whole body exercise were found in running time to exhaustion at 85% $\dot{V}O_2$peak.

3-5.5 Maximal performance

The improvements in respiratory muscle function observed in the present study did not translate into an improvement in either $\dot{V}O_2$peak or Wmax, which confirms previous findings on maximal exercise, with no studies reporting a change (Fairbarn *et al.* 1991; Hanel and Secher 1991; Inbar *et al.* 2000; Guenette *et al.* 2006; Downey *et al.* 2007). Healthy respiratory muscles do not limit $\dot{V}O_2$max, but rather are a part of it just as much as any other muscles used during exercise. Lowering the metabolic cost of ventilation would increase the exercise intensity required to elicit $\dot{V}O_2$peak, but $\dot{V}O_2$peak would not be changed unless oxygen were more efficiently extracted by the locomotor muscles compared with those of the respiratory system. This has been previously demonstrated using respiratory muscle unloading (Harms *et al.* 1998) with a greater power output preserving $\dot{V}O_2$peak, presumably because of the redistribution of blood flow (Harms *et al.* 2000). In our subjects, $\dot{V}O_2$peak measures pre-post IRL were not significantly different, and it is interesting to note that before conducting IRL, the maximum workload for the five subjects was 330W and post-loading was 350W.

3-5.6 Changes in $Tlim_{75}$

The observed improvements in respiratory muscle function represent a 36% significant increase in cycling endurance capacity with an increase in cycling time to exhaustion at 75% $\dot{V}O_2$peak from 60 ± 12 min pre-IRL to 82 ± 14 min post-IRL. This is inline with previous studies where an increase in cycling time to exhaustion has

been demonstrated using both VIH (Boutellier *et al.* 1992; Boutellier and Piwko 1992; Spengler *et al.* 1999; Markov *et al.* 2001; Stuessi *et al.* 2001; McMahon *et al.* 2002; Leddy *et al.* 2007) and IRL techniques (Caine and McConnell 1998; Guenette *et al.* 2006; Downey *et al.* 2007). During the trial, a decrease in both heart rate and ventilation was also apparent. Previously, a decrease in minute ventilation following a VIH intervention has been reported (Boutellier *et al.* 1992; Boutellier and Piwko 1992), but subsequent studies by the same group have failed to substantiate these findings (Spengler *et al.* 1999; Markov *et al.* 2001; Stuessi *et al.* 2001; McMahon *et al.* 2002). Leddy *et al.* (2007) have since repeated these findings of a decrease in ventilation (-7%) following VIH. A decrease in heart rate has been noted by a single study following a VIH intervention (Swanson 1998) and by one study examining IRL (Griffiths and McConnell 2007).

It is unlikely that the improvements observed in the present study were due to familiarisation, because the rigorous nature of our testing protocol which included taking the best result of two trials, both pre- and post-intervention, and excluding the results of the initial trial. Indeed, if familiarisation were a factor, despite our efforts, then we would have expected this to reveal itself as an improvement in $Tlim_{75}$ in the control group, which was not apparent. The inclusion of both a placebo and control group within the present study design allowed us to assess the magnitude of the placebo effect in our subjects. The improvements observed in our placebo group were not significant, and therefore not a major factor in the interpretation of this data.

We attempted to control for other possible explanations for the observed progress in $Tlim_{75}$, for example by providing our subjects with their previously recorded diets to follow in the 48-hr lead-up to a trial. Although the subjects did not record exercise intensity i.e. heart rate, whole body exercise training did not differ between or within

the three groups as determined by frequency and duration of training per wk. Training intensity during the intervention is a potentially confounding variable, but as all subjects understood the nature of the study, we do not consider it a major factor. For these reasons we believe that the observed affects can be explained exclusively by the IRL intervention.

The experimental intervention in the present study was therefore responsible for improvements in respiratory muscle function, which then, either directly or indirectly resulted in an increase in the cycling time to exhaustion with a concomitant decrease in exercising heart rate, ventilation, and rating of perceived exertion.

3-6 Conclusion

IRL using the TIRE device produced an increase in both the strength and endurance of the inspiratory muscles. These improvements resulted in an increase in cycling endurance at 75% $\dot{V}O_2$peak that was not apparent in either the placebo or control group. Familiarisation was kept to a minimum by the use of repeat trials, and the placebo effect was not a significant factor. This is the first study to adequately address the effect of IRL using both a placebo and control group. How these improvements influence actual competitive performance remains to be seen. The mechanisms behind the observed improvements have not yet received adequate attention, but continued studies within the area will hopefully provide the answer.

STUDY TWO – THE EFFECT OF NON-INVASIVE POSITIVE PRESSURE VENTILATION (NIPPV) ON RESPIRATORY MUSCLE STRENGTH AND ENDURANCE

4-1 Abstract

The aim of the present study was to assess the effect of domiciliary non-invasive positive pressure ventilation (NIPPV) on inspiratory muscle strength and endurance in patients with chronic stable type II respiratory failure.

Suitable patients (PaO_2 < 7.3 kPa (on air) & $PaCO_2$ > 6.5 kPa) were admitted to the ward for initiation of NIPPV. This patient group was studied prospectively (Group 1, n = 13, age = 64 ± 7) and a second group of long-term recipients of NIPPV were used as an historical group (Group 2, n = 10, 67 ± 12).

There were significant improvements in daytime blood gas tensions (PaO_2 pre = 6.24 ± 1.42 to post = 8.06 ± 1.24 kPa; $PaCO_2$ pre = 7.59 ± 0.87 to post = 6.45 ± 0.73 kPa) and respiratory muscle function (MIP pre = 37 ± 14 to post 53 ± 26 cm H_2O) when comparing Group 1 baseline to 3 months, and Group 1 to Group 2 (MIP = 52 ± 13 cm H_2O). Quality of life, oxygen saturation, and bicarbonate retention following 3 months domiciliary NIPPV was significantly better than when compared to baseline (Group 1).

Domiciliary NIPPV may be a useful method of treating type II respiratory failure patients by temporarily alleviating the load to which the respiratory muscles are exposed to and allowing adequate rest and relief for said muscles to adapt to the abnormal load which they are ordinarily exposed to on a daily basis.

4-2 Introduction

Respiratory failure is defined as a failure of maintenance of normal blood gas tensions, and may be further subdivided according to whether the arterial PCO_2 is normal / low (type I) or elevated (type II) (Lumb 2000).

The aetiology of respiratory failure is likely to be multifactorial with different factors assuming greater or lesser importance even in patients with the same condition. Furthermore, in an individual patient, there may be differences at various stages of the illness, and the same is true for the mechanism of benefit behind non-invasive positive pressure ventilation (NIPPV) (Elliott 1999).

NIPPV has been one of the major advances in respiratory medicine in the last decade (Elliott 2002). Its main aim is to give the patient ventilatory support using a face mask or nasal mask instead of an endotracheal tube (Brigg 1999). NIPPV is effective in patients with extra pulmonary restrictive disorders (Leger *et al.* 1994; Simonds and Elliott 1995), and a number of studies have shown that NIPPV is feasible at home in patients with COPD (Carroll and Branthwaite 1988; Elliott *et al.* 1992; Leger *et al.* 1994; Simonds and Elliott 1995; Kolodziej *et al.* 2007). During NIPPV, overnight abnormal physiology can be corrected, with improvements in gas exchange and sleep quality (Elliott *et al.* 1992), as well as improved exercise capacity and diurnal arterial gas tensions (Elliott *et al.* 1992; Sivasothy *et al.* 1998). Use of healthcare resources may also be reduced (Jones *et al.* 1998), with quality of life (Perrin *et al.* 1997) and functional score (Criner *et al.* 1999) improved.

For breathing to be effective, the respiratory muscle pump must have the capacity to sustain ventilation against a given load. It also requires neural drive from the respiratory centre in the brain stem. That is, there needs to be a balance between load,

capacity, and drive (Elliott 1999). Previously, Diaz *et al.* (2002) determined that the beneficial effects of NIPPV could be explained by a reduction in lung hyperinflation and therefore inspiratory loads. Their results are in agreement with those of Elliott *et al.* (1991) who found that the decrease of hypercapnia after NIPPV was related to a reduction in inspiratory loads, gas trapping, and central breathing drive.

In addition, recovery from respiratory muscle fatigue as measured as an improvement in respiratory muscle capacity has been investigated as a potential mechanism of benefit (Elliott 1999). However, Shapiro *et al.* (1992) concluded that respiratory muscle fatigue did not exist and that little was to be gained by resting the respiratory muscles. Though the outcome measure used in the study (6-min walking distance test) is an unconventional measure of respiratory muscle fatigue and is affected by other factors.

No long-term study has yet shown an improvement in measures of respiratory muscle strength and / or endurance following long-term NIPPV use. Therefore, the following study was designed to explore the effect of NIPPV on respiratory muscle capacity in hypercapnic respiratory failure.

4-3 Methods

4-3.1 Participants

Patients with type II respiratory failure were seen at the Chest Clinic, Nevill Hall Hospital and assessed for suitability of domiciliary NIPPV (PaO_2 < 7.3 kPa (on air) & $PaCO_2$ > 6.5 kPa). Those found suitable were admitted to the ward for initiation of NIPPV. This patient group was studied prospectively (Group 1, n = 13, age = 64 ± 7) and a second group of long-term recipients of NIPPV (>6 months) were used as an historical group (Group 2, n = 10, age = 67 ± 12). The local health authority ethics committee granted ethical approval for this study. Subject characteristics are illustrated in Table 4-1.

Table 4-1 Subject characteristics

	Group 1 (n = 13)	Group 2* (n = 10)
Males / Females	8 / 5	5 / 5
Age (yrs)	64 ± 7	67 ± 12
Stature (m)	1.64 ± 0.09	1.64 ± 0.13
Body Mass (kg)	87.0 ± 17.1	97.9 ± 12.4
BMI ($kg.m^{-2}$)	32.4 ± 6.5	36.5 ± 8.1

Mean ± SD is shown. *Group 2 data at >6 months. BMI = Body Mass Index.

Acute and chronic type II respiratory failure is prevalent in a range of disorders through thoracic cage restrictions (e.g. kyphoscoliosis), neuromuscular disease (e.g.

muscular dystrophy), sleep hypoventilation, obesity and chronic obstructive pulmonary disease (COPD). The diagnoses of the patients in the present study are illustrated in Table 4-2.

Table 4-2 Diagnosis of patients

	COPD	OH	CWD	NMD
Group 1	6	5	2	0
Group 2	2	4	3	1

COPD = chronic obstructive pulmonary disease; OH = obesity hypoventilation; CWD = chest wall disorder; NMD = neuromuscular disease.

All patients had to have received maximal medical treatment supervised by a chest physician with no hospital admission for at least one month before the study and to have remained free of an exacerbation during this time.

4-3.2 Procedure

Once admitted, patients were familiarised with the ventilatory equipment, fitting of facemask, and instructed in the use of the quick release strap. Patients were discharged on NIPPV (Sullivan VPAP II, ResMed, Sydney) once they were confident in its use. In contrast to negative pressure ventilation, NIPPV is usually delivered during sleep. All patients were then followed up in multidisciplinary clinics. The prospective group of patients had their spirometry, blood gases, and respiratory muscle strength and endurance measured at initiation of NIPPV (baseline data), and 3 months later. The historical group had their spirometry and blood gases checked at the

initiation of NIPPV and when their respiratory muscle function was tested. Patient perception of health was assessed at each time point using St George's Respiratory Questionnaire (SGRQ) and Short Form 36 (SF36).

Spirometry

The forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow rate (PEF) were measured (Masterscreen, Jaeger, Hoechberg) with three expiratory manoeuvres completed, the best of which were used for subsequent analysis (Quanjer *et al.* 1993). For more detail refer to section 2-2.2.

Blood gas data

A respiratory nurse specialist, experienced in the technique, sampled (earlobe) arterialised capillary blood on arrival at the clinic. The method of capillary sampling was as previously described (Spiro and Dowdeswell 1976) and involves spreading an earlobe (usually the patients left, with a right-handed operator) with nicotinate paste for at least 10 minutes to induce capillary vasodilatation. A stab incision is made in the inferolateral aspect of the pinna from which blood usually flows freely but may require a small amount of manual massage. The arterialised blood collects in a drop on the inferior aspect of the earlobe. It is drawn into a thin glass capillary tube by surface tension under the control of a gloved finger over the open end of the tube and then aspirated into the blood gas analyser (ABL500, Radiometer, Copenhagen). Blood gas tension, oxygen saturation, pH and bicarbonate retention were recorded.

Respiratory muscle function

Maximum inspiratory pressure (MIP) was used to indicate inspiratory muscle strength (Green *et al.* 2002). Pressure generated at the mouth whilst inspiring rapidly from residual volume was measured using a mouthpiece connected to a portable electronic manometer (RTSport, DeVilbiss, Sunrise Medical Ltd, Wollaston, UK). The mouthpiece has a fixed leak via a 2mm diameter aperture that prevented glottal closure during the inspiratory manoeuvre. The leak set a maximum flow during the inspiratory effort ($\approx$450 $ml.s^{-1}$) and allowed continuous measurement of pressure over the full range of vital capacity. MIP was the maximum pressure generated during the inspiration and was taken as the best of three inspirations (Green *et al.* 2002). The sustained maximum inspiratory pressure (SMIP) was also recorded and is reported as the area under the pressure time curve (Chatham *et al.* 1997). In addition, subjects completed the test of incremental respiratory endurance as described in Section 2-2.3. Briefly, inspiratory muscle endurance was assessed by repeating the inspiratory manoeuvre at a submaximal (80%) inspiratory pressure with ever decreasing intermittent rest periods between manoeuvres until task failure.

Health questionnaires

The SF36 is a short questionnaire with 36 items which measure eight multi-term variables: physical functioning (10 items), social functioning (two items), role limitations due to physical problems (four items), role limitations due to emotional problems (three items), mental health (five items), energy and vitality (four items), pain (two items), and general perception of health (five items). For each variable item scores are coded, summed, and transformed on to a scale from 0 (worst possible health state measured by the questionnaire) to 100 (best possible health state) with eight domain scores and overall physical and mental health component summary

scores reported. Normative data for this instrument has been established (Jenkinson *et al.* 1993), as has both the internal consistency and validity (Bousquet *et al.* 1994).

The St Georges Respiratory Questionnaire (SGRQ) is a respiratory-specific health related quality of life measure used to assess patients with respiratory disease. The measure consists of 50 (76 responses) items that produce three domain and one overall score as follows: Symptom (frequency and severity); Activity (activities that cause or are limited by breathlessness); Impacts (social functioning, psychological disturbances resulting from airways disease). Scores for each section and a total score range from 0 to 100 but in this case higher scores indicate poor health. Normative data, reproducibility and internal consistency for this instrument has been reported (Jones *et al.* 1991; Jones *et al.* 1992).

Questionnaires were completed in face-to-face interviews by the same interviewer each time (AG).

4-3.3 Statistical Analysis

Shapiro-Wilks tests were applied to data from each dependent variable and confirmed distribution normality. Student's paired-samples *t*-test was conducted to evaluate the impact of the NIPPV intervention on Group 1 (prospective group) patients. An independent-samples *t*-test was conducted to compare Group 2 data (historical group) to that of Group 1. An alpha of less than 0.05 was accepted as statistically significant in all tests. All data are presented as mean and 1 standard deviation (SD).

4-4 Results

Before any conclusions regarding NIPPV use can be drawn it is important to confirm that effective ventilation has been delivered. In the present study this was determined primarily by the extent of NIPPV use i.e. hours / day. The average use / compliance for our patients during the course of this study was 5.92 ± 2.5 hours / day taken from the NIPPV device itself.

Spirometry

There was no difference in lung function between the groups; however following three months of NIPPV, there was a significant increase in FEV_1 and FVC in group 1 patients (Table 4-3).

Table 4-3 Spirometry

	Group 1 (baseline) (n = 13)	Group 1 (3 month) (n = 9)	Group 2 (>6 month) (n = 10)
FEV_1 ($L.s^{-1}$)	0.83 ± 0.40	0.87 ± 0.48*	1.17 ± 0.56
FVC (L)	1.55 ± 0.60	1.61 ± 0.73*	1.87 ± 0.65
FEV_1/FVC%	56 ± 19	57 ± 18	61 ± 15
PEF ($L.s^{-1}$)	3.84 ± 1.92	3.55 ± 0.98	4.15 ± 1.54

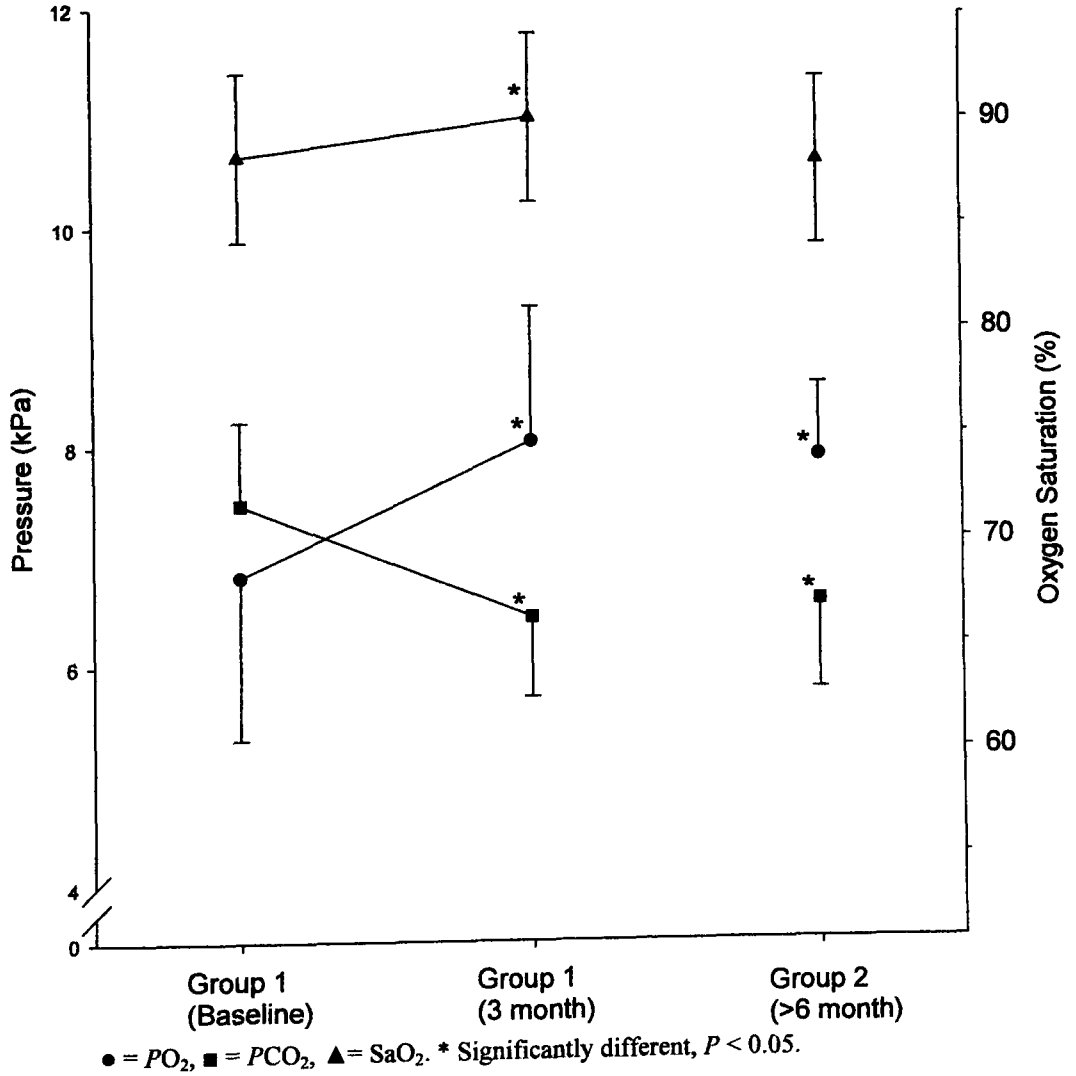
Mean ± SD is shown. * significantly different from Group 1 (baseline), $P < 0.05$. FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; PEF = peak expiratory flow.

Blood gas data

Group 2 patients who had used NIPPV for more than 6 months had significantly higher PO_2 and lower PCO_2 than group 1 patients. Similarly, both these measures

were greater in group 1 following 3 months of NIPPV (Figure 4-1). Oxygen saturation was improved at 3 months in group 1, but there was no difference in this measure between groups 1 and 2.

Figure 4-1 Blood gas tensions and O_2 saturation



● = PO_2, ■ = PCO_2, ▲= SaO_2. * Significantly different, $P < 0.05$.

In addition, bicarbonate retention was lower in group 1 patients following 3 months NIPPV, a trend which was not observed however for the group 2 patients. There was no change in pH for either of the groups studied (Table 4-4).

Table 4-4 Blood gas data

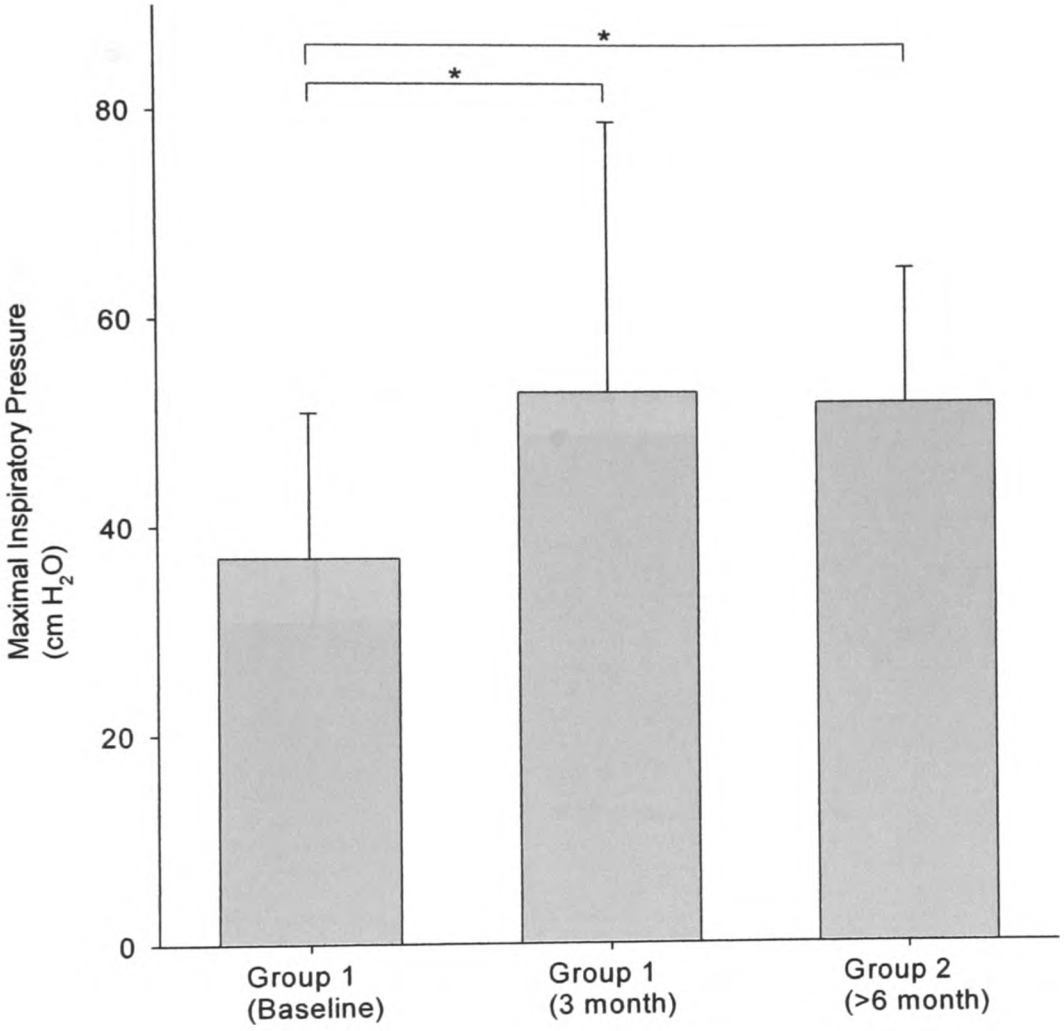
	Group 1 (Baseline) (n = 13)	Group 1 (3 month) (n = 9)	Group 2 (>6 month) (n = 10)
PO_2 (kPa)	6.24 ± 1.42	8.06 ± 1.24*	7.92 ± 0.67*
PCO_2 (kPa)	7.59 ± 0.87	6.45 ± 0.73*	6.59 ± 0.81*
SaO_2 (%)	87.1 ± 4.6	89.9 ± 3.7*	88.0 ± 4.4
pH	7.371 ± 0.511	7.384 ± 0.046	7.367 ± 0.046
HCO_3 ($mmol.L^{-1}$)	31.29 ± 2.59	25.79 ± 3.86*	28.67 ± 3.92

Mean ± SD are shown. * Significantly different from Group 1 (baseline), $P < 0.05$. Normal values: PaO_2 = 11-14kPa; $PaCO_2$ = 4.5 – 6 kPa; pH = 7.35 – 7.45; HCO_3 = 22-28 $mmol.L^{-1}$

Respiratory muscle function

Maximum inspiratory pressure (MIP), an indicator of inspiratory muscle strength, was significantly greater in group 2 compared with group 1, and also was higher when this measure was repeated 3 months later in group 1 (Figure 4-2).

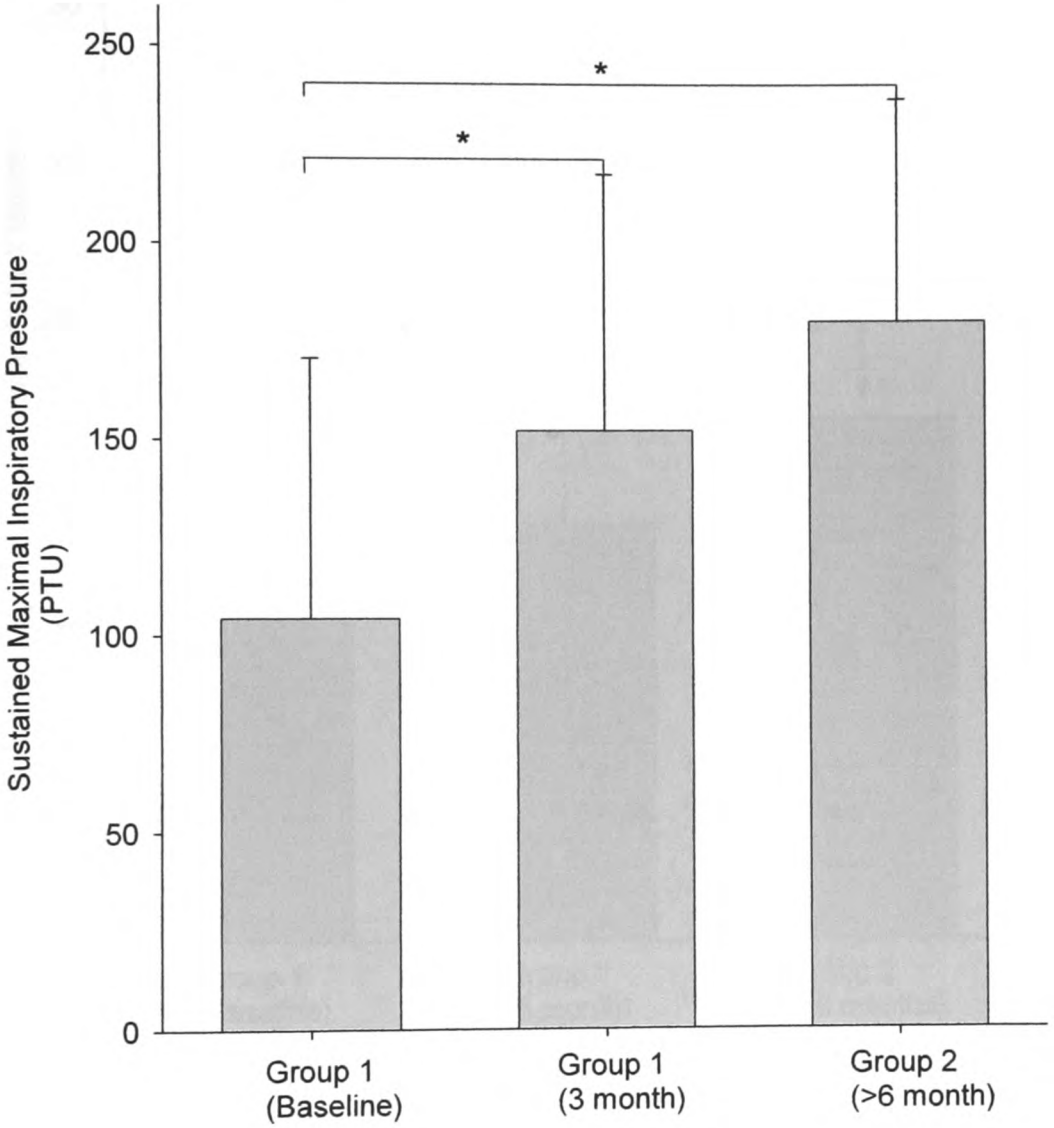
Figure 4-2 Effect of NIPPV on MIP



Data are mean ± SD. * significantly different from group 1 (baseline) (P<0.05).

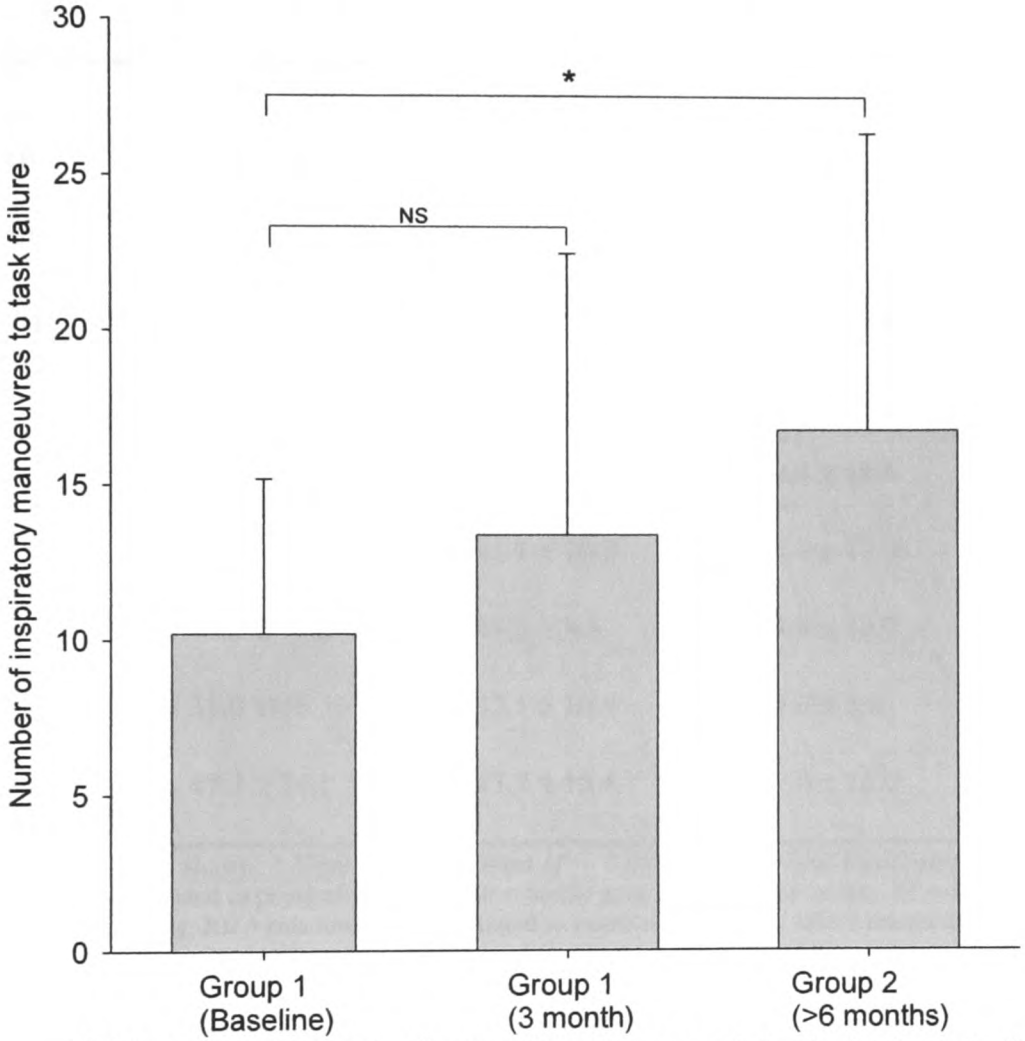
For the measure of sustained maximum inspiratory pressure (SMIP), the same pattern of improvement was observed with an increase reported following the 3 month NIPPV intervention for group 1 patients and a higher value demonstrated when comparing group 1 (baseline) to group 2 patients (Figure 4-3).

Figure 4-3 Effect of NIPPV on SMIP



Inspiratory muscle endurance was assessed as the number of inspiratory manoeuvres to task failure (inability to reproduce 80% of maximum). Group 1 patients after 3 months of NIPPV did not significantly increase their endurance (Figure 4-4). Group 2 patients had significantly higher inspiratory muscle endurance than group 1.

Figure 4-4 Effect of NIPPV on inspiratory muscle endurance



All data are mean ± SD. * significantly different from group 1 (baseline). NS not significantly different from group 1 (baseline) ($P<0.05$).

Health Questionnaires

With SF36 a higher score indicates an improvement in patient perception of health, however there was no change in either the eight domain scores or overall physical and mental health component summary scores after 3 months in group 1 patients or when comparing group 1 to group 2 (Table 4-5).

Table 4-5 Short Form 36 domain and summary scores

	Group 1 (baseline) (n = 13)	Group 1 (3 month) (n = 9)	Group 2 (>6 month) (n = 10)
PF	27.6 ± 11.5	26.5 ± 10.7	24.6 ± 9.0
RP	30.9 ± 11.6	34.1 ± 16.2	40.7 ± 12.9
BP	46.8 ± 15.0	49.6 ± 15.7	48.7 ± 9.1
GH	35.4 ± 11.6	32.7 ± 11.8	37.3 ± 10.3
VT	37.8 ± 12.4	41.8 ± 10.7	48.4 ± 14.4
SF	40.5 ± 16.8	38.9 ± 18.0	39.4 ± 18.6
RE	41.9 ± 18.6	41.1 ± 19.3	35.7 ± 13.9
MH	47.2 ± 13.2	47.5 ± 9.3	48.3 ± 12.7
PCS	31.0 ± 10.3	32.1 ± 10.9	35.6 ± 3.8
MCS	47.7 ± 14.1	47.7 ± 15.4	47.0 ± 15.0

Mean ± SD are shown. * Significantly different ($P < 0.05$). PF = physical functioning; RP = role limitations attributed to physical problems; BP = bodily pain; GH = general health; VT = vitality; SF = social functioning; RE = role limitations attributed to emotional problems; MH = mental health; PCS = physical component summary score; MCS = mental component summary score.

In the case of SGRQ, it is a lower score that indicates better patient perception of respiratory-specific health. Of the three domain and one overall score, no change was observed in symptoms or activity score but a significant reduction was observed for the impacts and total score when comparing group 1 patients at baseline to 3 months of NIPPV intervention (Table 4-6).

Table 4-6 St George's Respiratory Questionnaire Scores

	Group 1 (baseline) (n = 13)	Group 1 (3 month) (n = 9)	Group 2 (>6 month) (n = 10)
Symptoms	58.1 ± 20.1	55.9 ± 24.7	55.8 ± 20.0
Activity	80.4 ± 22.4	78.6 ± 19.8	73.4 ± 21.3
Impacts	57.5 ± 17.9	49.6 ± 15.3*	57.1 ± 18.6
Total	64.0 ± 16.7	59.9 ± 14.2*	62.0 ± 17.1

Mean ± SD are shown. * Significantly different from Group 1 (baseline), $P < 0.05$. Values for normal subjects: Symptoms = 12 ± 3; Activity = 9 ± 3; Impacts = 2 ± 1; Total = 6 ± 1.

4-5 Discussion

4-5.1 Main findings

The main findings of the present study are that the application of domiciliary NIPPV to stable type II respiratory failure patients: 1) improves daytime blood gases; 2) brings about an increase in both inspiratory muscle strength following 3 months NIPPV use, although improvements in inspiratory muscle endurance take longer to achieve (i.e. >6 months); and 3) improves patient perception of respiratory-specific health quality of life.

4-5.2 Respiratory function

We have observed a demonstrable effect of NIPPV on respiratory muscle function. As reviewed by Elliott (1999) small increases in mouth pressure have been cited previously, though in the absence of a control group these improvements may have been due to learning effects and better motivation. The same could be said of the present study if the group 1 patients are considered in isolation, however the group 2 patients also have a higher maximum inspiratory pressure, which is indicative of an actual change in inspiratory muscle capacity. That inspiratory muscle endurance, measured as the number of submaximal (80%) inspirations to task failure, is also greater in patients exposed to NIPPV for >6 months, supports this conclusion. In addition, the familiarisation and repeated manoeuvres performed by each patient should have removed the familiarisation component.

An increase in FEV_1 observed for group 1 patients is suggestive of a reduction in airway obstruction and is in agreement with previous findings (Elliott *et al.* 1991; Diaz *et al.* 2002). Changes in FEV_1 in the present study were proportional to those of FVC, and consequently the FEV_1/FVC ratio did not change, indicating that the improved maximal expiratory flow rates reflected increased volume recruitment i.e. RV was reduced more than TLC with a resultant increase in vital capacity (Diaz *et al.* 2002).

A change in lung volume recruitment has implications for the interpretation of the respiratory muscle function results. Is there an actual change in respiratory muscle strength / endurance or are the increases a result of the tasks being performed at a lower lung volume (thereby placing the inspiratory muscles at a greater mechanical advantage)?

On initiation to the project, lung function tests did not include measures of residual volume and functional residual capacity, however several of the group 1 patients did undertake these measures at baseline and 3 months later as part of a pilot study for a separate investigation. Although these data are not conclusive, subjects demonstrated a reduction in both of these measures, which indicates that lung volume recruitment may indeed be greater following application of NIPPV. This is in agreement with Diaz et al (2002) who observed a significantly lower RV and FRC following three weeks of NIPPV.

4-5.3 Blood gas data

Measurement of arterialised blood gas tensions is a routine part of the assessment of patients with acute and chronic respiratory disorders producing abnormalities of gas exchange. Blood sampling by direct arterial puncture is the accepted technique established in clinical practice despite requiring qualified medical staff to perform it and that it may result in significant discomfort and morbidity for the patient (Flenley 1988). Previous studies have demonstrated a close agreement between arterial and earlobe blood gas values in a normal population (Spiro and Dowdeswell 1976) and this accuracy appears to extend throughout the much wider ranges of arterial PO_2 and PCO_2 found in patients with respiratory disease (Pitkin *et al.* 1994).

Using the earlobe method, the present study demonstrated an improvement in daytime blood gases which is in agreement with the findings of Meecham-Jones *et al.* (1995) following a three month NIPPV intervention in addition to long term oxygen therapy. The significant reduction in bicarbonate in group 1 patients reveals a potential effect of NIPPV on the drive to breathe. If the nocturnal rise in CO_2 is prevented, bicarbonate retention will not occur, and if the CO_2 is lowered and a mild alkalosis

induced there will be excretion of bicarbonate and restoration of chemosensitivity to CO_2 (Elliott 1999). Indeed, Berthon-Jones and Sullivan (1987) demonstrated a left shift of the ventilatory response curve to progressive hypercapnia in patients with severe obstructive sleep apnoea after 90 days treatment with continuous positive airway pressure. Although not measured in the present study, our blood gas data is suggestive of an increase in the drive to breathe.

The mechanisms underlying changes in blood gas tensions induced by NIPPV are not completely understood (Diaz *et al.* 2002). It has been suggested that NIPPV may reduce the work of breathing, thereby facilitating recovery from inspiratory muscle fatigue (Mehta and Hill 2001). Several investigators have shown that the application of non-invasive ventilation, using both positive- and negative-pressure techniques, may produce significant reductions in diaphragmatic electromyographic (EMG) activity and work of breathing, implying muscle rest (Belman *et al.* 1990; Shapiro *et al.* 1992). However, this hypothesis has not been supported by the studies of Elliott *et al.* (1991) and Diaz *et al.* (2002) who concluded that the beneficial effects of NIPPV could be explained by a reduction in lung hyperinflation and inspiratory loads.

The present study is the first long-term study of NIPPV to demonstrate an improvement in measures of respiratory muscle strength and endurance; these data would support an increase in respiratory muscle capacity as a result of their being rested. However, as mentioned above, because our spirometry data suggested an increase in lung volume recruitment, it is not possible to conclude that this is the case. Further study is required to ascertain the mechanism behind changes following NIPPV, but it is unlikely that NIPPV brought about a change in lung volume recruitment great enough to account for the changes in inspiratory muscle strength.

4-5.4 Questionnaires

An improvement in PO_2 and PCO_2 is usually taken as a marker of successful ventilation (Elliott 1999), but patients receiving domiciliary ventilation often report an improved sense of well-being and better quality sleep as well as a reduction in the sensation of breathlessness. These improvements may occur with only small changes in arterial blood gas tensions. Indeed, in the present study we have observed a significant effect of NIPPV on the outcome measures for St George's Respiratory Questionnaire with specific decreases in both the Impacts domain score and overall Summary score.

Scores for SGRQ range from 0 to 100, with higher scores indicating poor health, have been established for normal subjects (Jones *et al.* 1991). Our patients have recorded much higher scores than is normal and reflects just how ill these type II respiratory failure patients are. Indeed, there are significant correlations between domain scores and other measures of disease activity i.e. FEV_1, FVC, SaO_2 at rest, and 6-min walk distance (Jones *et al.* 1992). Based on empirical data, a mean change score of 4 units is associated with slightly efficacious treatment, 8 units for moderately efficacious change and 12 units for very efficacious treatment (Jones *et al.* 1991; Jones 1994). Group 1 patients showed a mean change score of 7.9 ± 2 units for the Impacts domain score and 4.0 ± 1 units for Total score following a three month NIPPV domiciliary use. These data show that NIPPV is capable of bringing about a clinically significant change in the quality of life for patients suffering with hypercapnic respiratory failure and are in agreement with a previous study of NIPPV, where significant improvements in symptom, impacts, and total scores were reported (Meecham-Jones *et al.* 1995).

Like the SGRQ, Short Form 36 (SF36) is scored on a scale of 0 to 100, but in this instance a higher score indicates improved general health. Normative data for this instrument has been established (Jenkinson *et al.* 1993), and its responsiveness to changes in severity of disease reported (Ware *et al.* 1998). However, general health questionnaires by the nature of their design provide restricted coverage of the specific effects of a given disease and this may explain the lack of any changes in this measure. In addition, quality of life for type II patients tends to get worse over time so the fact that there was no change in these measures for our patients may have some clinical significance. A recent study of patients with restrictive ventilatory disorders undergoing positive pressure ventilation via nasal mask or tracheostomy found that quality of life at a given time was correlated with sleep quality (Pehrsson *et al.* 1994). Although this could not be confirmed in the present study, improvements in sleep following the NIPPV intervention in our patients may have contributed to the parallel improvements in quality of life.

4-6 Conclusion

In summary, these results show that application of non-invasive positive pressure ventilation to type II respiratory failure patients can significantly improve daytime blood gas tensions, and this observation coincides with an improvement in inspiratory muscle function. Quality of life is also better following three months of domiciliary NIPPV use. Further study is required to determine the mechanism behind these improvements but it is likely that it is due to an increase in the capacity of the respiratory muscles as a result of temporarily alleviating the load to which the

respiratory muscles are exposed to and providing the opportunity for these muscles to adapt.

STUDY THREE – EFFECTS OF LONG TERM ANABOLIC ANDROGENIC STEROID (AAS) ADMINISTRATION ON RESPIRATORY FUNCTION

5-1 Abstract

The purpose of this study was to investigate the effects of long-term anabolic androgenic steroids (AAS) administration on respiratory function.

Subject groups consisted of AAS users (n = 9) who were still using AAS at the time of testing (SU); AAS users (n = 6) who had been abstinent for a period greater than three months (SA), bodybuilding controls (n = 8) (BC), and (n = 8) sedentary male controls (SC). FEV_1, FVC, PEF were measured and reported as percentage of predicted values.

All subjects were within normal range, and there were no differences between groups. Maximum inspiratory pressure (MIP), a gauge of inspiratory muscle strength, and grip strength were both significantly greater in SU ($P < 0.05$) compared with SC (MIP: SU = 148 ± 24, SC = 98 ± 27 cm H_2O; Grip strength: SU = 62.0 ± 5.4, SC = 44.7 ± 5.7 Kgf), no significant difference was found in MIP or grip strength between the other groups. MIP and grip strength was significantly correlated (r = 0.57; $P < 0.05$). There were no differences in the breathing profiles of any of the groups studied.

The data from this study suggest that the combination of resistance training and AAS administration produce a significant increase in MIP in a cohort of long-term AAS users. Although the use of AAS is associated with a variety of potentially hazardous consequences on cardiovascular and hepatic health, we were unable to demonstrate any adverse effect of long term androgen administration on respiratory function. The efficacy of adopting AAS into clinical practice would seem to be plausible should further studies employing therapeutic doses of AAS sustain similar outcomes as demonstrated in the present investigation.

5-2 Introduction

Despite a lack of conclusive evidence, many athletes and sports-persons proclaim that anabolic androgenic steroids (AAS) have muscle building properties that result in improved physical performance, over and above that which can be achieved through natural means. An evaluation of the scientific literature would indicate the potential benefits for athletes engaged in power based sports, as improvements in reaction time (Ariel and Saville 1972), both isometric and isokinetic strength (Alén and Häkkinen 1987; Friedl *et al.* 1991) and muscle power (Alén *et al.* 1984) have been documented following AAS administration. There is no evidence regarding specific effects of AAS that would benefit performance of endurance athletes (Friedl 2000). In fact, AAS induced increases in body mass may hinder the performance of more aerobic based sports involving the propulsion of body mass.

The American College of Sports Medicine (ACSM), in conjunction with the majority of sports governing bodies, deplores the non-therapeutic use of AAS by athletes and sports-persons, because they contravene the fundamental rules and ethical principles of athletic competition. Indeed, the position of the ACSM is that although AAS can increase body weight (often in the lean body compartment), and increase gains in muscular strength above and beyond those obtained through high-intensity exercise and diet alone, their use is associated with adverse effects on liver, cardiovascular system, reproductive system, and psychological status (1987).

Reports of serious side effects related to the use of supraphysiological doses of AAS, such as liver disorders (Cabasso 1994), behavioural disorders (Bagatell and Bremner 1996), serious cardiovascular events (Huie 1994; Nieminem *et al.* 1996), including

sudden death (Dickerman *et al.* 1995) make it impossible to promote their use in many situations.

However, in some clinical situations, the potential for physiological doses of AAS to be beneficial for patients may outweigh their potential to cause harm. AAS have recently been approved for the treatment of cachexia associated with the Human Immuno Deficiency Virus (HIV) (Strawford *et al.* 1999), and more controversially in the treatment of aging males (Hayes 2000). In addition, several studies have shown the merits of using AAS in the treatment of weight loss in chronically ill patients with pulmonary disease (Schols 1995; Ferreira *et al.* 1998).

Malnutrition has been observed in 10 to 26% of outpatients with chronic obstructive pulmonary disease (COPD) and up to 47% of patients hospitalised with acute respiratory failure (Donahoe and Rogers 1990). These patients may benefit from anabolic treatments that lead to an increase in lean body mass. Indeed, AAS administration has been suggested as a way of alleviating muscle dysfunction in subjects with COPD (Casaburi 2000). In normal eugonadal men and in subjects with COPD, AAS have been shown to produce increases in lean body mass and muscle strength (Schols 1995; Bhasin *et al.* 1996). In addition, therapeutic doses of the AAS Nandrolone have been shown to increase maximal inspiratory pressure (MIP), a marker of the strength or force generating properties of the inspiratory muscles, by 10% in patients with tetraplegia (Spungen *et al.* 1999).

However, before these drugs can be adopted into clinical practice, additional studies are necessary to evaluate the effectiveness (Casaburi 2001) and more importantly, the safety (Dobs 1999) of prolonged AAS administration. Information regarding the long-term effects of AAS has both clinical and social relevance. Additionally, there is little available data on the effects of AAS on indices of respiratory function.

The aim of the present study was to examine the effects of supra-physiological AAS use on respiratory function in a group of male bodybuilders who used AAS over a prolonged period of time (approximately twenty years).

5-3 Methods

5-3.1 Foreword

It is important to emphasise that this was a monitoring study, which involved the recruitment of AAS users who had self-administered AAS for long periods of time and continued to do so. All AAS using subjects were counselled as to the hazardous effects of AAS use both before and after participating in the study. Subjects provided written informed consent before participation, which was ethically approved by the Bro-Taf health authority.

5-3.2 Participants

Subjects (n = 31, all male) were recruited to participate in the study. Participants were divided into four distinct groups which consisted of AAS users (n = 9), who were still administering AAS at time of testing (SU); AAS users (n = 6) who had been abstinent from AAS administration for a period greater than three months (SA); bodybuilding controls (n = 8) that did not use any pharmacological ergogenic aids (BC), and (n = 8) apparently healthy sedentary male controls (SC). The SA group had abstained from AAS use for a minimum of 12 weeks prior to examination [mean ± standard deviation (SD): 4.9 ± 1.8 months]. Subject characteristics are described in Table 5-1. The SA group allowed us to ascertain any acute effect of the presence of AAS on the

respiratory measures made, as three months is considered time enough for the bodybuilders to be considered free of administered AAS (Dickerman *et al.* 1999). The type, dosage and period of time that the bodybuilders had been taking AAS were determined using a drug history questionnaire.

Table 5-1 Training and drug history for AAS using vs. control groups

Group ID	**SU**	**SA**	**BC**	**SC**
	n = 9	n = 6	n = 8	n = 8
Variable				
Age (yrs)	41 ± 7	41 ± 11	38 ± 7	44 ± 8
Resistance Training (yrs)	24 ± 3	26 ± 3	17 ± 2	N/A
Training Sessions / Week	4.1 ± 1.2	3.8 ± 1.4	4.3 ± 0.7	N/A
Session Length (min)	58 ± 16	57 ± 12	59 ± 18	N/A
Length of AAS use (yrs)	21 ± 2	20 ± 3	N/A	N/A
Drugs other than AAS	N/A	N/A	N/A	N/A

Values are mean ± Standard Deviation (SD). N/A = Not Applicable. SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

Subjects were enrolled as part of a larger study in which cardiovascular and liver function were both assessed by Dr F Grace. All subjects were non-smokers and had no previous history of chronic respiratory or circulatory disease.

5-3.3 Procedure

Each subject arrived in the laboratory following an overnight fast, not having consumed any alcohol, or performed exercise for 36 hours prior to examination. Before testing, health questionnaires were completed (which included a family history) and concise drug histories were obtained. The most popular steroids used amongst these bodybuilders were: Nandrolone Decanoate, Testosterone, Methoandrostenolone, Oxymetholone, and Stanozolol. The AAS-using cohort administered doses far in excess of those approved for therapeutic purposes.

Anthropometrics

Skinfold measurements were used to determine body fat content. Measurements were taken on the right side of the body with the subjects in a relaxed position, at the medial aspect of the biceps, triceps, sub-scapular and suprailiac sites using calibrated Harpenden Skinfold callipers (British Indicators Ltd, London, UK). Skinfolds were taken in triplicate, with the mean skinfold thickness used as the criterion value. Body density was determined using the equations of Siri (1956), and percentage fat values were calculated using the equation of Durnin and Womersley (1974).

Blood Sampling

Venous blood was sampled from an anticubital vein following an overnight fast and 30 minutes supine rest (Pronk 1993) using the standard Venepuncture method. Blood samples were appropriately centrifuged and immediately stored at -70°C until analysis. Testosterone was analysed on a Bayer Advia Centaur immunoassay analyser, employing chemiluminescense detection (Bayer Diagnostics, Newbury,

Berkshire, UK). Sex hormone binding globulin (SHBG) was determined by enzyme immunoassay (IDS Ltd, Boldon, Tyne & Wear, UK).

Spirometry

The forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured using an online spirometer (Spirosense, Burdick Inc, Milton, WI, USA). Three expiratory manoeuvres were completed, the best of which were used for subsequent analysis (Quanjer *et al.* 1993).

Peripheral skeletal muscle function

Maximal grip strength was measured using an adjustable dynamometer (TKK 5001 Grip-A, Takei Scientific Instruments Co, Ltd, Japan). Participants held the dynamometer in line with the forearm at the level of the thigh and were instructed to squeeze vigorously and attempt to exert maximal force. Three attempts were made with each hand, with the highest score of either hand being recorded.

Inspiratory muscle function

Maximum inspiratory pressure (MIP) was used as an indicator of inspiratory muscle strength by measuring the pressure generated at the mouth when inspiring rapidly from residual volume. Pressure was measured using a mouthpiece connected to a portable electronic manometer (RTSport, DeVilbiss, Sunrise Medical Ltd, Wollaston, UK). The mouthpiece has a fixed leak via a 2-mm diameter aperture that prevented glottal closure during the inspiratory manoeuvre. The leak set a maximum flow during the inspiratory effort (~450 $ml.s^{-1}$) and allowed continuous measurement of pressure over the full range of vital capacity. Maximum inspiratory pressure was the maximum

pressure generated over 1s during the inspiration and was taken as the best of three inspirations (Green *et al.* 2002). The sustained maximum inspiratory pressure (SMIP) was also recorded and is reported as the area under the pressure time curve (Chatham *et al.* 1997). Extra care was taken to ensure that maximal inspiratory pressure was obtained because each subject was assisted by visual feedback on a computer screen and motivated by verbal encouragement.

Breathing Profiles

While relaxed and seated, each subject was asked to breathe through a mouthpiece interfaced to a screen pneumotachograph (MLT300L, Powerlab, ADInstruments, Hastings, UK) and connected to a computer. The timing indices and expiratory flow profiles were calculated from a 1-minute section of the flow recording (Williams *et al.* 2000).

5-3.4 Statistical Analysis

Data was analysed using the SPSS 10.0 for Windows statistical package. Data are presented as mean ± standard deviations (SD). Group differences were analysed using a one-way analysis of variance (ANOVA). A post-hoc Tukey test was employed to determine where statistically significant differences existed between pairs of mean values. Pearson product bivariate procedure was used to examine correlations between variables. Statistical significance was accepted at the $P < 0.05$ level.

5-4 Results

5-4.1 Subject anthropometrics and AAS use

There were no differences in age and height between the four groups, but the AAS using groups (SU; SA) were significantly heavier than the non-AAS users (BC; SC) (Table 5-1 and 5-2). There were no differences between the three bodybuilding groups for either the number or duration of weekly training sessions. Additionally, percentage body-fat was significantly higher in SC compared with the bodybuilding groups.

Table 5-2 Anthropometric and grip strength for AAS using vs. control groups

Group ID	**SU**	**SA**	**BC**	**SC**
	n = 9	n = 6	n = 8	n = 8
Variable				
Height (m)	1.72 ± 0.06	1.72 ± 0.09	1.73 ± 0.06	1.73 ± 0.05
Weight (kg)	102.9 ± 19.5**	104.8 ± 28.8**	80.5 ± 7.6	83.6 ± 13.3
BMI ($kg.m^{-2}$)	34.9 ± 2.7**	34.8 ± 3.7**	27.5 ± 2.2	27.3 ± 2.7
Body fat (%)	14.1 ± 3.3*	14.7 ± 3.0*	14.7 ± 1.3*	18.1 ± 3.6
Grip strength (Kgf)	62.0 ± 5.4*	56.0 ± 7.0	54.5 ± 5.7	44.7 ± 5.7

Values are mean ± SD * = $P<0.05$; ** = $P<0.01$. SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

Both Testosterone and Free Androgen Index were significantly higher ($P < 0.05$; $P < 0.01$ respectively), whilst SHBG was significantly lower ($P < 0.01$) in the SU group compared with controls ($P < 0.05$). The markedly altered hormonal profile displayed by the SU group is consistent with the use of exogenous androgens, and was used as confirmation of AAS use (Table 5-3).

Table 5-3 Male sex hormone data for AAS using vs. control subjects at time of physiological testing

Group ID	**SU**	**SA**	**BC**	**SC**
	n = 9	n = 6	n = 8	n = 8
Variable				
Testosterone ($nmol.L^{-1}$)	65 ± 66*	16 ± 4.8	17.1 ± 6.7	14.3 ± 3.8
SHBG ($nmol.L^{-1}$)	4.0 ± 2.9**	12.4 ± 8.4	23.7 ± 8.9	26.0 ± 15.2
Free Androgen Index	16.4 ± 7.8***	1.3 ± 1.5	0.71 ± 0.25	0.6 ± 0.2

Values are mean ± SD * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$. SHBG = Sex Hormone Binding Globulin. SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

5-4.2 Respiratory measures

There were no differences between groups for FEV_1, FVC, and PEF % predicted and in all cases the reported values were within normal limits (Table 5-4).

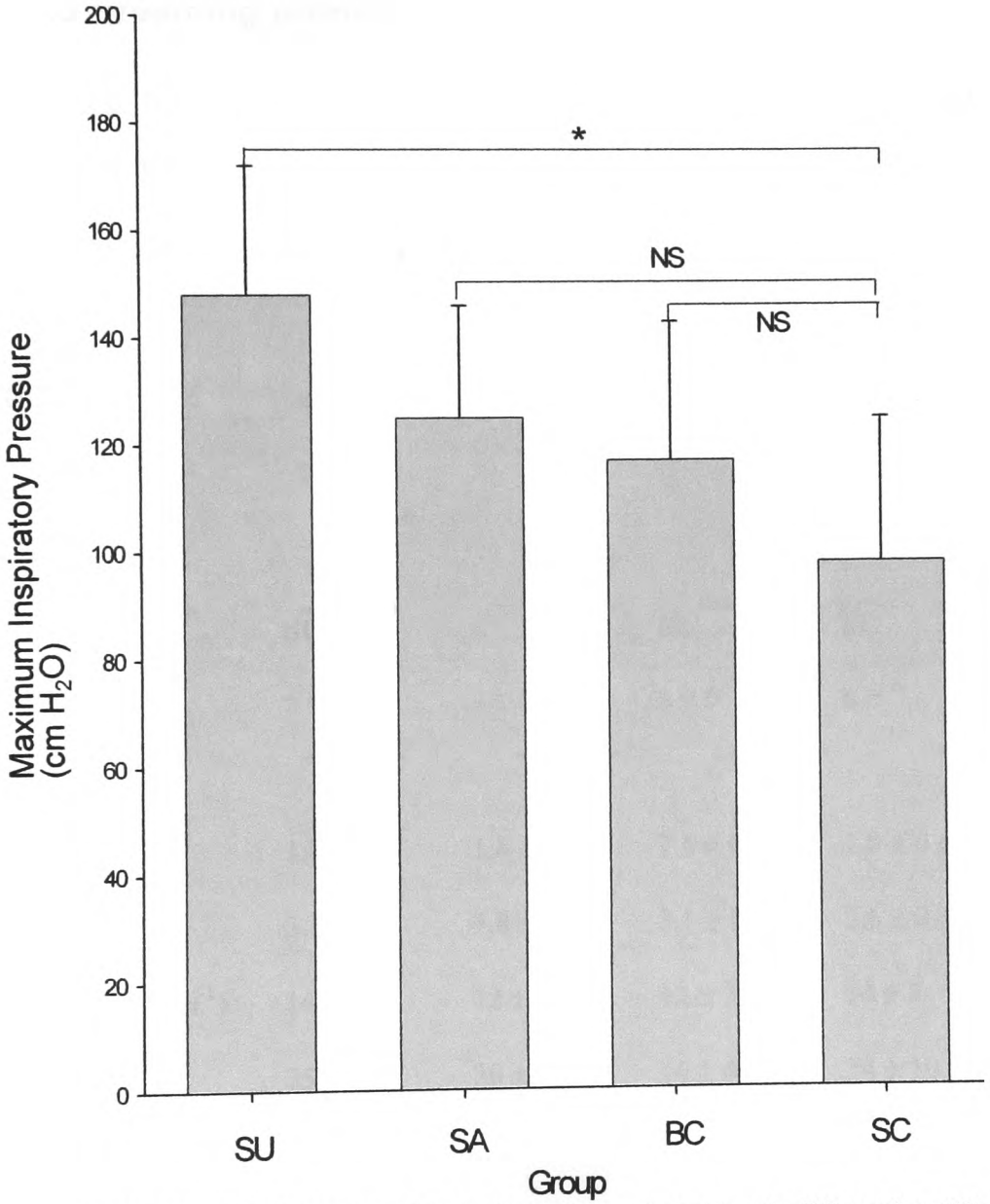
Table 5-4 Lung function data for AAS vs. control groups

Group ID	SU	SA	BC	SC
	n = 9	n = 6	n = 8	n = 8
Variable				
FEV_1 (%pred)	93 ± 17	102 ± 13	100 ± 7	96 ± 16
FVC (%pred)	99 ± 18	110 ± 9	105 ± 12	102 ± 17
PEF (%pred)	101 ± 16	96 ± 14	101 ± 12	94 ± 24
SMIP#	788 ± 266	786 ± 353	830 ± 266	724 ± 312

Values are mean ± SD. * Significantly higher than group SC. # Area under the time / pressure plot of sustained inspiratory flow (see text for details). SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

However, the SU group exhibited a significantly greater MIP when compared with SC ($P < 0.05$), but not with either SA or BC (Table 5-4; Figure 5-1). There were no differences in the sustainable inspiratory muscle pressure between groups (Table 5-4).

Figure 5-1 Maximum inspiratory pressure (MIP) values for AAS using vs. Control groups



* Significantly different from SC ($P < 0.05$). NS = not significantly different from SC.

Interestingly, grip strength was also significantly greater in SU compared with SC, but there was no significant difference in grip strength between any of the other groups.

There was a significant correlation between MIP and grip strength across all four groups ($r = 0.57$; $P < 0.05$).

5-4.3 Breathing profiles

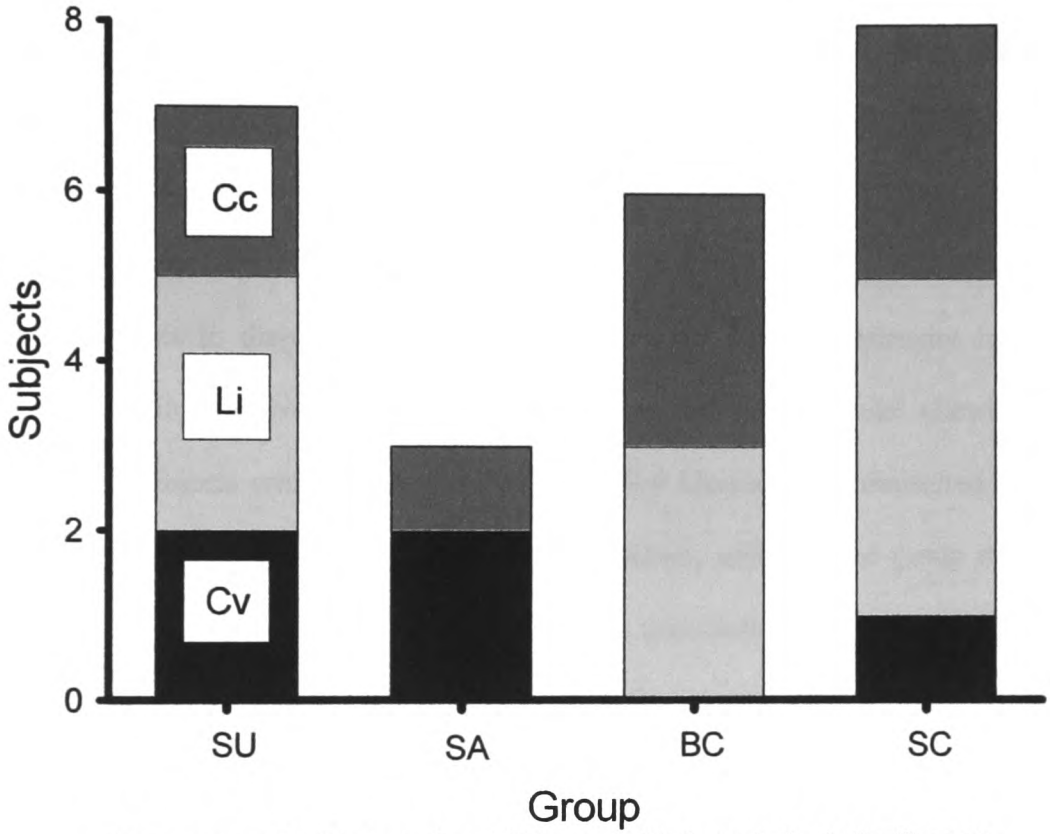
Breathing profile was unremarkable between groups showing breathing time, expiratory flow indices, or distribution of flow profiles to be unaltered by either resistance training alone or in combination with AAS use (Table 5-5; Figure 5-2).

Table 5-5 Breathing time and expiratory flow indices for AAS using vs. control groups

Group ID	SU	SA	BC	SC
	n = 7	n = 3	n = 6	n = 8
Variable				
t_I (s)	1.7 ± 0.4	1.8 ± 0.5	1.9 ± 0.5	2.0 ± 0.6
t_E (s)	3.1 ± 1.7	3.8 ± 2.0	3.1 ± 0.7	2.6 ± 0.6
Bf (breaths.min^{-1})	14 ± 5	12 ± 6	12 ± 3	14 ± 3
t_{PTEF} / t_E (%)	25 ± 7	30 ± 6	16 ± 6	24 ± 10

Values mean ± SD. The mean index from the sum of all breaths collected during the second minute recorded was used. t_I = inspiratory time; t_E = expiratory time; t_{PTEF} = time to peak expiratory flow. SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

Figure 5-2 Distribution of expiratory flow profiles for AAS using vs. Control groups



Cv, Convex profile (black shading; Li, Linear profile (grey shading); Cc, Concave profile (no shading). See text for details. SU = Using AAS at time of testing; SA = AAS users abstinent for 3 months; BC = bodybuilding controls; SC = sedentary controls.

5-5 Discussion

Lung function i.e. FEV_1, FVC, PEF % predicted, were within normal ranges for each of the four groups examined. This suggests that airway smooth muscle tone and subsequent lung mechanics is not altered by bodybuilding alone or when combined with AAS use. In tetraplegia however, one month's administration of Oxandrolone has been shown to provide improvements in lung function and MIP (Spungen *et al.* 1999).

Improvements in diaphragm contractility and muscle fibre hypertrophy has been observed following Nandrolone administration in the animal model (Lewis *et al.* 1999). In subjects with COPD, AAS (Nandrolone Deconate) administered with or without nutritional support resulted in MIP increases, solely in the group receiving AAS (Schols 1995). This suggests that AAS administration benefits inspiratory strength via the diaphragm or scalenus muscles (the primary muscles involved in this manoeuvre). Understanding of the underlying mechanism for muscular hypertrophy achieved through the use of exogenous androgens remains equivocal. Anabolic androgenic steroids in combination with resistance exercise have been shown to cause both hypertrophy (Bhasin *et al.* 1996) and hyperplasia (Kadi *et al.* 1999) of human skeletal muscle. In addition, proposed glucocorticoid antagonism (Hickson *et al.* 1990), or direct stimulation of IGF-I (Sheffield-Moore 2000) by AAS, would have benefits in arresting a multitude of chronic cachectic conditions.

The advantages of an improvement in respiratory muscle strength in healthy subjects are as yet undefined. But there is evidence of an increased sporting performance (cycling and rowing) following specific respiratory muscle training (Boutellier 1998; Volianitis *et al.* 2001; Romer *et al.* 2002). In addition, an increase in baseline

inspiratory muscle strength has previously been shown to result in a decrease in the extent of inspiratory muscle fatigue that occurs following exercise (McConnell *et al.* 1997). MIP in the AAS group was significantly greater than in the sedentary control group (Figure 5-1), but this was not observed in the group that had abstained from taking AAS for the three-month period.

Significantly greater diaphragm thickness and MIP values in resistance trainers compared with non-exercising adults has been reported by McCool *et al.* (1997), findings which were not substantiated in the present study. There were no differences between the bodybuilding groups for either the number or duration of training sessions per week. However, there exists the possibility that the AAS using group trained more intensively during workouts and had greater recovery. Neuropsychological benefits of AAS use in strength related exercise have been previously demonstrated (Kouri *et al.* 1995), and such an effect may account in part for MIP and grip strength differences between groups. Additionally, Bhasin *et al.* (1996) have suggested an additive effect of exercise and drug administration for greater strength gains in experienced lifters using high doses of testosterone enanthate (600 mg / wk) for 10 weeks. The present data suggests that the combination of AAS use and intensive resistance exercise produces an acute effect on MIP.

Bodybuilding alone or in combination with AAS use does not appear to alter breathing timing indices (Table 5-4). These indices, measured at rest, should be unaltered as none of the subjects reported any signs of dyspnoea. The AAS associated changes in lean body mass and increased respiratory muscle strength, did not alter expiratory airflow (Figure 5-2). If the subjects were to show any altered lung mechanics then t_{PTEF} would have decreased i.e. peak expiratory flow would occur sooner. In subjects with COPD, there is little post-inspiratory muscle activity and

expiratory flow peaks rapidly. This data supports previous studies showing t_{PTEF} correlates well with FEV_1 (Morris and Lane 1981). An unchanged expiratory flow profile also illustrates that despite the inspiratory muscles being stronger they do not alter airway tone or resistance. If for instance there were more post-inspiratory flow braking then more convex patterns would be seen (Williams *et al.* 2000).

Differences in grip strength between the four groups followed the same pattern as did MIP i.e. grip strength was significantly greater in SU compared with SC, but there was no difference between the other groups. We have observed an acute effect of AAS use on both MIP and grip strength that was not preserved after withdrawal of the anabolic steroids (SA group). Grip strength is a convenient measure of pre-pubertal strength gains but does not reflect changes in larger muscle groups (Johnson *et al.* 1994). Any prolonged AAS induced strength gains in the group of individuals we have examined may be confined to larger muscle groups. The rationale behind measuring grip strength in the present study was to evaluate any relationship between grip strength and MIP, both of which are accepted indices of strength in small muscle groups. Indeed, a significant correlation was observed between these two measures ($P < 0.05$), but the absolute r-value is too low (0.57) to be useful in predicting individual scores.

The therapeutic use of AAS supplementation to alleviate muscle dysfunction in subjects with COPD or in the treatment of cachexia associated with pulmonary disease have been tentative in the past due to the health effects associated with their use. There are undoubted health risks associated with supraphysiological AAS regimens, as used by bodybuilders and sportsmen. Such risks may be reduced with therapeutic doses of a specific AAS. For example, a recent meta-analysis (Whitsel *et al.* 2001) reviewed the effects of testosterone esters in 20 studies administering

therapeutic doses (1987-1999). Despite the dose-dependent decrease in HDL-cholesterol and concomitant declines in both total and LDL cholesterol, values were usually within normal limits and were far less dramatic than those experienced by bodybuilders administering relatively high doses of various AAS (Glazer 1991).

5-6 Conclusion

The present study demonstrated acutely higher MIP values when AAS use was combined with resistance exercise. In addition, no demonstrable adverse effects of supraphysiological doses of AAS were observed on respiratory function. Bearing in mind that AAS appears to be more effective in clinical situations (Ferreira *et al.* 1998), the efficacy of adopting AAS into clinical practice would seem to be plausible should further long term studies employing therapeutic doses of AAS sustain similar outcomes to those reported in the present investigation.

STUDY FOUR – EFFECTS OF GROWTH HORMONE ADMINISTRATION ON RESPIRATORY FUNCTION

6-1 Abstract

The aim of the present study was to determine the effects of 6 days recombinant human growth hormone (rhGH) administration, in an abstinent anabolic-androgenic steroid (AAS) using group on respiratory function compare with an abstinent AAS control group. Subjects were enrolled as part of a larger study investigating cardiac effects.

Male subjects (n = 48) were randomly divided, using a single blind procedure into two groups: 1) control group (EC) n = 24, means ± SD, age 32 ± 11 years; height 1.8 ± 0.06m; 2) rhGH using group (GH, 0.019mg.kg^{-1}.day^{-1}) n = 24; age 32 ± 9; height 1.8 ± 0.07m. Anthropometry and respiratory muscle function were investigated.

Forced expiratory volume in one second / forced vital capacity, maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) significantly increased when compared with the control group (P<0.05) (MIP: EC = 129 ± 28; GH ± 144 ± 24. MEP: EC = 157 ± 32; GH = 179 ± 35) . Body mass index and fat free mass index significantly increased whilst body fat significantly decreased within the GH group (Body fat: GH pre = 20.0 ± 6.0; GH dur = 19.0 ± 6.0; GH post = 19.1 ± 5.8, P<0.05).

The present study demonstrated an improvement in respiratory muscle strength and body composition following short-term high dose rhGH administration in former AAS users.

6-2 Introduction

Growth hormone, also called *somatotrophic hormone* or *somatotropin,* is a small protein molecule that contains 191 amino acids. Growth hormone is secreted by the anterior pituitary. It causes growth of almost all tissues of the body that are capable of growing (Rincon-Limas *et al.* 1993). The rate of growth hormone secretion increases and decreases within minutes usually in relation to the person's state of nutrition or stress, such as during 1) starvation, 2) hypoglycaemia, 3) exercise; 4) excitement; and 5) trauma (Guyton and Hall 1996). The first two hours of deep sleep correspond to increases with a level as high as 30 $\mu g.L^{-1}$ (Melmed 2006). Aging and obesity are associated with suppressed secretory bursts of the hormone (Iranmanesh *et al.* 1991). *Panhypopituitarism* is a term which describes decreased secretion of all the anterior pituitary hormones (which includes growth hormone). The decrease may be congenital (present from birth), or it may occur suddenly or slowly at any time during the life of the individual (Guyton and Hall 1996). If growth hormone deficiency occurs during childhood, generally the features of the body develop in proportion to one another, but the rate of growth is greatly decreased. If growth hormone deficiency occurs during adulthood individuals are overweight, with reduced lean body mass, increased fat mass and reduced strength and exercise capacity (Salomon *et al.* 1989; Cuneo *et al.* 1991; Amato *et al.* 1993; Bengtsson 1993; Beshyah *et al.* 1995).

Merola *et al.* (1995) evaluated lung volume and respiratory muscle strength in patients diagnosed as growth hormone deficient. This study reported impairment of ventilatory function and a decreased respiratory muscle pressures when compared with healthy subjects. The same research group then examined growth hormone deficient patients before and after 6 and 12 months of recombinant growth hormone treatment (Merola *et al.* 1996). Merola at el. (1996) demonstrated that after 12 months of recombinant

growth hormone treatment, lung impairment and reduced respiratory muscle pressures can be reversed.

Growth hormone excess results in the clinical condition known as acromegaly. Acromegalics have an increased risk of diabetes mellitus, hypertension and premature mortality due to cardiovascular disease (Bengtsson 1993). Increase in lung size has been described in acromegalic patients (Iandelli *et al.* 1997), however in the same study inspiratory and expiratory muscle force was below normal limits.

Study three investigated the effect of long term AAS use on respiratory function and demonstrated no adverse effects of supraphysiological doses on respiratory function. In fact, an increase in inspiratory muscle strength was apparent in AAS users which could prove important in clinical settings. Growth hormone supplementation has also been suggested to be of ergogenic potential for treating patients with advanced COPD, especially those presenting loss of muscle mass or peripheral muscle weakness (Villaca *et al.* 2006).

6-3 Methods

Individuals who were previous participants in AAS related studies were used as subjects. The subject contact details were held in a database used by the Health & Exercise Science Research unit at the University of Glamorgan (Grace *et al.* 2001). All subjects had abstained from taking AAS for a period of more than 12 weeks, prior to the present study and were observed in a normal training situation. All subjects provided written, informed consent and were informed that they were free to withdraw from the investigation at any time.

Subjects (n = 48) were recruited to participate in the study. Participants were divided into two distinct groups: The first group were weight lifters using recombinant human growth hormone (rhGH) (GH) (n = 24); the second group were weight lifting controls, who did not use any pharmacological ergogenic aids (EC) (n = 24). Table 5-6 describes subject characteristics. GH group subjects self-administered rhGH, under supervision, by subcutaneous abdominal injection, in a controlled hygienic environment, for six consecutive days in a dosage of 0.58 international units / kg / day (0.019 $mg.kg^{-1}.day^{-1}$). The inclusion of a placebo to a group that was self-administering was considered unethical.

Subjects were examined prior to the commencement of rhGH administration (day one). All subjects were then examined on day seven followed by a period of abstinence for one week and re-examination on day fourteen.

Subjects were enrolled as part of a larger study in which cardiovascular function, including pulse-wave velocity, venous blood sampling, and 12-lead ECG were assessed previously. All subjects were non-smokers and had no previous history of chronic respiratory or circulatory disease.

Each subject arrived in the laboratory following an overnight fast, not having consumed any alcohol, or performed exercise for 36 hours prior to examination.

A drug screen was performed on each subject prior to the start of the investigation to exclude potential confounding of results by surreptitious use of anabolic steroids or other agents. Urinalysis was performed at a World Anti-doping Agency / International Committee (IOC) accredited laboratory. In total nine subjects were excluded after failing this initial drug screen leaving the cohort number at twenty-four in each group.

Anthropometrics

See Methods 2-2.1 for details of body fat measures.

Fat free mass was calculated by subtracting fat mass from total body mass. Fat free mass index was calculated by dividing the subject's fat free mass in kilograms by the square of the subject's height in metres.

Spirometry

The forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured using an online spirometer (Spirosense, Burdick Inc, Milton, WI, USA). Three expiratory manoeuvres were completed, the best of which were used for subsequent analysis (Quanjer *et al.* 1993).

Inspiratory muscle function

Maximum inspiratory pressure (MIP) was used as an indicator of inspiratory muscle strength by measuring the pressure generated at the mouth when inspiring rapidly from residual volume. Pressure was measured using a mouthpiece connected to a portable electronic manometer (RTSport, DeVilbiss, Sunrise Medical Ltd, Wollaston,

UK). The mouthpiece has a fixed leak via a 2-mm diameter aperture that prevented glottal closure during the inspiratory manoeuvre. The leak set a maximum flow during the inspiratory effort (~450 $ml.s^{-1}$) and allowed continuous measurement of pressure over the full range of vital capacity. Maximum inspiratory pressure was the maximum pressure generated over 1s during the inspiration and was taken as the best of three inspirations (Green *et al.* 2002). The sustained maximum inspiratory pressure (SMIP) was also recorded and is reported as the area under the pressure time curve (Chatham *et al.* 1997). Extra care was taken to ensure that maximal inspiratory pressure was obtained because each subject was assisted by visual feedback on a computer screen and motivated by verbal encouragement.

Statistics

Shapiro-Wilks tests were applied to data from each dependent variable and confirmed distribution normality. A two-way repeated measures analysis of variance (ANOVA) was used to test or between and within group differences. A Tukey *post-hoc* test was used to locate specific significant differences. An alpha of less than 0.05 was accepted as statistically significant in all tests. All data are presented as mean and 1 standard deviation (SD).

6-4 Results

There were no differences in age and height between the two groups, but the GH group (administering rhGH) demonstrated increased body mass, body mass index and fat free mass index when taking rhGH and a significant decrease in the same measures on cessation of the drug. Body fat significantly decreased within the GH group; this

measure remained decreased on cessation of the drug (Table 6-1). There were no differences within the control group for these measures ($P<0.05$).

There were no differences between or within groups for FEV_1, FVC, or FEV_1 / FVC and in all cases the reported values were within normal limits (Table 6-2).

However, the GH group exhibited a significantly greater MIP and MEP both within the group and when compared to the EC group (Table 6-2; Figure 6-1).

Table 6-1 Effect of rhGH and subject information

Variables	Exercise Control Group (EC)			Treatment Group (GH)		
	(n = 24)			(n = 24)		
Age (Yrs)	32 ± 11			32 ± 9		
Height (m)	1.8 ± 0.06			1.8 ±0.07		
				PRE-GH	DUR-GH	POST-GH
	1	7	14	1	7	14
BM (kg)	89.8 ± 12.7	89.6 ± 12.6	89.5 ± 12.7	86.1 ± 12.0	86.7 ± 12.1*	85.5 ± 11.8
BMI ($kg.m^{-2}$)	28.0 ± 3.1	27.9 ± 3.1	27.9 ± 3.1	27.5 ± 3.0	27.7 ± 3.1*	27.3 ± 3.0*
Body Fat (%)	21.9 ± 3.8	21.7 ± 3.8	21.6 ± 4.0	20.0 ± 6.0	19.0 ± 6.0*	19.1 ± 5.8*
FFMI ($kg.m^{-2}$)	21.8 ± 2.1	21.8 ± 2.1	21.8 ± 2.1	21.9 ± 1.9¥	22.3 ± 1.9	22.0 ± 1.9¥
RT (Yrs.)	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6	12.2 ± 3.6
WT (Sessions / Wk)	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1	4.4 ± 1.1
TT (min)	47 ± 15	47 ± 15	47 ± 15	47 ± 15	47 ± 15	47 ± 15
Energy Intake (020 J / day)	18050 ± 4100	18100 ± 2020	18175 ± 3100	17900 ± 3020	18450 ± 3900	18100 ±
Protein Intake (g / day)	205 ± 60	195 ± 55	213 ± 45	207 ± 35	217 ± 65	210 ± 50

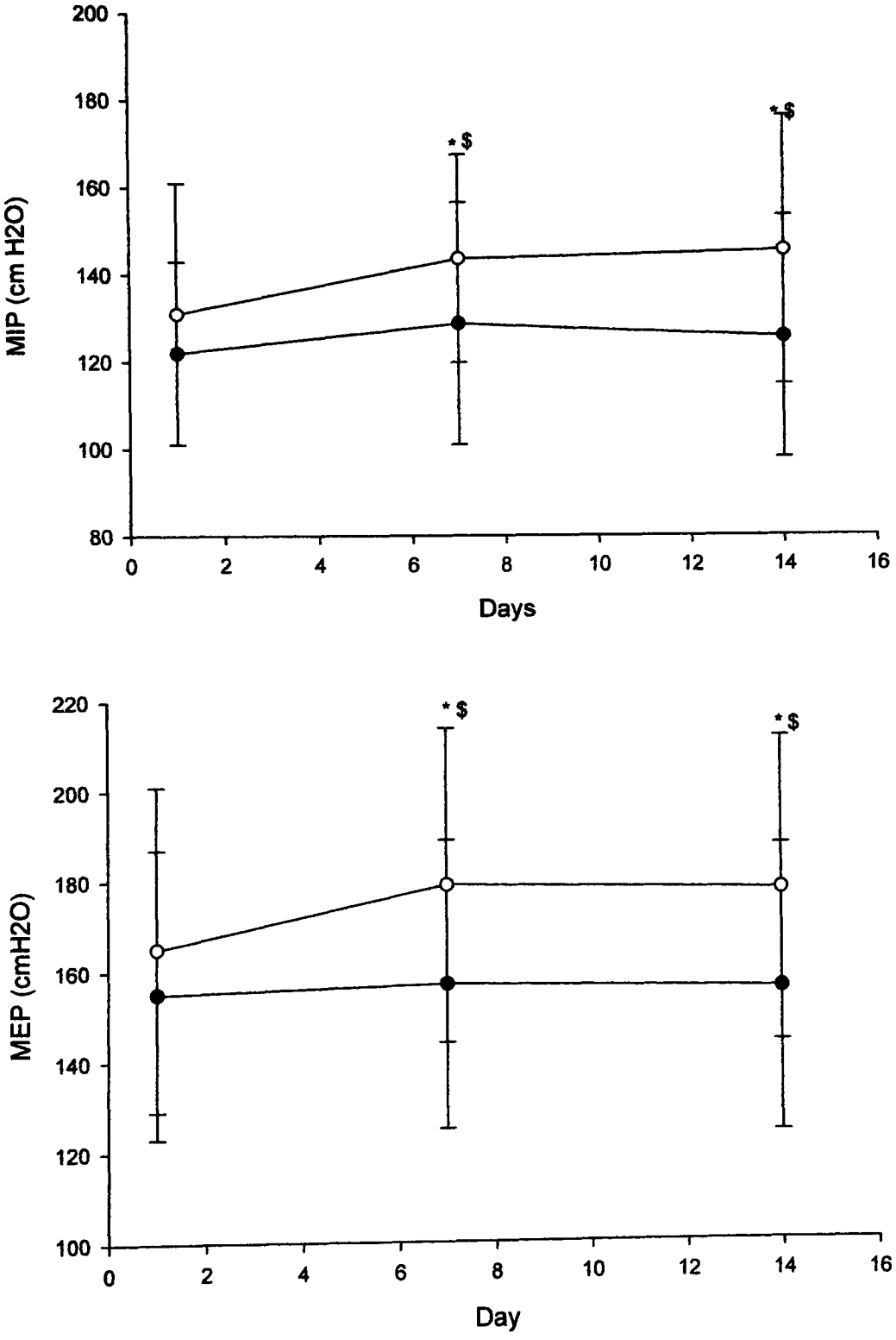
Mean ± SD are shown. * Significantly different to PRE-GH. ¥ Significantly different to DUR-GH. BM = Body Mass; BMI Body Mass Index; FFMI = Fat Free Mass Index; RT = Resistance Training; WT = Weight Training; TT = Training Time. GH Growth Hormone.

Table 6-2 Effect of Growth Hormone on Respiratory Function

Variables	Exercise Control Group			Treatment Group		
				PRE-GH	DUR-GH	POST-GH
	1	7	14	1	7	14
FEV_1 (L)	4.4 ± 0.6	4.4 ± 0.6	4.4 ± 0.6	4.2 ± 0.6	4.3 ± 0.6	4.2 ± 0.6
FVC (L)	5.3 ± 0.8	5.2 ± 0.7	5.3 ± 0.9	4.9 ± 0.8	4.9 ± 0.8	4.9 ± 0.8
FEV_1/FVC (%)	83 ± 6	82 ± 5	82 ± 5	84 ± 7	85 ± 6	85 ± 6
PEFR (L / min)	623 ± 46	617 ± 61	623 ± 64	627 ± 78	632 ± 59	632 ± 65
FIV_1 (L)	4.8 ± 0.8	4.8 ± 1.0	4.9 ± 0.8	4.6 ± 0.8	4.6 ± 0.8	4.4 ± 1.1
FIVC (L)	5.1 ± 0.8	5.1 ± 0.9	5.2 ± 0.9	4.9 ± 0.8	4.9 ± 0.7	4.9 ± 0.8
PIFR (L / min)	439 ± 112	449 ± 119	471 ± 111	493 ± 98	506 ± 111	480 ± 129
MVV (L)	165 ± 23	160 ± 25	166 ± 28	162 ± 24	161 ± 18	157 ± 22

Mean ± SD are shown. * Significantly different to PRE-GH ($P < 0.05$). § Significantly different to Exercise Control group (P 0.05). FEV_1 = Forced Expiratory Volume in 1 second; FVC = Forced Vital Capacity; PEFR = Peak Expiratory Flow Rate; M = Maximum Inspiratory Pressure; MEP = Maximum Expiratory Pressure; FIV_1 = Forced Inspiratory Volume in 1 second; FIV = Forced Inspiratory Vital Capacity; PIFR = Peak Inspiratory Flow Rate; MVV = Maximum Voluntary Ventilation; VO_{2PEAK} Peak Oxygen uptake.

Figure 6-1 Effect of Growth Hormone administration on MIP and MEP



Values are mean ± SD. * Significantly different from day one. $ Significantly different from EC. ($P < 0.05$). Shaded symbols, EC; unshaded symbols, GH.

6-5 Discussion

Lung function, as measured by FEV_1, FVC, FEV_1 / FVC, PEFR, FIV_1, FIVC, PIFR, and MVV, were within normal ranges for the two groups examined. This suggests that airway smooth muscle tone and subsequent lung mechanics are not altered by weight lifting alone or when combined with rhGH administration.

In a study administering rhGH to adult onset growth hormone deficiency patients, no effect on lung volume was shown (Merola *et al.* 1996), but an improvement in respiratory muscle strength after 12 months of rhGH therapy was established. The present study also demonstrated a significant increase in both maximum inspiratory and expiratory pressures after just seven days rhGH administration. An effect that was apparent on cessation of the drug for a further 7 days. However, a dosage of 11 $IU.day^{-1}$ to underweight patients with COPD has no effect on maximal respiratory pressures compared to a placebo group after 21 and 81 days (Burdet *et al.* 1997).

An increase in both muscle mass and maximal tetanic tension induce by rhGH administration and exercise has been demonstrated previously in the animal model (Anderson *et al.* 2000). These improvements have not been demonstrated in humans (Yarasheski 1994; Frisch 1999).

The present study is the first to demonstrate, in a normal population i.e. growth hormone levels, an increase in inspiratory and expiratory muscle strength with rhGH administration. As previously discussed in the case of AAS, an increased MIP has been suggested to improve sporting performance (Boutellier 1998; Volianitis *et al.* 2001; Romer *et al.* 2002) and to decrease the extent of inspiratory muscle fatigue that occurs following exercise (McConnell *et al.* 1997).

6-6 *Conclusion*

The present study clearly demonstrated acutely higher MIP and MEP values following six days rhGH administration in combination with resistance exercise. No demonstrable effects of short-term, high dose rhGH administration were observed on respiratory function. In addition, improvements in body composition were also noted. The suggestion that growth hormone supplementation for treating patients with advanced COPD, especially those presenting with loss of muscle mass or peripheral muscle weakness seems plausible providing further studies sustain similar outcomes to those reported here.

STUDY FIVE – EFFECTS OF DIFFERENT INSPIRATORY RESISTIVE LOADING INTENSITIES ON EXERCISING HEART RATE AND PERCEIVED EXERTION

7-1 Abstract

This study was designed to investigate the relationship between the intensity of an inspiratory resistive loading (IRL) programme and its effect on respiratory muscle strength, exercising heart rate and ratings of perceived exertion.

A total of 66 subjects were randomly assigned to one of three groups. One group trained at 100% of maximum inspiratory pressure (MIP) for six weeks (MAX, n = 22). A second group performed six weeks of inspiratory muscle training at 80% MIP (SUB, n = 21), and a third control group received no inspiratory training (CON, 23).

Both the MAX and SUB training groups improved maximum inspiratory pressure relative to the control group (32 ± 19 cm H_2O, $P = 0.01$; 37 ± 25 cm H_2O, $P = 0.001$, respectively). A significant decrease in heart rate (-6 ± 9 beats.min^{-1}, $P = 0.02$) and rating of perceived exertion (-0.5 ± 1.4, $P = 0.04$) was observed for the MAX group only.

It is concluded that six weeks of both MAX and SUB training were sufficient to improve inspiratory muscle strength. However, exercising heart rate and perceived exertion decreased with MAX training only.

7-2 Introduction

One reason why some studies have shown increases in respiratory muscle function, but no change in exercise responses may be related to the intensity of the IRL programme. Sheel (2002) suggested that studies utilising the highest percentage of MIP also report the greatest improvement in MIP. However, no studies have compared IRL protocols at high intensities most typical of such strength training regimes, on changes in MIP and exercising responses i.e. heart rate and ratings of perceived exertion. We hypothesised that higher training intensities will result in a greater training stimulus and result in more marked changes in MIP and exercise responses. Therefore, the aim of this study was to compare the effects of six weeks of IRL at two relatively high intensities on changes in MIP and exercise responses.

7-3 Methods

7-3.1 Participants

Sixty-six apparently healthy subjects, consisting of 40 males and 26 females, with a mean ± 1 standard deviation (SD): age 21.8 ± 3.9 yr; stature 1.73 ± 0.1 m; body mass 74.9 ± 12.0 kg, were assigned randomly to one of two IRL training groups or a control group (Table 7-1). Written informed consent was obtained from all subjects before commencing the experiment, which had local ethical committee approval. Subjects were instructed to maintain both their usual diet and training regime during the intervention period, and to abstain from strenuous physical activity before the exercise test.

Table 7-1 **Anthropometrical data for maximal (MAX), sub-maximal (SUB), and control (CON) training groups**

Parameter	MAX n = 22	SUB n = 21	CON n = 23
Sex (♂/♀)	16/6	12/9	12/11
Age (yr)	21.9 ± 3.3	21.3 ±1.8	21.8 ± 5.1
Stature (m)	1.74 ±0.1	1.70 ± 0.1	1.71 ± 0.1
Body Mass (kg)	76.1 ± 11.4	70.0 ± 12.4	74.1 ± 12.2

Values are mean ± SD. No significant differences were observed in these basal measures. MAX = trained at 100% MIP; SUB = trained at 80% MIP; CON = no training.

7-3.2 Procedure

Lung function spirometry, including MIP, and maximum expiratory pressure (MEP), were assessed before and after a six week inspiratory training or control period. In addition, changes in exercising heart rate (HR) and Borg's (1974) rating of perceived exertion (RPE) were measured at the end of a 5 min, constant workload cycling bout. The work rate for this exercise was set at 2 W per kg body mass for males, and 1.5 W per kg body mass for females and pedalling frequency was maintained at 60 rpm throughout.

7-3.3 Lung Function

Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), peak expiratory flow (PEF) was determined by spirometry using a computerised spirometer (Spirosense Spirometry System, Burdick Inc, Milton, USA). The best of three

measurements were used for subsequent analysis as recommended by Quanjer *et al.* (1993).

Mouth pressures were measured with a portable hand-held mouth pressure meter (Micro Medical Ltd, Rochester, Kent, UK). Subjects were encouraged to make maximum inspiratory and expiratory efforts at or near residual volume and total lung capacity respectively. The maximum of three manoeuvres was recorded as recommended by Green *et al.* (2002).

7-3.4 Respiratory Training

All training was conducted three times per week for six weeks. The maximum group (MAX) consisted of 22 subjects who trained by reproducing 100% MIP 10 times with 20 s recovery between each effort. The sub-maximum group (SUB) consisted of 21 subjects who completed a sub-maximum training protocol of "through-range" inspirations, from residual volume to total lung capacity, at 80% of their maximum effort. Subjects were allowed 20 s recovery between each of the 10 attempts. The control group (CON) consisted of 23 subjects who did not complete any respiratory training between pre- and post-testing. All inspiratory muscle training was conducted using the RT2 training device (DeVilbiss Sunrise Medical Ltd, Wollaston, UK), which is flow-resistive in nature with subjects having to breathe through a 2 mm leak, present to prevent glottal pressure. The measured resistance was approximately 270 cm $H_2O.L^{-1}.s^{-1}$. The pressure generated at the mouth is recorded by computer and displayed in real time. Subjects perform three maximal inspirations from residual volume to total lung capacity and the user selects the highest of the three recorded. Depending on the group, the computer then re-draws an on-screen template at 100% (MAX group) or 80% (SUB group) of this maximum which the subject must

reproduce at the end of each 20s rest period. In this way it is possible to ensure that the subjects are training at their individually prescribed intensity. The maximal template is re-assessed at each IRL session.

7-3.5 Statistics

Shapiro-Wilks tests were applied to data from each dependent variable and confirmed distribution normality. A one-way analysis of variance (ANOVA) was used to test the differences in the training-induced changes between training and control groups (Post-pre test scores). A Tukey *post-hoc* test was used to locate any significant differences between groups. Pearson's product moment correlation coefficient was used to establish if changes in MIP data were related to HR and RPE changes. An alpha of less than 0.05 was accepted as statistically significant in all tests. All data are present as mean and 1 standard deviation (SD).

7-4 Results

7-4.1 Lung Function

There was no difference in basal measures for age, stature, or body mass for the three groups (Table 7-1). Respiratory data including lung function as assessed by FEV_1, FVC, FEV_1 / FVC %, and PEF did not change differently for the MAX, SUB, and CON groups (Table 7-2). However, the increase in MIP was significantly greater for the MAX (+32 ± 19 cm H_2O, $P = 0.011$) and SUB (+37 ± 25 cm H_2O, $P = 0.001$) training groups when compared with the CON (+12 ± 21 cm H_2O, $P > 0.05$) (Figure 7-1). The change in MEP was not different between any of the groups (Table 7-2).

7-4.2 Exercise Responses

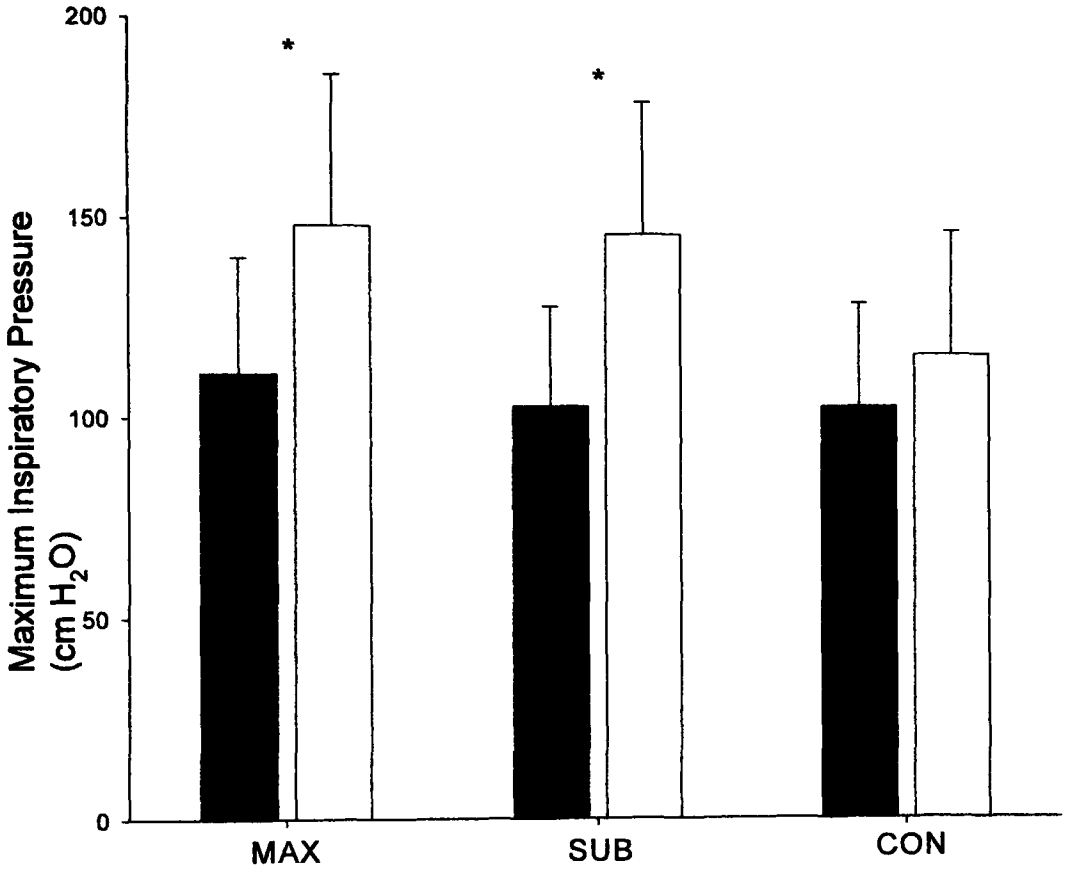
The change in MAX exercising HR (-6 ± 9 beats.min^{-1}), was significantly different from CON (+2 ± 11 beats.min^{-1}) (P = 0.02). However, the change in SUB exercising HR (-3 ± 9 beats.min^{-1}), was not different from either group. The reduction in RPE for the MAX group (-0.5 ± 1.4) was significantly different (P = 0.04) from both the SUB and CON group (0.4 ± 1.5 and 0.6 ± 1.5, respectively) (Table 7-3).

Table 7-2 **Changes in respiratory data from pre- to post-training for maximum (MAX), sub-maximum (SUB), and control (CON) training**

Parameters	MAX (n = 22)			SUB (n = 21)			CON (n = 23)		
	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff
MEP ($cm\ H_2O$)	108 ± 25	110 ±29	3 ±22	96 ± 28	100 ± 32	4 ± 18	97 ± 22	97 ± 24	-0.2 ± 21
FEV_1 (L)	3.8 ± 0.6	3.7 ± 0.7	-0.1 ± 0.2	3.8 ± 0.6	3.7 ± 0.8	-0.1 ± 0.2	3.7 ± 0.7	3.5 ± 0.7	-0.2 ± 0.2
FVC (L)	4.6 ± 0.7	4.4 ± 0.8	-0.2 ± 0.2	4.4 ± 1.0	4.3 ± 1.0	-0.1 ± 0.2	4.4 ± 0.9	4.2 ± 0.8	-0.2 ± 0.2
FEV_1/FVC%	83 ± 6	84 ± 6	0.4 ± 3	87 ± 6	86 ± 7	-0.4 ± 3	84 ± 6	83 ± 7	-1.0 ±4
PEF ($L.s^{-1}$)	8.0 ± 1.6	7.5 ± 1.4	-0.5 ± 1.0	7.5 ± 1.8	7.6 ± 1.6	1.1 ± 1.0	7.9 ± 1.6	7.7 ± 1.8	-0.2 ± 0.9

Values are mean and SD. MEP: maximum expiratory pressure, FEV_1, forced expiratory volume in 1s; FVC, forced vital capacity; PEF, peak expiratory flow, Diff: difference post – pre. * Significantly different from control, ($P < 0.05$). MAX = trained at 100% MIP; SUB = trained at 80% MIP; CON = no training.

Figure 7-1 **Effects of six weeks of maximal (MAX), submaximal (SUB), or control (CON) intervention on maximum inspiratory pressure (MIP).**



Values are mean ± SD. * = change significantly different from control ($P < 0.05$). Pre-training = black bars, Post training = clear bars.

Table 7-3 **Changes in exercise data from pre- to post-intervention for maximum (MAX), sub-maximum (SUB), and control (CON) training**

Parameters	MAX			SUB			CON		
	n = 22			n = 21			n = 23		
	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff
HR (beats·min^{-1})	157 ± 19	151 ± 17	-6 ± 9*	154 ± 19	151 ± 19	-3 ± 9	159 ± 17	161 ± 17	2 ± 11
RPE	13.0 ± 1.7	12.5 ± 1.4	-0.5 ± 1.4*	12.5 ± 1.1	12.8 ± 1.7	0.4 ± 1.5	12.7 ± 1.1	13.3 ± 1.6	0.6 ± 1.5

Values are mean (SD). MIP: maximum inspiratory pressure, MEP: maximum expiratory pressure, HR: heart rate, RPE: rating of perceived exertion, Diff: difference post – pre. * Significantly different from control, ($P < 0.05$). MAX = trained at 100% MIP; SUB = trained at 80% MIP; CON = no training.

7-5 Discussion

7-5.1 Main Findings

The main findings of this study were that MIP increased significantly following six weeks of training for both the MAX and SUB training groups. However, exercising HR and RPE were significantly lower for the MAX group only. Changes in expired volumes and associated flow rates were unchanged for all three groups. Nevertheless, MIP, an indicator of voluntary inspiratory muscle strength, showed a 29% and 38% improvement in the MAX and SUB training groups respectively. The magnitudes of these increases are in agreement with previous studies (Clanton *et al.* 1985; Inbar *et al.* 2000; Volianitis *et al.* 2001; Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; Downey *et al.* 2007; Griffiths and McConnell 2007; Wylegala *et al.* 2007).

7-5.2 Respiratory Function

MIP is a simple way to gauge inspiratory muscle strength (Green *et al.* 2002), however as discussed previously, an improvement in exercise performance is not always associated with an increase in this measure. As performance was not measured in the present study, the effects of the observed increases in MIP cannot be ascertained. The observed reduction in HR and RPE for the MAX group suggests a performance benefit may accompany this training however. McConnell *et al.* (1997) demonstrated that exercise-induced fatigue (measured as a reduction in MIP) was greatest in subjects with the lowest inspiratory muscle strength. Therefore, an improvement in MIP through an inspiratory muscle training protocol may improve performance by attenuating exercise-induced respiratory muscle fatigue (Coast *et al.*

1990; Johnson *et al.* 1993) and prolong sub-maximal exercise such as marathon running (Loke *et al.* 1982). Furthermore, two recent studies have demonstrated a decrease in respiratory muscle fatigue following IRL (Volianitis *et al.* 2001; Romer *et al.* 2002). After IRL, these authors also found an improvement in rowing and cycling performance respectively. However, out of the above-mentioned studies only Johnson *et al.* (1993) really demonstrated diaphragmatic fatigue (they used phrenic nerve stimulation) whilst the remaining studies all assessed respiratory muscle fatigue as a reduction in MIP post exercise. The significance of a change in this measure is reduced when it is considered that our own control group demonstrated a test-retest variation of 12%.

Both the MAX and SUB groups completed a total of 18 IRL training sessions at 100% and 80% of MIP respectively. Both of these training interventions resulted in significant improvements in MIP, which did not differ between groups. Clearly, the difference in training intensity was not sufficient to alter the magnitude of the induced changes in inspiratory muscle strength. Whether these represent optimal training loads remains to be determined, as lower training intensities previously used to successfully improve MIP were not included in the present study (Volianitis *et al.* 2001; McConnell and Sharpe 2005). The significant increase in MIP of approximately 30%, despite the relatively short six week IRL training programme, suggests that inspiratory training in the region of 80-100% MIP is very effective. Volianitis *et al.* (2001) found a 10-week IRL programme resulted in a 45% improvement in MIP, whereas Sonetti *et al.* (2001) reported only an 8% increase following a 5-week training programme. However, Sonetti *et al.* (2001) employed a mixed VIH / IRL training programme which Romer *et al.* (2002) have suggested may not be as effective. The present study also demonstrated that significant changes in MIP and

exercising HR can be brought about with relatively short IRL sessions lasting around 10-15 min. This contrasts with previous studies where IRL sessions commonly last around 30 min (Chatham *et al.* 1999).

The finding that MEP was not changed for any of the groups probably demonstrates the specificity of the effects of inspiratory training on expiratory lung function. The finding that FEV_1 and PEF were unchanged may also be explained in the same manner. Whilst FVC is generally held to be largely dictated by an individual's stature and not influenced by training. Consequently, any observed increase in this was not a significant factor in the present study.

7-5.3 Exercise Response

Although, both the MAX and SUB training groups increased MIP, only the MAX group experienced a significant decrease in exercising HR. Swanson (1998) also found HR was lower following a six-week VIH training intervention (n = 4), however, no other study investigating VIH training have corroborated these findings. Harms *et al.* (1997) have shown that reducing the cost of breathing (by 50%) leads to an increase in blood flow to exercising skeletal muscle (by 5-7%). However, we are not aware of any published studies reporting the effects of redistribution of blood flow following RMT at present. Indeed, it is highly unlikely that an RMT program will reduce the cost of breathing to the extent that a ventilator does and hence, the concomitant increase in exercising skeletal muscle will be correspondingly small and potentially difficult to detect. An increase in stroke volume following a respiratory training intervention would explain the heart rate observations. However, Markov *et al.* (2001) have demonstrated that VIH does not appear to alter stroke volume. Consequently, the mechanism behind the observed decrease in exercising heart rate

for the MAX group remains to be determined. It is tempting to suggest that the mechanism behind the observed improvements after an IRL intervention are different to that following VIH. Indeed O'Kroy and Coast (1993) demonstrated that VIH training improves both flow and resistive tests whilst IRL appears to affect only strength and resistive measurements, so the possibility does exist. Therefore, we suggest that the mechanism behind improvements following VIH and IRL interventions deserve individual investigation in their own right.

The RPE was significantly decreased in the MAX group only. This decrease in RPE may be related to the same factors associated with the decrease in HR, and / or to an altered perception of breathing effort. Perceived exertion has previously been demonstrated to correspond to HR and blood lactate response (Borg *et al.* 1987). However, no significant correlations were found between changes in HR and RPE in the present study. A reduction in RPE has been reported previously following IRL (Kellerman *et al.* 2000; Volianitis *et al.* 2001). Consequently, the precise mechanism by which IRL lowers RPE remains to be determined, but it seems likely that an alteration in the perception of respiratory muscle work may play a role.

In the present study, the SUB group were required to inspire from residual volume through to total lung capacity, whereas the MAX group simply repeatedly reproduced 100% MIP. The reason for this was that subjects attempting to inspire at 100% MIP fell short of the through volume manoeuvre. Through lung-volume training has previously been suggested to be superior to other methods of RMT (Chatham *et al.* 1999). This was made following the observation that RMT performed at different lung volumes exhibited the greatest effect within the lung volume in which the training was prescribed (Tzelepis *et al.* 1994). The present study confirms that inspiring from

residual volume to total lung capacity is not necessary to bring about significant adaptations in the respiratory muscles provided the intensity is high enough to compensate. Moreover, only the MAX group, who did not inspire through volume, experienced a significant decrease in exercising HR and RPE. These findings also indicate that pressure-threshold devices, that close when the target pressure can no longer be maintained (Romer *et al.* 2002; Romer *et al.* 2002; Romer *et al.* 2002; McConnell and Sharpe 2005; Guenette *et al.* 2006), are therefore likely to be as effective in increasing MIP.

7-6 Conclusion

This study has demonstrated that six weeks of inspiratory muscle training is sufficient to induce a significant increase in MIP in both MAX and SUB training groups. However, only the MAX training group resulted in a significant decrease in HR and RPE during exercise. The mechanisms responsible for these changes in exercise responses observed with the MAX group remain to be elucidated, but may differ from those involved in VIH training.

STUDY SIX - A Potential Mechanism?

8-1 Implications for Cardiac Change

Markov *et al.* (2001) tested whether the increased cycling endurance observed after respiratory muscle training (RMT) in healthy sedentary humans was associated with a training-induced increase in cardiac stroke volume (SV) during exercise, similar to the known effect of endurance training. The authors reported a significant effect of RMT on cycling endurance at 70% of maximum aerobic power (W_{MAX}). Despite a 24% increase in cycling endurance there was no change in stroke volume during exercise. A group performing aerobic endurance training (whole body) demonstrated an increase in both cycling endurance and stroke volume.

It was concluded by Markov *et al.* (2001) that the increased cycling endurance observed after RMT is not due to cardiovascular adaptations, and that the results provide evidence for the role of the respiratory system as an exercise-limiting factor. However, the type of RMT used for this study was voluntary isocapnic hyperpnoea (VIH) so it should be concluded that the increased cycling endurance is not due to cardiovascular adaptations following VIH training. This does not preclude the possibility that resistive inspiratory muscle training could improve cycling endurance through cardiovascular changes. To this end it was decided to investigate the possible cardiovascular adaptations to respiratory muscle training using ultrasound echocardiography.

8-2 Measurement of Stroke Volume

Stroke volume (SV) was derived echocardiographically using a HP Sonos 5500 Ultrasound System (Hewlett Packard, Andover, MA) with a 2.5Hz transducer. A 2-D sector scan of the left ventricle outflow tract was gained from a parasternal long axis view. Placement of a cursor line at the level of the valve allowed an M-mode trace of the aortic tract and delineation of the opening and closure of the aortic valve leaflets. Placement of the ultrasound transducer in the suprasternal notch allowed a 2-D sector scan of the ascending aorta. The sample volume was oriented parallel to flow in the aorta to facilitate pulsed-wave Doppler echocardiographic representation of the aortic flow waveform. From the M-mode trace, the diameter (D) of the annulus of the aortic valve was measured. The cross-sectional area (CSA) of the open valve was then determined using the following equation: $(\pi D^2) / 4$. From the Doppler trace, the outline of the waveform was digitised to calculate flow velocity integral (FVI, cm). The FVI is calculated by the computer of the echo machine as the area under the curve from the continuous wave Doppler of aortic outflow (Figure 8-1).

The FVI and CSA measurements permitted the calculation of stroke volume according to the following equation: SV = CSA x FVI. All measurements and calculations were made by an experienced echocardiographer.

Figure 8-1 Flow velocity integral (FVI) of aortic flow (shade area)

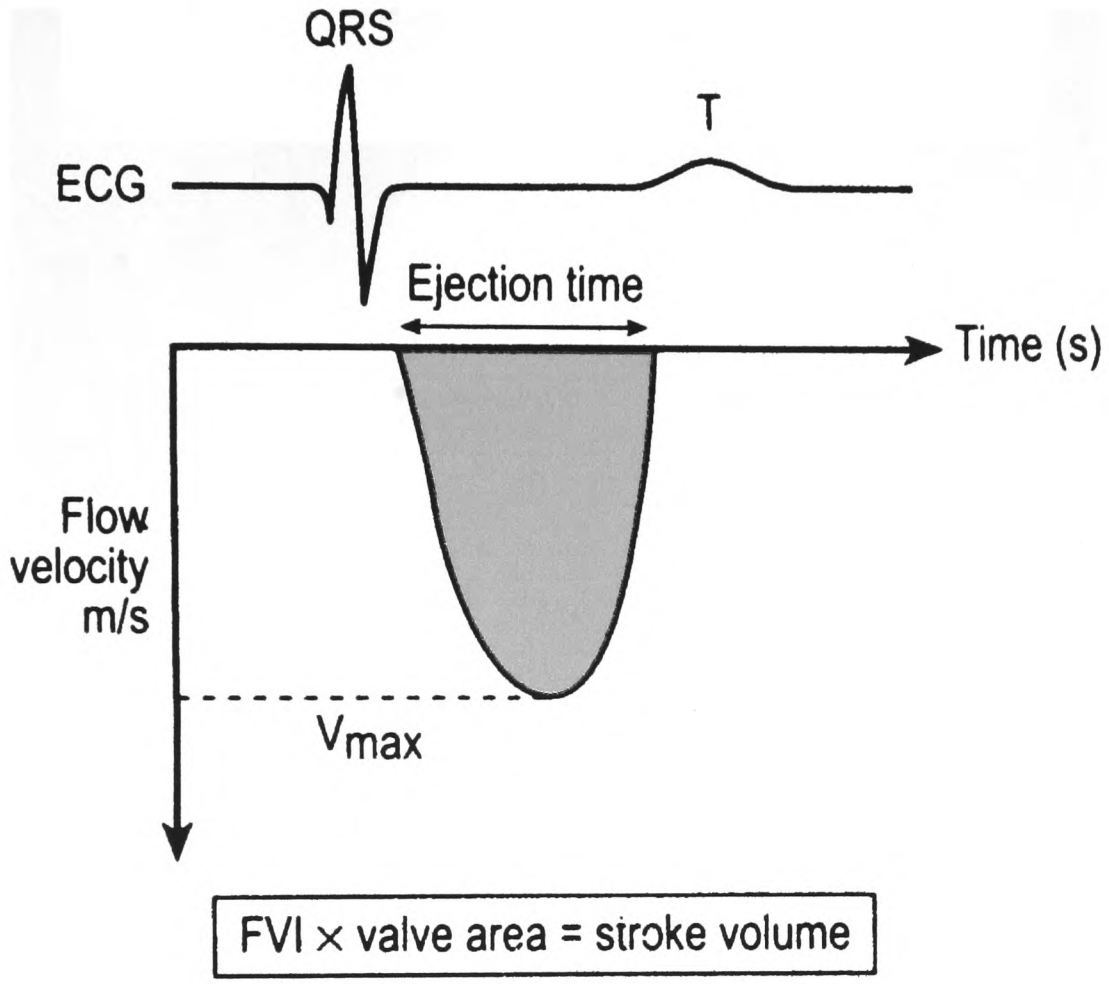
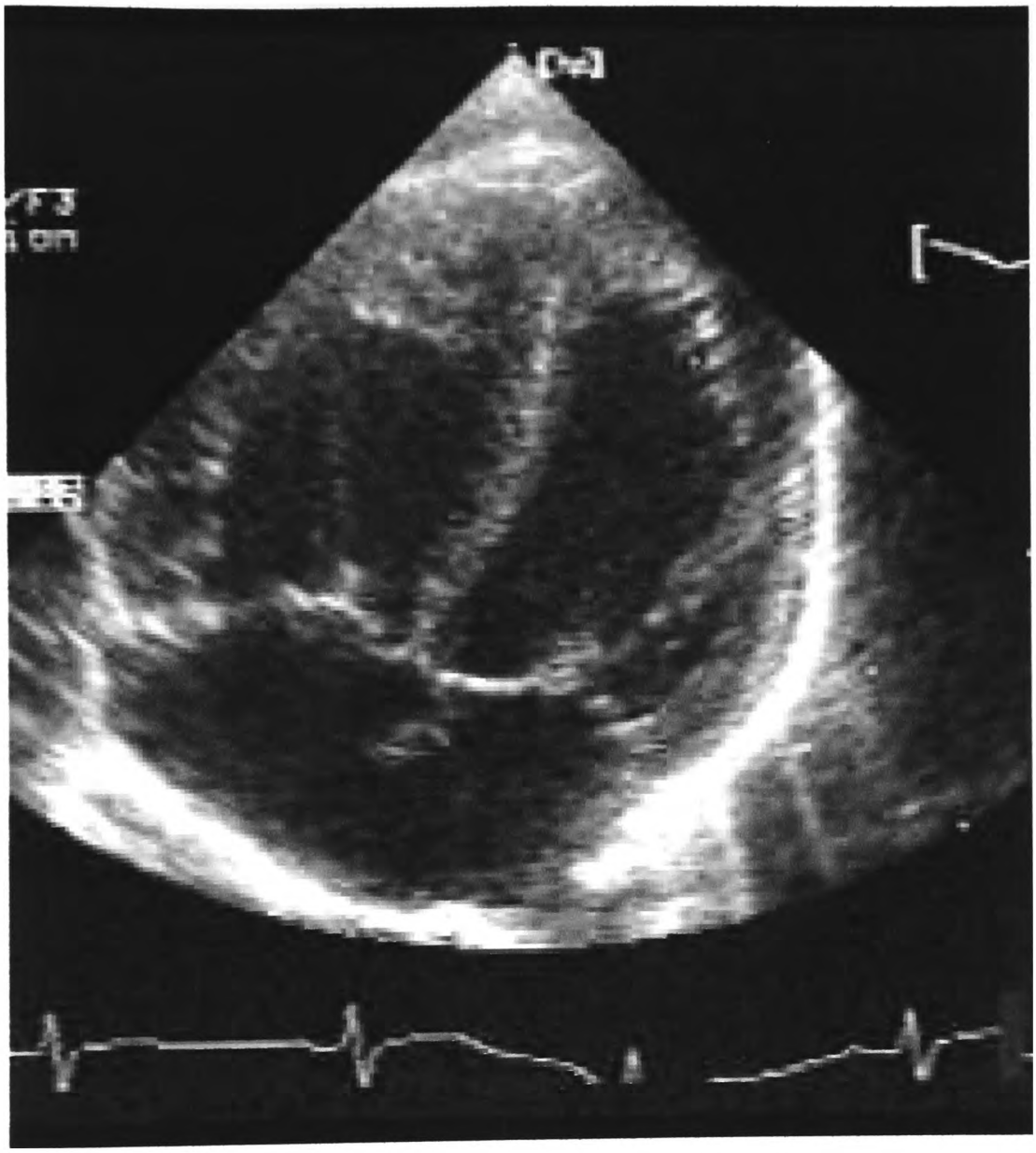


Figure 8-2 Echocardiograph



8-3 Experimental Design

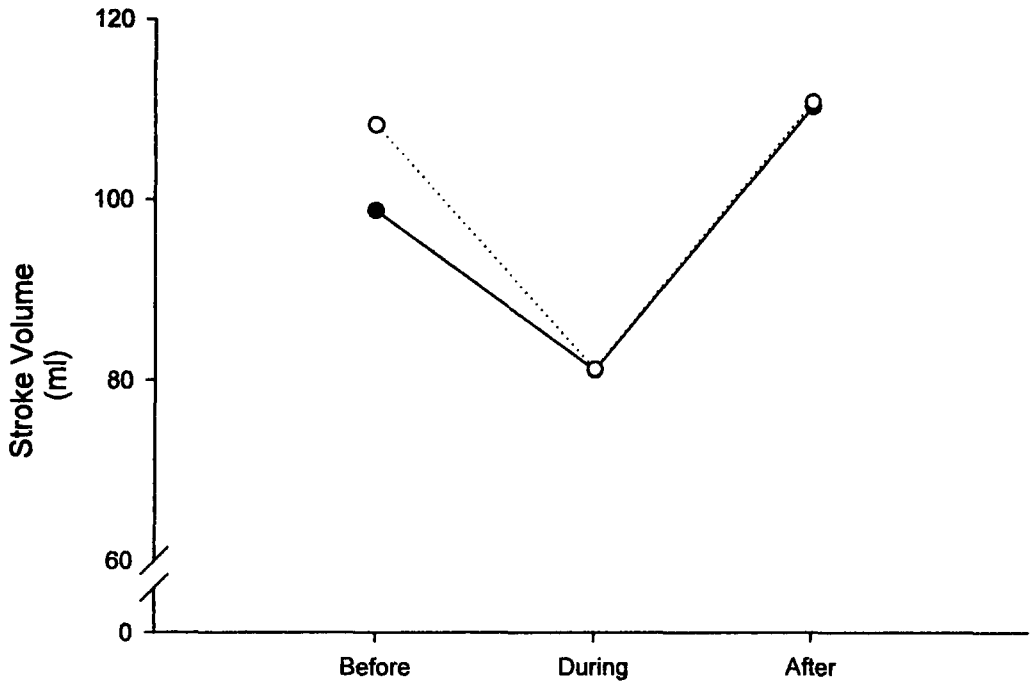
Two apparently healthy male subjects were invited to the Cardiac Rehabilitation Unit, Royal Glamorgan Hospital.

The aim of this pilot study was to determine stroke volume before, during and after a maximal inspiratory manoeuvre from RV to TLC against resistance (TIRE). Recordings of consecutive cardiac cycles were taken with points at which inspiration against resistance began and ended marked using the echo machine. Measurements were repeated to ensure accurate and reliable data. Table 8-1 shows the stoke volume results as calculated by the echocardiographer:

Table 8-1 Stroke volume before, during and after a maximal inspiration

Subject	Stroke Volume (ml)		
	Before	During	After
AG1	108.0	77.6	110.0
AG2	90.0	85.1	112.0
GE1	111.0	82.6	115.0
GE2	106.0	80.3	108.0

Figure 8-3 **Mean stroke volume before, during and after a maximal inspiration against resistance**



At rest, in healthy untrained subjects, stroke volume has been reported at 80 ml increasing to 100 – 110 ml at work rates 53, 68, and 83% $\dot{V}O_2$peak during upright cycling (Goodman *et al.* 2005). Subjects in the present study are supine for the measurements so an increased stroke volume at rest would be expected due to an increased venous return (Miller *et al.* 2005).

8-4 Discussion

A fall in arterial blood pressure during inspiration was first reported as early as 1733 with an augmentation in venous return to the right ventricle reported eighteen years later (Olsen *et al.* 1985). Subsequent investigators have confirmed this phenomenon documenting an inspiratory decline in left ventricular stroke volume (Goldblatt *et al.* 1963; Ruskin *et al.* 1973; Buda *et al.* 1979). Stroke volume appears to be reduced during active inspiration, however on cessation of this manoeuvre a large and potentially significant increase in stroke volume, greater than whilst at rest, is observed. Olsen *et al.* (1985) observed a diminished stroke volume during inspiration in conscious dogs, which Karam et el. (1984) demonstrated this in humans concluding it was due to a transient impediment to left ventricular ejection. Scharf et el. (1981) have previously used the Mueller manoeuvre as a controlled stress to demonstrate the presence of abnormal wall motion in patients with coronary artery disease. Loaded breathing might be used as a simple and safe method of subjecting the heart to controlled stress as a diagnostic tool in the left ventricular pressure-volume relationships (Karam *et al.* 1984). Chronic exposure to these manoeuvres, which are a feature of an IRL intervention, will potentially have a profound effect on the heart itself through the Frank-Starling relationship. We suggest a training effect similar to the responses of a normal exercise regime could be expected from the heart responses we have recorded, as first proposed by the work of Swanson (1998). Although, Markov *et al.*, (2001) reported no change in stroke volume following an isocapnic hyperpnoea intervention, the effects of a single inspiration against the same external resistance used in the present study have noted a significant effect on stroke volume (n = 2) as measured by echocardiography (unpublished observations). Further study

incorporating accurate appraisal of stroke volume following a resistive type intervention is required.

CHAPTER 4 - GENERAL DISCUSSION

9-1 Overview

As initially stated in section 1-7, the primary aims of this study were:

- To determine the functional significance of IRL training on cycling endurance capacity, whilst accounting for both familiarisation and placebo effects.
- To establish the relationship between the intensity of IRL (as a percentage of MIP) and duration of an IRL training intervention on the respiratory muscles.
- To investigate a possible mechanism behind improvements following IRL such as diaphragm thickness and stroke volume.
- To investigate the effect of an inspiration against resistance on stroke volume.
- To examine and evaluate the effect of non-invasive ventilation on respiratory muscle performance in a group of patients suffering with type II respiratory failure.
- To determine the long term effects of AAS use on respiratory system including respiratory muscle performance.
- To determine the effects of growth hormone on respiratory function.

To accomplish these aims, six separate studies have been successfully carried out, generating original data for this interesting area of respiratory muscle physiology.

Study one was the first of two studies designed to investigate the effects of specifically training the inspiratory muscles to produce increases in both strength and / or endurance. Firstly, this study confirmed that the intervention was successful with marked increases in both strength and endurance reported in the trained group. In addition, these increases coincided with dramatic improvements (36%) in cycling

endurance capacity at 75% $\dot{V}O_2$peak. Furthermore, heart rate, ventilation and RPE were all reduced during the exercise bout in the trained group. Neither the control group, who performed no training, or the placebo group, who performed sham training, demonstrated significant improvements in the same parameters. These findings demonstrate a significant effect of inspiratory resistive loading that cannot be explained by the effects of familiarisation or placebo.

Study two demonstrates a significant effect of long-term domiciliary NIPPV in type II respiratory failure patients on blood gas data, lung function, and quality of life that are associated with improvements in inspiratory muscle function that suggests these improvements are a result of alleviating the load to which these muscles are exposed, therefore providing adequate time the muscles to adapt.

Study three provides information regarding the effects of long-term (>20 years) AAS use on respiratory function. Here, indices of respiratory volume and flow remain unaffected by the use of these drugs. Subjects actively taking AAS possessed a higher inspiratory muscle strength when compared to control groups, which was correlated with grip strength.

Study four investigated the effects of short-term recombinant human Growth Hormone (rhGH) on the respiratory system. Here, body composition and respiratory muscle strength were both significantly improved following 14 days of rhGH administration.

Study five reported the results of the second IRL study conducted, this one designed to explore the effects of the intensity of the intervention on firstly inspiratory muscle function and secondly, heart rate and RPE responses to exercise. Here it was found that although training at both 100% and 80% of maximum (inspiratory pressure) brings about improvements in inspiratory muscle strength, only the maximum training intensity resulted in improvements in heart rate, and ratings of perceived exertion during cycling exercise.

Study six, a pilot study (n = 2), demonstrated that during an inspiration against the resistance used in the respiratory training studies stroke volume is reduced and immediately after stroke volume significantly increases.

9-2 Summary of findings

Respiratory muscles and NIPPV

The role that the respiratory (inspiratory) muscles play in everyday life is not recognised in a normal population, even during exercise. However, in a clinical situation when the load experienced by these muscles is increased, and their ability to ventilate the lungs impaired, their role becomes more obvious. Study two demonstrated that unloading the respiratory muscles in patients with type II respiratory failure results in improvements that are not apparent with optimal therapy prior to NIPPV use. The rationale for using NIPPV is to relieve the respiratory muscles from the increased loads to which they are exposed though changes in airways resistance, lung hyperinflation, or chest wall compliance. The principles of training suggest that by alleviating the respiratory muscles from this load, the muscles will adapt. The present research was the first to investigate the long-term effect of NIPPV on respiratory muscle strength and endurance. Furthermore, the improvements in inspiratory muscle strength and endurance observed following three months of domiciliary NIPPV use (5.9 ± 2.5 hrs use / day) coincided with improvements in blood gas tensions, oxygen saturation, bicarbonate retention, and quality of life. In addition, significant increases in FEV_1 and FVC were reported indicative of an increase in lung volume recruitment, which may have also influenced the measures of inspiratory muscle performance. Even if true, it is doubtful that the changes in lung volume could account fully for the improvements in strength and endurance. The data

generated from this study provides a rationale for conducting specific respiratory muscle training in this group of patients.

Respiratory muscles and AAS

The purpose of this investigation was to determine the effects of anabolic androgenic steroids on respiratory function in male body builders. The primary variable of interest was maximal inspiratory pressure. This study demonstrated no adverse effect of AAS use on respiratory function as measured by spirometry, expiratory flow profiles, and maximum inspiratory pressures. Inspiratory muscle strength was greater in the group of bodybuilders actively taking AAS at the time of testing when compared to a group who had been abstinent for a three-month period, a group of bodybuilders who had never used AAS, or a sedentary group. In addition, the same pattern was true for the measure of grip strength.

However, to suggest that the results from the present study can be extrapolated to patient populations may be too large a leap in reasoning, but what it does provide is information of the effects of long-term users on respiratory function in subjects with no history of respiratory disease. These data would help in the design of future studies aimed at examining directly the effects of AAS in a clinical setting as a way of offsetting muscle dysfunction in subjects with COPD (Casaburi 2000).

Respiratory muscles and rhGH

Patients deficient in growth hormone have impaired ventilatory function and a decrease in respiratory muscle pressures when compared with healthy subjects (Merola *et al.* 1995). The same group reported rhGH administration reversing these

impairments (Merola *et al.* 1996). We have demonstrated improvements in body composition and respiratory muscle strength and if duplicated in a clinical setting could prove to be of great benefit in alleviating conditions associated with COPD i.e. loss of muscle mass or peripheral muscle weakness (Villaca *et al.* 2006).

Respiratory muscle training

Studies one and five examined the effects of specifically training the respiratory muscles on whole body exercise. The first of the two studies provides evidence that specific IRL improves whole-body endurance capacity in subjects of average fitness. The novel aspects of the study are the inclusion of both a control and placebo group; and this study provides data on a range of physiological and perceptual variables under identical conditions exercise pre- and post-intervention (10 weeks IRL).

Significant improvements in inspiratory muscle strength and endurance, induced using the TIRE regime, coincide with increases in cycling endurance capacity at 75% $\dot{V}O_2$peak (by 36%) and reductions in exercising heart rate, ventilation, and perceived exertion during the exercise bout.

Study five compared two training regimes (100% MIP vs. 80% MIP) and a control group. Although both training intervention resulted in an increase in MIP, only the maximal group demonstrated a decrease in exercising heart rate and perceived exertion during a constant load exercise bout.

Future studies examining IRL should incorporate a maximal program (100% MIP) for a duration of six weeks.

Mechanism of action

Research into the mechanism that may be behind respiratory training has focused on VIH training. Several groups have systematically examined some of the possible mechanisms that could bring about the improvements in cycling endurance. To date, they have shown that the improvements in cycling endurance are not due to a change in stroke volume, (Markov *et al.* 2001), or to an increased oxygen supply as measured by blood gas concentrations (Stuessi *et al.* 2001). A decrease in blood lactate during endurance and following incremental exercise has been observed by some (Spengler *et al.* 1999; Romer *et al.* 2002), but not all (Sonetti *et al.* 2001).

A decrease in the rating of perceived exertion, as found in the present study, is a candidate for the observed improvements in cycling endurance capacity (study one). Previously, Volianitis *et al.*, (2001) and Kellerman *et al.*, (2000) reported a decrease in the perception of respiratory effort following a respiratory training intervention. In an outcome measure such as cycling time to exhaustion, which is by definition motivationally dependent, a decrease in the perception of exertion will likely have a profound effect on increasing $Tlim_{75}$.

Another possibility to explain the effects of RMT is altered ventilatory efficiency. The reduction in ventilation that the present study and others (Boutellier *et al.* 1992; Boutellier and Piwko 1992) have observed could contribute to the improvements in fixed work rate tests. The decrease in ventilation for a given workload will reduce the metabolic requirements of the respiratory muscles and result in diminished competition for blood flow requirements between the respiratory muscles and locomotor muscles (Sheel 2002). This factor is cited by some studies that have examined IRL (Volianitis *et al.* 2001; Romer *et al.* 2002) although it has not been investigated directly. It follows recent work where reducing the work of breathing

using a proportional assist ventilator during cycling exercise resulted in an increase in the blood flow to the legs. An average 50% reduction in the work of breathing results in a 5-7% increase in leg blood flow (Harms *et al.* 1997), and this translates into a 15% increase in endurance performance (Harms *et al.* 2000). However, it is very unlikely that the adaptations from a 10-wk respiratory training protocol will decrease the work of breathing anywhere near to the 50% decrease achieved using a ventilator. Therefore although changes in leg blood flow may occur, they are likely to be very small and difficult to detect (CA Harms, Personal Communication). Certainly any improvement in oxygenation or blood perfusion to the locomotor muscles would likely enhance performance (Harms *et al.* 2000), but to what extent this is occurring following RMT (VIH or IRL) remains to be investigated. This phenomenon, if it does exist following IRL would most likely be manifest during maximum exercise. As discussed earlier, an increase in $\dot{V}O_2$peak was not an expected outcome of the IRL intervention, but if blood flow redistribution was occurring then an increase in power output, and hence leg blood flow, would be required to attain the previously measured $\dot{V}O_2$peak, as has been demonstrated using respiratory muscle unloading (Harms *et al.* 1998). In the present study, no change in either $\dot{V}O_2$peak or maximum power output was observed suggesting that either blood flow redistribution does not occur, or it is too small to detect in terms of power output.

Two studies have attempted to examine respiratory fatigue following IRL and both have shown that an intervention such as used in the present study can attenuate respiratory muscle fatigue (Volianitis *et al.* 2001; Romer *et al.* 2002). However, although respiratory muscle fatigue (decrease in breathing endurance time) has been demonstrated following exercise at 65, 75, 85, and 95% $\dot{V}O_2$peak (Perret *et al.* 2000),

Johnson *et al.*, (1993) reported, specifically diaphragm fatigue (as shown by a reduction in transdiaphragmatic twitch pressure) only at intensities of exercise >85% $\dot{V}O_2$peak. The contradictory evidence reported probably results from the involvement of extra-diaphragmatic muscles in addition to the diaphragm during the resistive breathing endurance tests (Perret *et al.* 2000). It is reasonable to suggest that the IRL-interventions used in the present study likely resulted in an attenuation of respiratory muscle fatigue, and is a possible contributing factor to the improvements in cycling time to exhaustion.

A significant effect of an IRL intervention on exercising heart rate has not been previously reported. How the attenuation in heart rate observed in the present study may have been brought about is not really known and requires further investigation. Although, Markov *et al.*, (2001) reported no change in stroke volume following an isocapnic hyperpnoea intervention, our own group, examining the effects of a single inspiration against the same external resistance used in the present study have noted a significant effect on stroke volume (n = 2) as measured by echocardiography (unpublished observations). Stroke volume appears to be reduced during active inspiration, however on cessation of this manoeuvre a large and potentially significant increase in stroke volume, greater than whilst at supine rest, is observed. Indeed loaded inspiration (inspiration against resistance) has been suggested as a method of subjecting the heart to controlled stress as a diagnostic tool in the evaluation of left ventricular pressure-volume relationships (Karam *et al.* 1984). Chronic exposure to these manoeuvres, which are a feature of a IRL intervention, will have a profound effect on the heart itself. We suggest a training effect similar to the responses of a normal exercise regime could be expected from the heart response we have recorded,

as first proposed by the work of Swanson (1998). Further study incorporating accurate appraisal of stroke volume following a resistive type intervention is required.

Romer *et al.*, (2002) suggested that the mechanism behind the improvements in exercise performance was multifactorial in nature, with each of the above exerting some effect. We would agree with this statement but would emphasise that the exact proportion that each of the above plays remains unknown.

9-3 Future Research

Each of the six studies conducted in the course of this study have contributed to the body of knowledge and helped generate potential lines of enquiry for future research in this interesting area.

- The mechanism behind improvements following domiciliary NIPPV use can be teased out by including measures of lung volume and bilateral phrenic nerve stimulation. This approach would factor out motivation issues apparent with voluntary measures of respiratory muscle strength and would help confirm whether improvements in respiratory muscle function are a contributor to the other improvements brought about by NIPPV.
- If this proves to be the case, the next step would be to include specific inspiratory resistive loading in the rehabilitation program for type II respiratory failure patients. By carefully prescribing the work/rest ratios in these patients, it may be possible to adequately improve their respiratory muscle performance so that they can cope more easily on a day-to-day basis the loads to which they are exposed.
- The effect of AAS in a group of sedentary males and would make an interesting comparison with the bodybuilding groups. Most likely, with no stimulus for the muscles to adapt, there would be no change in whole body strength. This is suggested by the fact that although our body builders had been taking AAS for >20 yrs, no specific training was applied to the respiratory muscles, and therefore they did not adapt.

- AAS administration in populations where the load experienced by the respiratory muscles is great such as COPD, kyphoscoliosis, and obesity merits investigation as the increased load will likely result in increases in respiratory muscle strength.
- Respiratory muscle function is greater in trained athletes, but by no means optimal. The effect of IRL in a group of highly trained individuals is needed.
- Changes in stroke volume during respiratory manoeuvres against resistance with an increased number of subjects.
- The mechanism behind IRL interventions appears to be multifactorial with many potential candidates capable of explaining the observed improvements that are reported in the present study. A series of investigations designed specifically to elucidate the mechanism is required and should include measurements of:
    - blood flow redistribution during exercise.
    - oxygen cost of breathing during ventilatory manoeuvres.
    - carbon dioxide sensitivity.
    - stroke volume.

The present study has shown that the respiratory, particularly inspiratory, muscles play a much greater role in health and exercise than was previously suspected. It will take a further study incorporating the above-suggested studies to unravel exactly how IRL brings about the findings reported here.

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