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The utility of surface parasternal intercostal electromyography in the assessment of paediatric respiratory disease

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The utility of surface parasternal intercostal electromyography in the assessment of paediatric respiratory disease

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Author Statement

I declare that the work contained within this thesis is my own. I have prepared this thesis under the supervision of Dr Gerrard Rafferty and Professor Anne Greenough. Alan Lunt provided valuable support with pulmonary function testing at the outset of the studies. Methacholine challenge testing was performed in conjunction with Ashraf Ali. Claire Pringle and Keith Sharp undertook EMG signal analysis for the data contained within Chapters Four and Five.

Access to patients and healthy subjects was facilitated by collaboration with clinical colleagues at King's College Hospital and research fellows within both the London Respiratory Muscle Group and Professor Anne Greenough's neonatal research group.

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Abstract

Respiratory disease is the most common cause of both acute and chronic illness in children. Optimal management of respiratory disease relies on accurate assessment of disease severity and response to interventions. Current measures of pulmonary function are both unsuited to use in pre-school age children as well as poorly representing the range of pathophysiological changes occurring across the spectrum of lung diseases in children. Electromyography of the parasternal intercostal muscles (EMG_{para}), as a marker of respiratory system load-capacity balance, may represent a novel, effort-independent method for assessment of respiratory disease in paediatric populations.

The studies within this thesis assessed the application of EMG_{para} in evaluating response to clinical interventions in children with asthma and cystic fibrosis, as well as obtaining values of EMG_{para} values in healthy children, and comparing these measures of EMG_{para} to those obtained in the children with respiratory disease. This thesis also further investigated the relationship between EMG_{para} and conventional measures of pulmonary function in both adult and paediatric subjects.

Reductions in EMG_{para} activity were demonstrated following administration of bronchodilator in children with asthma, and with resolution of an infective exacerbation in children hospitalised for an acute exacerbation of cystic fibrosis lung disease. Data from a large cohort of healthy children, as well as highlighting technical and developmental considerations relevant to clinical application of the EMG_{para} technique, also demonstrated higher levels of EMG_{para} in children with respiratory disease compared to healthy controls. The absence of statistically significant correlations between EMG_{para} and standard pulmonary function variables from the studies conducted in both children with respiratory disease and in adult subjects undergoing incremental induced bronchoconstriction indicated the complex, multifactorial relationship between respiratory muscle activity and pulmonary function. While EMG_{para} cannot be viewed as a substitute for conventional lung function techniques, the data from

the studies contained within this thesis support further investigation and development of this novel method to assess of respiratory disease.

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List of abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATS	American Thoracic Society
AU	Arbitrary units
AUC	Area under the curve
AX	Reactance area
β_2 agonist	Beta-two receptor agonist
BD	Bronchodilator
BMI	Body mass index
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
CV	Coefficient of variation
DRG	Dorsal respiratory group
ECG	Electrocardiogram
EELV	End expiratory lung volume
EMG	Electromyogram
EMG _{di}	Diaphragm electromyogram
EMG _{di} %max	EMG _{di} as a percentage of maximum
EMG _{para}	Parasternal intercostal electromyogram
EMG _{para} %max	EMG _{para} as a percentage of maximum
EOT	End of test
ERS	European Respiratory Society
FEF ₂₅₋₇₅	Forced expiratory flows between 25 and 75% of FVC
FEV _{0.5}	Forced expiratory volume in 0.5 seconds
FEV _{0.75}	Forced expiratory volume in 0.75 seconds
FEV ₁	Forced expiratory volume in one second
FFT	Fast Fourier transform
FRC	Functional residual capacity
FVC	Forced vital capacity

IC	Inspiratory capacity
IOS	Impulse oscillometry
LCI	Lung clearance index
log	Logarithm
logEMGAR	Logarithm of the ratio of EMG measured at two time points
μV	Microvolt
maxEMG _{di}	Maximum diaphragm electromyogram signal
maxEMG _{para}	Maximum parasternal intercostal electromyogram signal
mBorg	Modified Borg breathlessness scale
MCT	Methacholine challenge test
MBW	Multiple breath washout
MyHC	Myosin heavy chain
NA	Nucleus ambiguus
NRA	Nucleus retroambiguus
NRD	Neural respiratory drive
NTS	Nucleus tractus solitarius
NVE	Neuroventilatory efficiency
NVE _{di}	Neuroventilatory efficiency of the parasternal intercostals
NVE _{para}	Neuroventilatory efficiency of the diaphragm
P _{0.1}	Pressure generation within 1 st 100ms of airway occlusion
PC20	Provocative concentration of methacholine inducing a 20% fall in FEV ₁
pCO ₂	Partial pressure of carbon dioxide
P _{di}	Transdiaphragmatic pressure
PEEP	Positive end-expiratory pressure
PEEPi	Intrinsic positive end-expiratory pressure
PEF	Peak expiratory flow
PFT	Pulmonary function testing
P _{gas}	Gastric pressure
P _{I,max}	Maximal inspiratory pressure
pO ₂	Partial pressure of oxygen
P _{oes}	Oesophageal pressure
PRG	Pontine respiratory group

PRO	Patient-reported outcome
R5Hz	Oscillometric resistance at five Hertz
R20Hz	Oscillometric resistance at twenty Hertz
RAR	Rapidly adapting stretch receptor
REM	Rapid eye movement
RF	Resonant frequency
R_{int}	Respiratory interrupter technique
RMS	Root mean square
ROC	Receiver operator characteristic
Rrs	Respiratory system resistance
SAR	Slowly adapting stretch receptor
SD	Standard deviation
SNIP	Sniff nasal inspiratory pressure
TLC	Total lung capacity
VRG	Ventral respiratory group
WHO	World Health Organisation
Xrs	Respiratory system reactance
Z5Hz	Oscillometric impedance at five Hertz
Zrs	Respiratory system impedance

1. Chapter One: Introduction

Chronic respiratory disease in childhood places a significant burden on healthcare systems both in the United Kingdom and internationally. Optimal management of respiratory disease requires clinicians to be able to accurately assess disease severity, progression and response to treatment. Current routine measures of pulmonary function such as spirometry require volitional manoeuvres from the subject and are therefore inappropriate for use in younger age groups. Even in older age groups able to comply with the complex manoeuvres required, such tests also only assess individual aspects of respiratory function and may therefore underestimate the full impact of the range of pathophysiological changes. Airway narrowing, distal airways collapse, hyperinflation and alterations in respiratory pattern serve not only to increase the respiratory load but also to decrease the capacity of the respiratory muscles, reducing their ability to respond to this load. Electromyographic measurement of parasternal intercostal muscle (EMG_{para}) activity provides both a marker of load on the respiratory muscles as well as an index of capacity and therefore offers a potential non-invasive, effort-independent method to assess the respiratory load-capacity balance.

1.1 Respiratory disease in childhood

Respiratory disease is the most common cause of both acute and chronic illness in children in the United Kingdom, accounting for 14% of all hospital consultant appointments and 42% of all inpatient admissions [1]. While the majority of acute episodes of respiratory illness will resolve spontaneously with no long-lasting effects [2], chronic disease places a substantial burden on healthcare systems worldwide and results in significant morbidity. It is also well recognised that much of the burden of disease later in life has its origins in early childhood [3-6]. This thesis will examine the performance of a novel measure of

disease severity and control in three common lung diseases of childhood, namely asthma, pre-school wheeze and cystic fibrosis.

1.1.1 Childhood asthma

Asthma is the most common chronic disease in childhood, and the leading cause of morbidity in children [7]. Asthma is responsible for a significant burden on both primary and secondary care worldwide, and in many countries the prevalence is rising [7-9]. Asthma is characterised by the presence of increased airway smooth muscle activity and chronic airway inflammation, resulting in airway epithelial swelling, mucous hypersecretion, and airways obstruction.

The diagnosis of asthma in children is principally clinical, and there exists no standardised definition of the type, severity or frequency of symptoms, nor of the investigative findings necessary for a formal diagnosis [10]. Nonetheless, there are common features to all definitions, namely the presence of more than one of wheezing, chest tightness, cough and breathlessness or difficulty breathing, as well as of variable airflow obstruction and bronchial hyperresponsiveness [7-9, 11, 12].

In older children, a detailed clinical history directly from the patient can ascertain the presence, frequency and severity of the symptoms of asthma. In younger children, however, this is more challenging and relies on parental report. Although cough can easily be noted by a caregiver, wheeze is commonly used by parents to describe any of a range of abnormal respiratory sounds, and thus the use of parent-reported wheeze may be poorly specific for asthma. Chest tightness and breathlessness are subjective sensations and obtaining an accurate indication of the presence of these symptoms is challenging in younger children [13].

Determining the presence of variable airflow obstruction requires objective assessment of pulmonary function. In children over the age of five, spirometry or peak expiratory flow can be used to assess presence, severity, variability and

reversibility of airflow obstruction [11, 14], and consequently assist in diagnosis and guide treatment decisions. International guidelines recognise that there may be little opportunity to obtain such measures in children under the age of five [7, 8, 11]. Management recommendations for children in this age group are therefore based on analysis of the probability of the child having asthma, taking into account factors such as family history, age, sex and presence or absence of atopy. Suggested treatment options include either “watchful waiting”, which may result in delayed treatment for children later shown to be asthmatic, or a trial of treatment, which risks exposing children without asthma to the potential side effects of medication [10, 11].

1.1.2 Pre-school wheeze

In the pre-school age group, recurrent wheeze is a commonly reported problem with a wide spectrum of presentations and proposed aetiologies. Various large birth cohort studies have attempted to classify wheezing phenotypes. Between three and six phenotypes of wheezing illness [15-19] have been identified based on factors such as coexisting conditions (such as atopy, allergic rhinitis and eczema), family history, age at onset and frequency and severity of symptoms. In practice, however, classification into these phenotypes is dependent on the child’s long-term outcome and hence can only be assigned retrospectively, making their clinical utility extremely limited. In order to address this, the European Respiratory Society (ERS) Task Force on pre-school wheeze has proposed a symptom-based classification, which divides wheezing illnesses in this age group into two categories: episodic viral wheeze, occurring only with viral respiratory infections and with no interval symptoms, and multiple trigger wheeze, whereby triggers such as allergens, tobacco smoke and exercise, as well as viral illness, may result in wheeze [20]. While there is evidence that this classification is useful in predicting treatment response [13], it has been criticised due to the lack of evidence that these two phenotypes truly represent different pathological processes or result in different outcomes; indeed children have been shown to exhibit both phenotypes within a time course as short as one year [21]. It has also been noted that the absence of reliable objective

markers of pulmonary function for use in this age group may contribute to the difficulty in classifying wheezing phenotypes [13]. Due to the complexity of identifying aetiologies and subtypes of wheezing illness, the phrase “pre-school wheeze” will be used in this thesis to denote any chronic wheezing illness occurring in children under the age of five years.

1.1.3 Cystic fibrosis

Cystic fibrosis (CF) is the most common life-threatening inherited disease in the United Kingdom. CF is caused by mutations in the CF transmembrane conductance regulator gene, resulting in disruption of sodium and chloride channels in secretory epithelial cells [22]. This causes multisystem dysfunction, including pancreatic insufficiency, liver disease, poor gut motility, elevated sweat electrolytes, and highly viscous mucous in the lungs. The increased viscosity of pulmonary secretions leads to a tendency to pulmonary infections, resulting in a vicious cycle of endobronchial infection and concomitant inflammation of the small airways [23]. This results in progressive pulmonary destruction and eventual respiratory failure, which is the cause of death in the majority of patients with CF [24].

The substantial advances in CF diagnosis and care have resulted in significant improvements in maintenance of pulmonary function and associated increases in quality of life for patients with CF. Life expectancy for a child diagnosed with CF at birth now extends into the fifth decade, and the majority of children with CF will experience long intervals between hospitalisations [25]. Nonetheless, structural changes have been shown to be present in the airways of infants with CF [26] and CF-related deaths continue to be reported in children prior to secondary school age [25]. Monitoring pulmonary function is essential in facilitating optimal management [27], with maintenance of good pulmonary function during childhood having been shown to be associated with improved outcomes later in life [28].

1.1.4 Pathophysiological changes in obstructive lung disease

Obstructive lung disease may result in a wide variety of changes within the respiratory system. The exact presentation will vary between diseases but all obstructive lung diseases can be said to share a number of common pathophysiological features.

Airway narrowing is a predominant feature of all such diseases. It may occur at any level within the bronchial tree, as a result of hyperactivity and hypertrophy of smooth muscle, airway oedema, chronic airway remodelling, and mucous hypersecretion [29]. Although much emphasis is placed on the obstructive defects seen in the larger proximal airways, small airway dysfunction is also of great significance. It has been suggested that up to 90% of the airflow limitation present in asthma occurs beyond the 8th generation of the airways [30] and the earliest pathophysiological changes occurring in cystic fibrosis-related lung disease occur in the smaller, distal airways [31].

Obstruction of distal airways may result in their collapse during expiration. Such collapse leads to gas trapping within the lung and hyperinflation, defined as an abnormally high volume of air remaining in the lungs at the end of expiration. Hyperinflation has been demonstrated both during acute exacerbations of respiratory disease and chronically, even in the absence of severe airflow limitation [32]. Hyperinflation imposes an elastic load on the respiratory muscles, due to the added stretch on the lung tissues as a result of the elevated lung volume. Hyperinflation also leads to intrinsic positive end-expiratory pressure (PEEPi) causing a threshold load which must be overcome before negative pressure can be generated and air can flow into the lungs [33].

An increase in end-expiratory lung volume also causes the diaphragm to operate at a mechanical disadvantage. As end expiratory lung volume increases, the diaphragm becomes progressively flattened and shortened, resulting in decreased force generating capacity [34]. At higher lung volumes, the zone of apposition is also reduced and the costal diaphragm fibres insert at a less acute angle, causing the costal portion of the diaphragm to have an expiratory, rather

than inspiratory, effect [34]. Other inspiratory muscles must therefore be recruited to effect the intrathoracic pressure change required for ventilation.

The degree of expiratory airflow limitation seen in moderate to severe airways obstruction may alter the normal respiratory pattern, moving expiration from a passive process, mediated by the elastic recoil of the lung and chest wall, to one requiring active contraction of the expiratory muscles. Recruitment of these muscles, particularly the large abdominal muscles, will increase the body's metabolic requirements and consequently the demands on the respiratory system will be further increased.

1.2 Structural anatomy of the respiratory system

The lungs are a pair of passive distensible organs, located, with the heart, within the thoracic cage. The thoracic cage is comprised of the thoracic portion of the vertebral column, the sternum, and twelve pairs of ribs, each articulating dorsally with a thoracic vertebral body and ventrally with the costal cartilage [35]. The orientation of the ribs in adults is such that the axis of rotation is orientated laterally, dorsally and caudally. The ribs themselves are not horizontal, but slope inferiorly and anteriorly from their articulations with their respective vertebral bodies. This combination results in lateral and anterior displacement of the anterior ends of the ribs occurring simultaneously with their elevation, as well as cranial and anterior movement of the sternum. This results in increases in both the antero-posterior and lateral dimensions of the ribcage [36], often described as the 'pump-' and 'bucket-handle' motions of the ribs respectively.

The lungs are surrounded by a serous membrane (visceral pleura) which folds back on itself to form a two layered structure [35]. The outer pleural membrane (parietal pleura) is attached to the inside of the rib cage. Surface tension of the small amount of fluid between the pleural membranes maintains close

apposition of the two pleurae, opposing the elastic recoil of the lung parenchyma, preventing lung collapse and maintaining lung inflation.

1.2.1 Development and maturation of the respiratory system

The data obtained from studies in paediatric populations must be placed into context with the substantial anatomical and physiological changes that occur throughout childhood. As the studies contained within this thesis include children of less than a year of age, developmental changes from infancy have been considered. At birth, the human infant has lungs that are structurally similar to the fully developed adult lung, with the adult number of airways and all components of the airway wall being present [37]. Development of the lungs is rapid in the first few months of life, but continues throughout childhood and adolescence. At birth, up to 150 million alveoli are present [37]. It was until recently thought that alveolarisation was complete by seven to eight years of age, with alveolar numbers of between 200 and 600 million reported at this age [38-40]. More recent data has suggested, however, that alveolarisation continues throughout adolescence, with a combination of increases in both alveolar number and size proposed to be responsible for the near-quadrupling of lung volume from age seven to adulthood [41]. Gas exchange per unit of body mass also increases during childhood [42], mainly as a result of increases in the number of pulmonary capillaries [37]. The airways increase in size, resulting in decreased airways resistance [43-45], with an associated increase in lung compliance [46, 47].

Structural changes in the thoracic cage and torso also contribute to changes in respiratory system efficiency. The infant ribcage is highly compliant, with a greater proportion of cartilage to mineralised bone [48]; ribcage ossification continues until as late as 25 years of age [35, 49]. Stiffening of the chest wall occurs during infancy as a result of both ribcage ossification [48] and increases in muscle mass. Chest wall compliance in the infant is up to three times greater than that in the adult [50], which would result in a relaxed lung volume lower

than airway closing volume, potentially predisposing the infant to atelectasis and poor oxygenation. Functional residual capacity (FRC) is actively maintained above closing volume in infants by initiating inspiration prior to reaching relaxed lung volume and by a combination of post-inspiratory action of the inspiratory muscles and expiratory “braking” by the upper airway muscles [48]. Passive maintenance of FRC above closing volume is achieved by the end of the second year of life, and active efforts at maintaining appropriate end-expiratory lung volumes are no longer observed [51].

In infancy, the ribs are in a more perpendicular orientation relative to the vertebral column. The rib cage is more circular than in adults [52] and the upper ribs are distinctly shorter than the lower ribs, giving a triangular shape to the thoracic cage, in contrast to the dome shape seen in adults [53]. Upon adoption of the upright posture at around six months [22] the ribs start to slope downward. By the age of ten years, the inferior sloping of the ribcage has reached adult configuration [54]. As thoracic length increases with age, the height of the abdominal hydrostatic column increases, which results in a greater gravitational pull on the abdominal contents. This augments the inspiratory action of the diaphragm, thus resulting in reductions in the required effort to effect volume change of the lungs [48].

The horizontal orientation of the ribcage in infants and young children results in a flattened diaphragm with a corresponding smaller zone of apposition and less of the abdominal contents being encased by the lower ribcage. The ‘bucket-’ and ‘pump-handle’ effects of diaphragm contraction are therefore reduced in the infant [53], meaning that rib cage motion contributes little to tidal volume [55]. This deleterious effect is somewhat offset by the smaller radius of curvature of the diaphragm in infants and children, enhancing the force-generating ability of the diaphragm through the law of Laplace [56]. The greater chest wall compliance results in less efficient translation of reductions in pleural pressure changes to lung volume changes, with discrepancies of up to sevenfold between diaphragm pressure-volume work and pulmonary work being reported [57].

1.2.2 The respiratory muscles

Volume change of the lungs, and hence pulmonary ventilation, occurs as a result of contraction of the respiratory muscles [58] which work in concert to increase thoracic volume, expand the rib cage, lowering pleural and intrapulmonary pressure and causing air to flow into the lungs. Effective function of the respiratory muscle pump is essential to maintain adequate gas exchange and comprises both inspiratory and expiratory muscles. The inspiratory muscles include the diaphragm and external and parasternal intercostal muscles as well as accessory muscles of the upper chest and neck. The expiratory muscles are comprised of the internal intercostal muscles and abdominal muscles (rectus abdominus, internal and external obliques, and transversus abdominus).

1.2.2.1 Respiratory muscles: the diaphragm

The diaphragm is the primary muscle of respiration performing approximately 70% of the work of breathing in healthy adults [33]. It is a large dome shaped muscle that sits between the thoracic and abdominal compartments, innervated by the phrenic nerves, which originate from the third, fourth and fifth cervical segments [36]. The two halves of the diaphragm (left and right hemidiaphragms) are innervated by their respective phrenic nerves. The fibres of the diaphragm originate from the postero-inferior portion of the sternum, the inner surfaces of the lower six ribs and costal cartilages, and the lumbar vertebrae and insert into a central tendon [35]. Contraction of the diaphragm results in flattening of the muscular sheet, compressing the abdominal contents and causing the volume of the thoracic cavity to be increased. The increase in intra-abdominal pressure causes outward movement of the lower rib cage. Furthermore, the orientation of the fibres of the diaphragm where they attach to the lower rib cage (the 'zone of apposition') means that contraction of the muscle results in an upward and lateral movement of the lower ribs [59], thus

increasing the lateral and antero-posterior diameter of the rib cage. It can thus be seen that the 'bucket-handle' motion of the ribs depends on the angle of insertion of the costal diaphragm muscle fibres (and thus the size of the zone of apposition) being maintained.

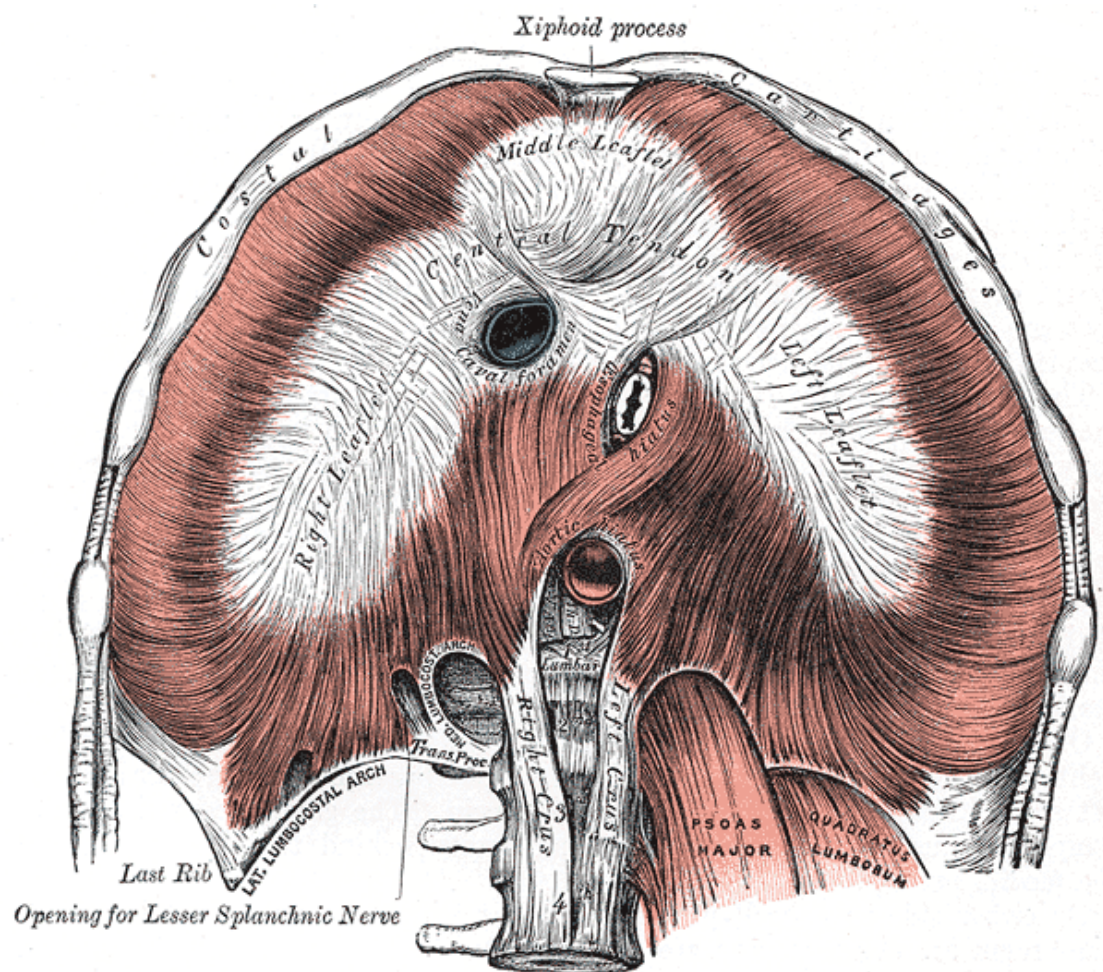


Figure 1 Diagram of the human diaphragm. Reproduced from Gray, H. (1918) *Anatomy of the human body*, Philadelphia: Lea and Febiger [35].

1.2.2.2 Respiratory muscles: intercostal muscles

There are two layers of intercostal muscles: external and internal. The external intercostal muscles are located superficially to the internal intercostal muscles [35]. The internal and external intercostal muscles between each pair of ribs are innervated by the corresponding intercostal nerve [35]. The fibre direction of each of the two muscle layers is shown in Figure 2.

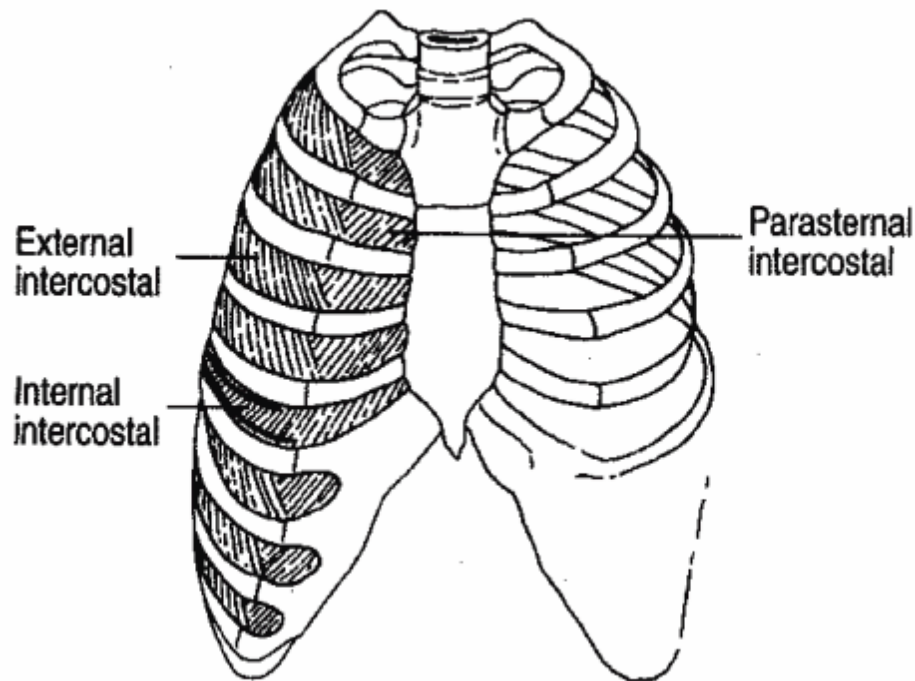


Figure 2 Fibre direction of the intercostal muscles [60].

Both groups of muscles play a significant role in respiratory function [61, 62]. The internal intercostal muscles are orientated laterally and inferiorly from the cranial to the caudal rib, and have a predominantly expiratory action, contraction causing collapse of the rib cage. The external intercostals extend medially and inferiorly from the superior to the inferior rib in each interspace, and act to raise the inferior rib, consequently having an inspiratory effect. Both the internal and external intercostal muscles also have a postural role, being particularly active during rotation of the trunk [63, 64]. Between the sternum and costo-chondral junction in the first five interspaces ventrally, the external

intercostals are replaced by a fibrous aponeurosis. The remaining internal intercostals in this area are termed the parasternal intercostal muscles.

1.2.2.3 Respiratory muscles: the parasternal intercostal muscles

The parasternal intercostal muscles are anatomically similar to the internal intercostal muscles, but are functionally distinct. Unlike the internal intercostal muscles, the parasternal intercostals have an inspiratory role, and have been termed the 'inspiratory intercostals'. Similarly to the diaphragm, the parasternal intercostal muscles are composed of predominantly slow twitch, oxidative fibres, making them resistant to fatigue [65]. A number of studies in dogs and human subjects have resulted in a detailed understanding of the role of this muscle group. Contraction of the parasternal intercostal muscles increases both the lateral and, to a lesser extent, the anteroposterior diameters of the chest wall [66]. Denervation or thoracic epidural block of these muscles in anaesthetised dogs results in significant decreases in tidal volume, minute ventilation and increases in $p\text{CO}_2$ [67, 68], with marked reductions in cranial rib displacement despite substantial compensatory increases in external intercostal muscle activity [69]. In contrast, severing of the canine external intercostal muscles in the presence of preserved parasternal intercostal muscle function results in only moderate reductions in tidal volume with no increase in parasternal intercostal activity [70]. Studies involving selective denervation of canine chest wall muscles suggest that the parasternal intercostal muscles are responsible for approximately 80% of rib motion during quiet breathing [69], though this may not be the case in humans.

Wilson *et al* [71] applied the Maxwell reciprocity theorem to the human respiratory system in order to calculate indirectly the mechanical advantage of several rib cage muscles. The authors state that the effect of a respiratory muscle's contraction (airway pressure change, ΔP_{ao}) is related to the mass of the muscle (m), the maximum active muscle tension per unit cross sectional area (σ), and the fractional change in muscle length ($\Delta L/L$) per unit volume increase of the chest wall (ΔV_L)_{Rel}, giving the equation:

$$\Delta P_{ao} = m\sigma(\Delta L/(L\Delta V_L))_{Rel}$$

The mechanical advantage of a basic lever is defined as the ratio of force delivered to the force applied, and when applied to the respiratory muscles can be defined as the change in airway pressure divided by the total respiratory muscle tension required to effect the change ($\Delta P_{ao}/m\sigma$). Using the above equation, the mechanical advantage can be calculated by measuring $(\Delta L/(L\Delta V_L))_{Rel}$. By assessing changes in intercostal muscle length induced by passive lung inflation during computed tomography scanning, together with cadaveric studies, these authors demonstrated that the parasternal intercostal muscles shorten during passive lung inflation and can therefore be said to have an inspiratory mechanical advantage, but that this is less than that of the external intercostal muscles [71, 72]. The mechanical advantage of the human parasternal intercostal muscles is fourfold greater in the second than in the fifth interchondral space [72].

While the parasternal intercostal muscles, when activated in isolation, do effect ribcage motion and volume change of the thoracic cavity, their role in the fully intact respiratory system is also dependent on their interaction with other muscle groups, particularly the external intercostal muscles and diaphragm [73]. It is noted that, while the parasternal intercostal muscles move the upper ribs laterally and cranially, and contraction of the external intercostal muscles results in cranial rib displacement, contraction of these two muscle groups enhances lateral rib motion, but with the additional effect of caudal displacement of the sternum [74].

Tetraplegic patients with preserved parasternal intercostal muscle activity demonstrate normal lung compliance, FRC and transpulmonary pressures. By comparison, those with non-functional parasternal intercostal muscles demonstrate reductions in these parameters, supporting the premise that these muscles also assist in stabilising the upper chest wall against the potentially

deforming action of the diaphragm [75]. Studies in dogs have also demonstrated the stabilising role of the parasternal intercostal muscles, showing dorsal and caudal displacement of the ribs during inspiration in dogs with flail chest in whom the parasternal intercostal muscles had been denervated [76].

The parasternal intercostal muscles are consistently active during inspiration, only inactive when the inspired volume is minimal [62, 77-79]. They are activated in concert with the diaphragm [79, 80] and a strong agreement between both the amplitude and timing of peak diaphragm and parasternal intercostal electrical activity has been observed [81, 82].

The neural drive to the parasternal intercostal muscles demonstrates a cephalocaudal gradient, with higher neural drive being delivered to those muscles with the greatest mechanical advantage [61, 80, 83, 84]. Timing of the activation of the parasternal intercostal muscles also shows a cephalocaudal gradient, with the muscles in the first to third interspaces being activated within the first 10% of inspiratory time, and those in the fourth and fifth interspaces commencing activity approximately one-third of the way through an inspiratory effort [80]. A mediolateral gradient of activation has been found in dogs [85], though this is not the case in human subjects [80]; it is suggested that these differences occur due to the differences in rib cage geometry and muscle fibre orientation between the two species.

1.2.2.4 Respiratory muscles: sternocleidomastoid

The sternocleidomastoid muscle is an accessory muscle of inspiration, originating from the lateral surface of the mastoid and occipital bones, and inserting *via* two heads into the upper part of the anterior surface of the manubrium sterni (sternal head) and the medial surface of the clavicle (clavicular head) [35]. Unilateral activation of the sternocleidomastoid acts to flex the head laterally and rotate it to the contralateral side, or bilateral

activation acts to flex the cervical spine. If the head is fixed, however, contraction of the sternocleidomastoid muscles causes elevation of the sternum and upper ribs, and assists in stabilising the upper rib cage against the action of the diaphragm that, if unopposed, would deform the ribcage. The sternocleidomastoid is only tonically active during resting breathing but is recruited when ventilatory requirements increase, such as during exercise or in the presence of lung disease [86].

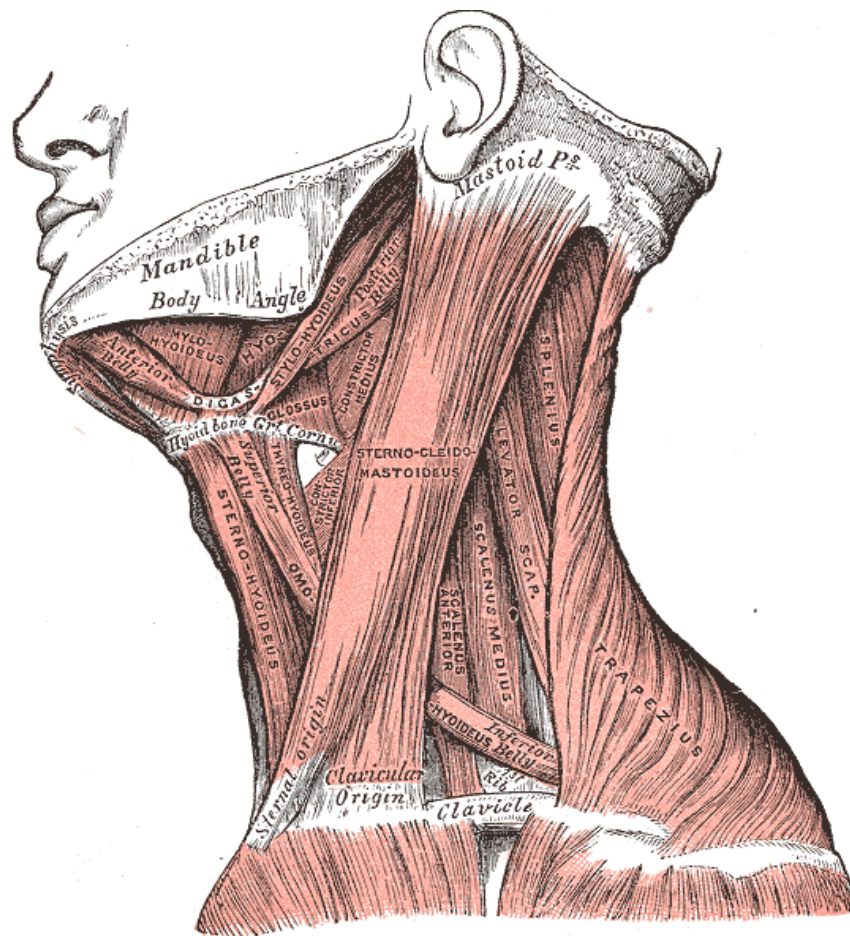


Figure 3 Diagram of the human sternocleidomastoid muscle. Reproduced from Gray, H., (1918) *Anatomy of the human body*, Philadelphia: Lea and Febiger [35].

1.2.2.5 Respiratory muscles: the scalenes

The scalene group comprises three segments: anterior, medial and posterior. These muscles arise from the transverse processes of the second to the seventh cervical vertebrae and insert into the first and second ribs. The scalenes act to elevate the first and second ribs and as such serve as inspiratory muscles [35]. The scalenes have been shown to be consistently active during inspiration in humans, even when the inspired tidal volume is small [79, 87].

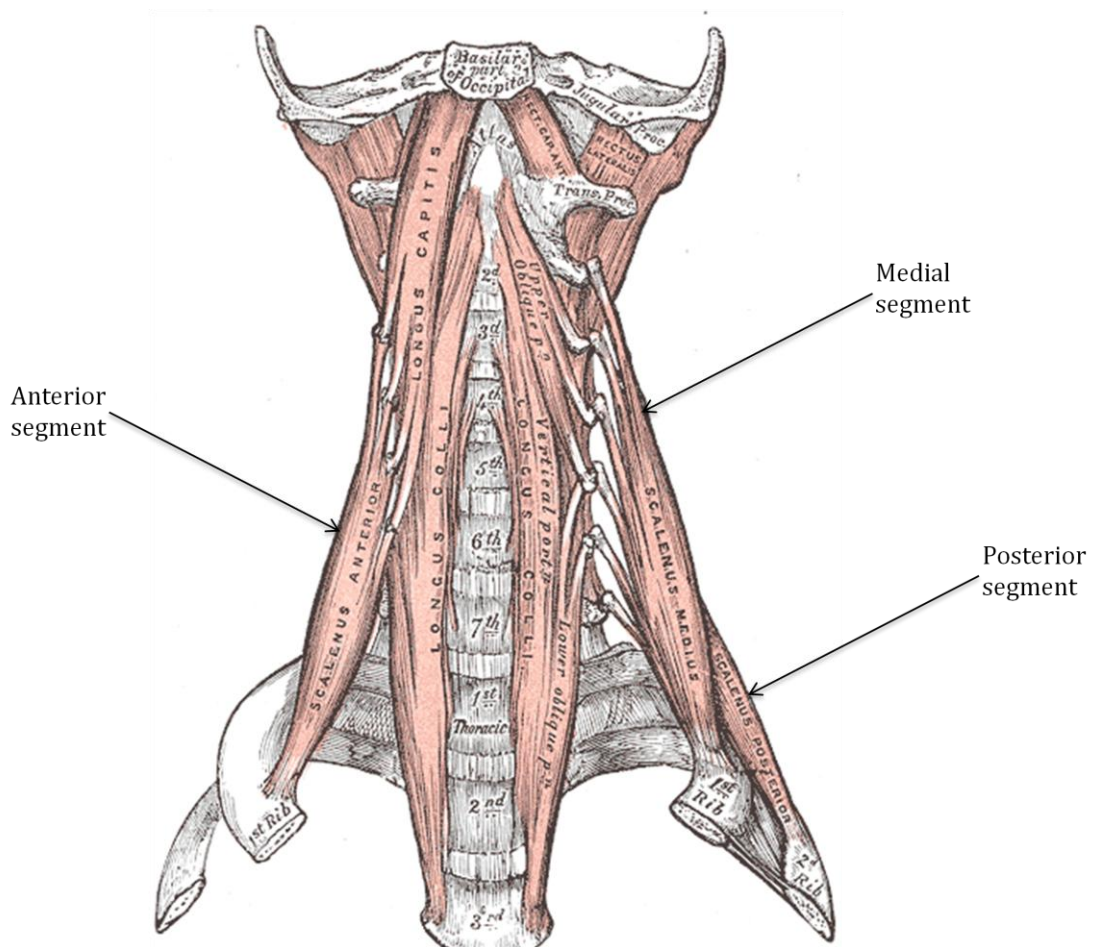


Figure 4 Diagram of the human scalene muscles (anterior, medial and posterior segments indicated with arrows). Adapted from Gray, H., (1918) *Anatomy of the human body*, Philadelphia: Lea and Febiger [35].

1.2.2.6 Respiratory muscles: the abdominal muscles

The abdominal muscles comprise a group of four separate muscles: transversus abdominus, internal and external oblique, and rectus abdominus [86]. Transversus abdominus is a thin, flat, horizontally-orientated sheet of muscle, and is the deepest of the four abdominal muscles. The internal oblique muscle sits superficial to transversus. Both muscles originate from the lumbodorsal fascia, the inner lip of the iliac crest and the lateral aspect of the inguinal ligament, with transversus having fibres additionally originating from the inner surfaces of the cartilage of the lower six ribs. Both muscles insert via an aponeurosis into the pubis, pectineal line and linea alba. The internal oblique also has attachments into the seventh to twelfth ribs, with those fibres inserting into the lower three ribs being continuous with the internal intercostal muscles. The external oblique is the largest of the three flat abdominal muscles. It arises from the external and inferior surfaces of the lower eight ribs, inserting via an aponeurosis into the linea alba and inguinal ligament. Rectus abdominus is a long flat muscle, extending along the length of the front of the abdomen. It originates from the crest of the pubis and the pubic symphysis, and inserts into the cartilages of the fifth to seventh ribs and into the body of the fifth rib [35].

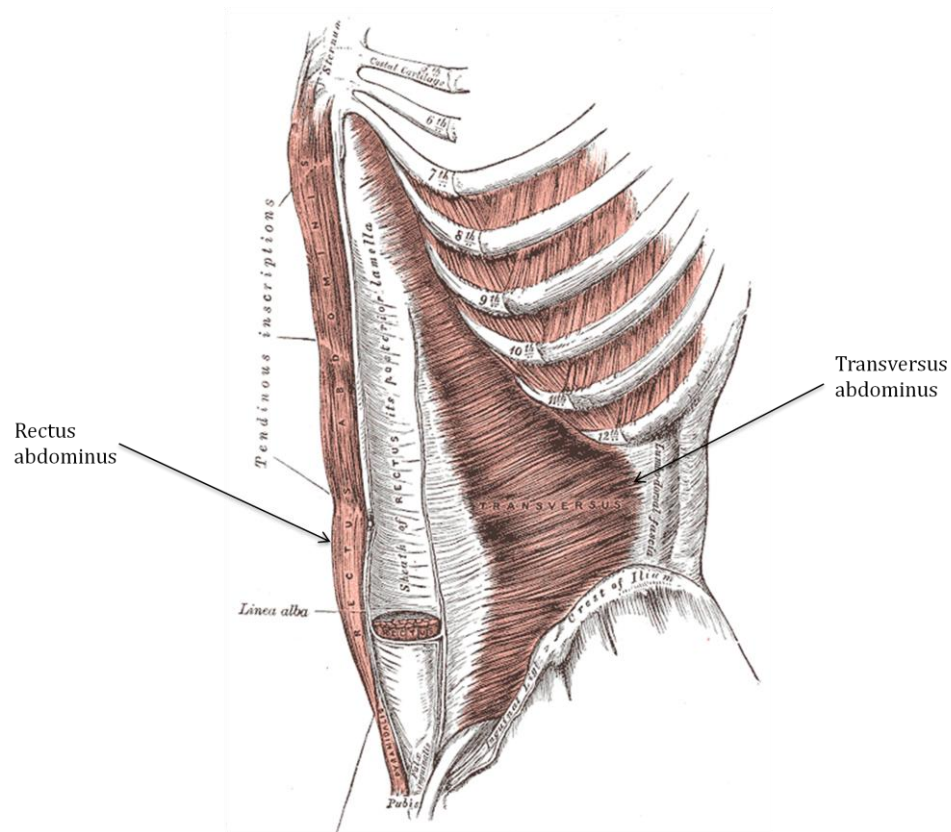


Figure 5 Diagram of the human rectus abdominus and transversus abdominus muscles. Adapted from Gray, H., (1918) *Anatomy of the human body*, Philadelphia: Lea and Febiger [35].

Contraction of the abdominal muscles pulls the abdominal wall inward, which acts on the abdominal contents, displacing the diaphragm upward, and also causing the lower ribs to move caudally [58]. The resultant increases in intra-abdominal and intra-thoracic pressures causes air to move out of the lungs. Contraction of the abdominal muscles may also serve an accessory inspiratory role as active expiration to lung volumes below FRC augments the subsequent inspiratory effort through elastic recoil of the chest wall [36].

1.2.3 Respiratory muscle structure and function

The respiratory muscles are skeletal muscles, demonstrating common structural and functional characteristics with peripheral muscles. They are composed of individual motor units, each consisting of a group of muscle fibres innervated by a motor neurone [86]. Human skeletal muscle fibres are classified into type 1 (slow twitch) or type 2 (fast twitch) fibres on the basis of their functional properties. Myosin is the protein responsible for conversion of chemical energy, in the form of adenosine triphosphate (ATP), into mechanical energy, and as such the myosin isoform that predominates in any given myofibril determines the functional properties of that myofibril. Three myosin heavy chain (MyHC) isoforms are expressed in adult human skeletal muscle: slow (type 1), fast 2A and fast 2X [88]. In addition, a neonatal MyHC isoform is observed but rapidly differentiates into the adult forms after birth [89].

Slow fibres reach peak tension more slowly and have a slower maximum shortening velocity, as well as generally achieving lower tension during an isometric contraction, but demonstrate much greater resistance to fatigue than type 2A and 2X fibres. These fibres consume relatively small amounts of ATP, with their consumption being matched by aerobic oxidative ATP production as a result of large numbers of mitochondria. Type 1 fibres also show high capillary density, are typically small in diameter, and are supplied by motor neurones that fire at low frequency and have slower conduction velocities. In contrast, type 2X fibres are large in size, have few mitochondria, and are supplied by large-diameter motor neurones that fire at high frequencies and have rapid conduction velocities. Type 2X fibres demonstrate very high anaerobic metabolic power but, since they derive much of their ATP from anaerobic glycolysis, the cytosol rapidly becomes acidic due to lactate production, inhibiting the contractile response and leading to fatigue. Type 2A fibres are intermediate to types 1 and 2X, able to derive ATP from both oxidative and glycolytic pathways, and hence are able to produce relatively high force over prolonged periods, demonstrating moderate fatigue resistance [86, 88, 90].

Due to the requirement to contract rhythmically and repeatedly throughout life, the respiratory muscles must demonstrate high levels of resistance to fatigue. The human diaphragm is composed of approximately 55% type 1 fibres, 21% type 2A and 24% type 2X [91], with the intercostal muscles demonstrating a slightly higher ratio of slow oxidative fibres (greater than 60%) [92]. A lower proportion of slow oxidative fibres (25%) are present in the infant diaphragm at birth, reaching adult proportions within the second year after birth [93]. During quiet breathing, mainly slow fibres are active, with a shift to fast twitch fibres observed as respiratory frequency increases [94, 95]. Diaphragm muscle fibres have also been shown to have a smaller cross-sectional area than limb muscle fibres, resulting in a smaller diffusion distance and increased efficiency of oxygen delivery to the contracting cells [96].

1.2.3.1 Physiology of muscle contraction

An action potential arises in the motor neurone supplying the motor unit, resulting in release of acetylcholine into the neuromuscular junction and release of sodium ions at the motor end plate. This depolarisation of the muscle fibre triggers a muscle action potential, which propagates along the sarcolemma and through the t-tubule system, resulting in release of calcium ions from the sarcoplasmic reticulum. At rest, troponin-tropomyosin complexes are bound to the myosin receptor sites of the actin filament, preventing cross-bridge formation. Hydrolysis of the ATP molecule into adenosine diphosphate (ADP) energises the myosin head. Following the arrival of an action potential, the troponin-tropomyosin complexes bind with the released calcium ions, allowing the energised head of the myosin filament to attach to the actin filament and form a cross bridge. The myosin head rotates, sliding the actin filament towards the centre of the sarcomere, and releasing the ADP molecule and the phosphate group. Following this 'power stroke', a new ATP molecule binds to the myosin head, releasing it from its attachment to the actin filament. If ATP remains available and calcium is still present in the sarcoplasm, myosin receptor sites of the actin filament will remain available for cross bridge formation and a further

contraction cycle will be initiated. Although the motion of an individual myosin head effects only a very small change in sarcomere length, the repeated actions of many actin-myosin complexes results in large displacements of the filaments [86, 90, 97].

1.2.3.2 Factors affecting muscle contraction

The force generated by an individual muscle during a contraction is dependent on the length of the muscle at the point of stimulation, the number of muscle fibres stimulated, the frequency at which they are stimulated, and the velocity of muscle fibre shortening. These factors are also closely related with specific changes in muscle length, load, firing frequency or velocity of shortening rarely occurring in isolation.

The length-tension relationship of skeletal muscle is a function of the degree of overlap of the actin and myosin filaments (Figure 6). Under circumstances of excessive stretch, fewer myosin heads are able to make contact with actin filaments; once a muscle fibre reaches approximately 170% of its optimal length, no overlap occurs and no tension can be generated. If a muscle is shortened to substantially below its resting length, the myosin heads become compressed against the Z discs at the end of the sarcomere, again reducing the number of myosin heads available for cross-bridge formation [90]. In respiratory muscles, this effect becomes apparent under circumstances of lung hyperinflation, whereby the respiratory muscles become shortened with a consequent reduction in force-generating ability [98]. Muscle length change in the human body is limited by the points to which the muscle is attached and therefore rarely exceeds 30% change from optimal length [90].

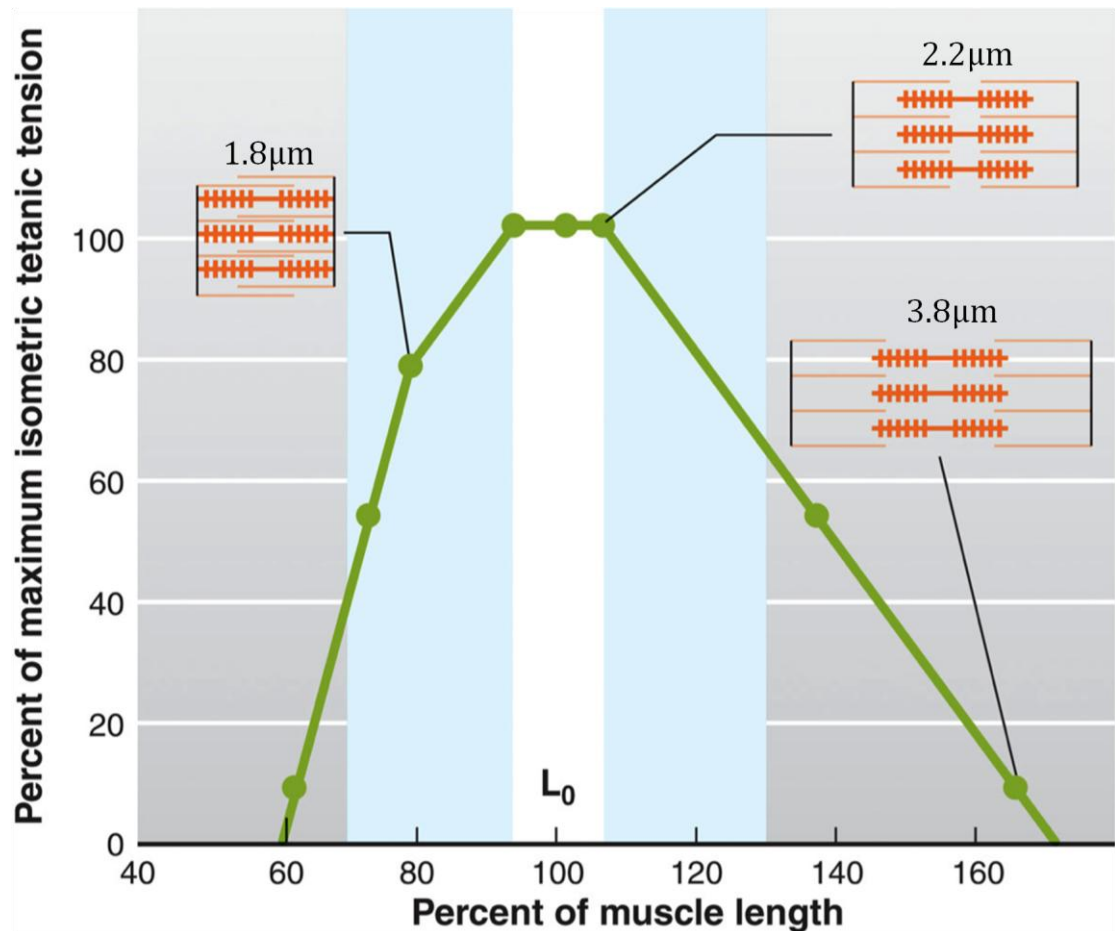


Figure 6 Length-tension relationship in a skeletal muscle fibre. Reproduced with permission and adapted from [90]. L_0 = optimal muscle length.

The frequency of stimulation also affects the force generated by the muscle through summation of isometric contractions [90]. Maximal force is generally achieved in response to 100Hz stimulation, though high frequencies are rarely seen during voluntary contractions, with motor neurone firing frequencies of 5-30Hz observed during normal muscle activity [99]. The nature of the force-frequency curve varies between different skeletal muscles [100], with muscles comprised predominantly of fast-twitch fibres requiring higher stimulation frequencies to achieve maximal force.

The force and velocity of a muscle's contraction demonstrate an inverse hyperbolic relationship, with maximal shortening velocity achieved under a condition of zero load. The shortening velocity is determined by the rate of

actin-myosin cross-bridge cycling and ultimately by the rate of ATP hydrolysis [90].

1.3 Assessment of respiratory function

It is well recognised that optimal treatment of respiratory disease requires accurate assessment of respiratory function, with maintenance of good pulmonary function in childhood being associated with improved outcomes into adulthood [27, 101, 102]. Early intervention is therefore recognised as being of major importance in childhood lung disease [103]. Many of the pharmacological treatments available to treat lung disease, while demonstrating good safety profiles, are not without side-effects and thus overtreatment must be avoided [11]. Management of respiratory disease also places a significant burden on patients and their families [104, 105], which further emphasises the importance of making treatment decisions with objective evidence of their efficacy in mind.

Clinician opinion is a significant factor in assessment of lung disease, particularly in the younger age groups where objective measures are lacking. Physicians are often, through clinical assessment alone, able to identify an obstructive pattern of lung disease [106], but are generally unable to accurately quantify the severity of airflow obstruction [107]. Clinical signs, such as oxygen saturation, respiratory rate and asthma severity scores have been shown to correlate poorly with severity of airways obstruction and need for hospitalisation in children with asthma [108]. Equally, underestimation of disease severity by children, parents and carers has been implied to be a possible contributing factor in severe exacerbations [109, 110]. More detailed objective measures that assess pulmonary function are required to facilitate optimal management.

1.3.1 Spirometry

Spirometry is currently viewed as the cornerstone of pulmonary function testing in both adults and children. The key parameters used are the forced vital capacity (FVC), and the expired volume in the first second of a forced exhalation (FEV₁). Other parameters may be of interest to clinicians or researchers but are not routinely used in clinical practice and hence will not be discussed in detail.

While spirometry has long been recommended as the key tool with which to assess disease severity and guide treatment decisions in a wide range of lung diseases, it carries a number of drawbacks. Chief among these in paediatric respiratory care is the level of cooperation and understanding required from the subject. Some authors have suggested that reliable results may be obtained from children as young as three years old with appropriate coaching and encouragement [111], but in practice this is frequently not feasible [31]. Reflecting this, national and international guidelines do not recommend the use of spirometric measures to guide treatment in children under the age of five years [7, 8, 11, 14, 112]. A major drawback associated with spirometry is the reliance of the technique on the subject's own motivation and cooperation in order to obtain appropriate maximal efforts. Factors such as fatigue, breathlessness and cough, frequently observed in patients with lung disease, may also impair patients' ability or desire to comply with the demands of the test and hence result in values that are submaximal or of poor quality and technically unacceptable [14, 113].

In addition to the volitional nature of spirometry, variability of the measure can impact on diagnostic accuracy. Normal variability of spirometry is approximately 2-3% [114], but in the presence of obstructive airways disease has been shown to be two- to three-fold higher [113]. The greater variability of spirometric measures in diseased populations results in a reduced sensitivity of

the test to small changes in pulmonary function that can occur early in the disease process and may result in delays in commencing treatment.

1.3.2 Peak expiratory flow

Peak expiratory flow (PEF) can be derived from spirometry or can be measured independently using a small hand-held meter. PEF is widely used in the home and primary care settings for ongoing monitoring of asthma in particular, and is recommended in national and international guidelines as an appropriate monitoring tool [8, 11]. However, PEF values have been shown to demonstrate little relationship to other spirometric parameters [115] and the values obtained from home measurement of PEF are potentially unreliable [116] and bear little relation to symptoms or disease severity [117].

1.3.3 Forced oscillation technique

The forced oscillation technique (FOT) superimposes small oscillations of pressure over a range of frequencies onto tidal respiratory flow and uses the resultant pressure-flow responses to determine a range of pulmonary impedance characteristics [118], including resistive, inertive and elastic properties of the respiratory system.

FOT requires only passive cooperation from the subject and thus is suitable for use in pre-school children. Nonetheless, difficulties in cooperation have been reported, including refusal to breathe through a mouthpiece or wear a noseclip, inability to maintain a leak-free seal around the mouthpiece, or difficulty maintaining consistent tidal breathing in the presence of the imposed pressure signals [119]. Such difficulties may also be encountered when attempting to use this technique with patients who are breathless, coughing frequently or who are reliant on supplemental oxygen, with measurement success rates of as low as

20% reported in acutely unwell pre-school children [120]. FOT has also been shown to be unable to discriminate between healthy pre-school children and those with cystic fibrosis lung disease, bringing into question the ability of the technique to detect early, milder disease [121]. Without sedation, this technique is also unsuitable for use in infants.

1.3.4 Body plethysmography

Patients with obstructive lung disease have increased airways resistance, which in turn can lead to suboptimal lung emptying and gas trapping resulting in increases in end-expiratory lung volume. Body plethysmography allows accurate measurement of intrathoracic gas volumes and airways resistance, which can be used to assess obstructive lung disease severity. In order to satisfy the conditions of Boyle's Law [122], which underpins the technique itself, the subject must remain in a sealed rigid chamber, such that changes in intrathoracic pressure can be inferred from changes in chamber pressure [123]. Enclosure in a plethysmograph for several minutes, is often unacceptable to younger children [124] and the manoeuvres are complex, and therefore rarely successful in this age group.

1.3.5 Respiratory interrupter resistance

The respiratory interrupter resistance technique (R_{int}) is a non-invasive method for measuring respiratory system resistance. It applies a brief (100msec) occlusion to airflow during inspiration or expiration during which mouth pressure equilibrates with alveolar pressure. Respiratory system resistance is calculated by back extrapolation to obtain airway pressure at the point of occlusion and dividing this by the respiratory flow at that time [125-127]. The technique is effort-independent and requires only passive cooperation from the subject. It does, however, require the subject to breathe through a mouthpiece

and wear a noseclip, which is unnatural for most children and is difficult during an acute exacerbation, particularly if the child is oxygen-dependent. In addition, the child's cheeks must be supported to prevent their influence on resistance measurements. R_{int} can also underestimate airways resistance in severe airways obstruction [128] due to impaired pressure transmission across the airways to the mouth. While studies have demonstrated good short-term repeatability, long-term variability of the measurement is much greater, limiting its application clinically [129].

1.3.6 Multiple breath washout

Multiple breath washout (MBW) techniques use inert gases to measure FRC and have recently been further developed to assess gas mixing within the lung. The lung clearance index (LCI) is the number of lung volume turnovers required to reduce the concentration of the test gas to one 40th of the equilibration concentration achieved during the test gas wash-in period. Such measures of ventilation inhomogeneity have been suggested to provide a sensitive marker of airways disease [119]. These techniques can be performed using a facemask or mouthpiece and noseclip and require only tidal breathing. Only passive cooperation is therefore required from the subject and as such the technique can be performed in children of any age, though infants may still require sedation [130]. Although requiring the subject to breathe through a mouthpiece or facemask, the major barrier to wider use of MBW techniques is the lack of agreement regarding standardisation of equipment and methodology and the relative lack of commercially available equipment [119]. Much research to date has focussed on the use of MBW techniques in cystic fibrosis but there is a lack of data in the literature regarding the utility of these techniques in other obstructive lung conditions [130]. Recent work has shown MBW to be of little use in assessing response to bronchodilator medication in pre-school children. Using this technique, it was not possible to discriminate between healthy children and those with recurrent wheeze, with ventilation inhomogeneity

increasing in some subjects, which the authors suggested was due to inability of the bronchodilator medication to permeate poorly ventilated lung areas [131].

1.3.7 Infant pulmonary function testing

A variety of pulmonary function tests are available for use specifically in infants such as the rapid raised thoraco-abdominal compression technique and infant plethysmography. However, both of these techniques are limited by the size and age of the child due to constraints imposed by the equipment used and the requirement that the child be sedated. Such techniques are therefore not used in routine clinical assessment and are generally restricted to specialist centres [132, 133].

1.3.8 Summary

Although a range of pulmonary function testing modalities have been developed that may be suitable for use in the paediatric population, few are suitable for routine use, particularly in pre-school children. Infancy and early childhood is a critical time for lung development, and insults to the lung sustained during this period can have a significant impact on pulmonary function through later life. The need for improved techniques to accurately assess lung function and allow both longitudinal monitoring to track disease progression and the effects of treatment interventions particularly during acute exacerbations of lung disease in this younger age group is therefore apparent. Objective measures that are applicable across a wide range of ages and abilities in children in both acute and chronic disease situations are required.

1.3 Control of respiration

Control of the respiratory cycle is mediated primarily by the respiratory centres in the medulla and pons of the brainstem. These areas act to regulate ventilation and allow respiratory homeostasis to be maintained under varying conditions. Axons from respiratory centres within the brain travel down the spinal cord to innervate the respiratory muscles via motor nerves.

Breathing movements are produced by this spatially distributed ponto-medullary respiratory network that generates rhythmic patterns of alternating inspiratory and expiratory activities that drive and coordinate the activity of spinal and cranial motoneurons. The respiratory motor pattern originates and is controlled by inputs within interconnected bilateral columns of medullary neurons. The functional role of the pons in the generation and control of respiratory rhythm and pattern has not been fully established, although pontine regions have been shown to interact with multiple medullary compartments which interact and provide strong modulation of medullary respiratory network activity and control respiratory phase transitions.

1.3.1 Medullary respiratory centres

Groups of cells demonstrating respiratory related activity are found throughout the medulla in number of different nuclei, however the major respiratory neurones are concentrated into four main nuclei: the dorsal respiratory group (DRG) within the nucleus tractus solitarius (NTS); the ventral respiratory group (VRG), containing the nucleus ambiguus (NA) and nucleus retroambiguus (NRA); the pre-Bötzinger complex; and the Bötzinger complex, located in and near the nucleus retrofacialis.

The pre-Bötzinger complex is thought to be a key centre of respiratory rhythmogenesis, containing neurons with intrinsic pace-making capabilities that demonstrate cyclical firing in phase with respiratory activity without

additional synaptic input [134]. The Bötzinger complex lies rostral to the NA and is composed of expiratory neurones and vagal and glossopharyngeal motor neurones [135]. The NTS is the main site receiving input from lung and peripheral chemoreceptor afferents and provides sensory afferent input to the Bötzinger complex [136]. Both the Bötzinger and pre-Bötzinger complexes provide input to the VRG and DRG [137].

The DRG is located within the dorsomedial area of the medulla and comprises only inspiratory neurones, which fire immediately prior to the onset of inspiration and relay activity to the phrenic nerves to initiate diaphragm contraction [138]. This activity lasts for approximately two seconds in adults under resting conditions, before ceasing to allow passive expiration [36]. The DRG is responsive to afferent input from chemoreceptors and lung mechanoreceptors via the ninth and tenth cranial nerves and spinal cord, as well as descending afferents from higher brain centres. The DRG inspiratory neurones inhibit the expiratory neurones of the VRG and pontine respiratory group (PRG) [136].

The VRG lies ventrolaterally in the medulla. This area behaves similarly to the DRG, though contains both inspiratory and expiratory neurones, with the inspiratory and expiratory neurons demonstrating reciprocal inhibition [139]. The NA contains premotor inspiratory neurones that supply the external and parasternal intercostals, accessory muscles of inspiration and the laryngeal motor neurones [140], as well as supplying parasympathetic input to the heart and bronchioles [141]. The rostral portion of the NRA contains inspiratory neurones, and the caudal portion expiratory neurones that activate expiratory muscles under conditions of active expiration, as well as sending inhibitory activity to suppress inspiratory neurones, acting as an inspiratory “off switch” [136].

1.3.2 Pontine respiratory group

The pontine respiratory group (PRG) has reciprocal connections with the respiratory centres within the medulla [137]. In the absence of any influence from the pons, the medullary respiratory centres generate a slow, rhythmic, gasping breathing pattern [142]. The PRG consists of expiratory neurones in the medial parabrachial nucleus and inspiratory neurones in the lateral parabrachial nucleus and Kölliker-Fuse nucleus [143]. Heightened activity in the PRG reduces the duration of activity of inspiratory neurones in the medulla, reducing inspiratory time and resulting in earlier initiation of expiration [86]. This “phase-switching” allows respiratory frequency to increase. Inferior areas of the pons exert an excitatory influence on inspiratory neurones, though the activity of this area is usually suppressed by the PRG. In the absence of any input from the PRG or lung stretch receptors, breathing will cease in full inspiration [142].

1.3.3 Cortical control of breathing

Descending influences from the cerebral cortex to the medullary respiratory centres allow volitional control of breathing for tasks such as speech, laughter, defaecation and childbirth. Impulses involved in the voluntary control of breathing bypass the medullary and pontine respiratory centres and synapse directly with spinal respiratory motor neurones [86]. This is demonstrated in the case of rare brainstem lesions in which cortical control of breathing remains intact with loss of autonomic control, such that patients are able to maintain adequate ventilation while awake but control is lost during sleep [144]. Pain, temperature and emotion also demonstrate an effect on ventilation, mediated via input from the hypothalamus, reticular formation and limbic system [86, 145].

1.3.3.1 Respiratory chemoreceptors

The chemoreceptors involved in respiratory control respond to changes in pH, pO_2 and pCO_2 . The responses to changes in O_2 and CO_2 are quantitatively and qualitatively different. Relatively minor increases in CO_2 partial pressures result in significant and rapid alterations in ventilation [146]. In contrast, the ventilatory response to decreasing arterial oxygen pressures is hyperbolic under isocapnic conditions; large changes in pO_2 are initially required to mediate a change in ventilation but thereafter the increase in ventilation is rapid [147].

Central chemoreceptors are found in several locations within the brain, including the ventrolateral medulla, NTS, VRG, the locus ceruleus, the caudal medullary raphé, and the fastigial nucleus of the cerebellum [146]. These receptors are located beyond the blood-brain barrier and therefore do not respond directly to changes in blood gas levels, but rather require changes in cerebrospinal fluid pH to occur (via changes in hydrogen ion concentration), which are subsequently sensed by the chemoreceptors [148].

Peripheral chemoreceptors are located within the carotid bodies at the bifurcation of the carotid arteries and in the aortic bodies above and below the aortic arch. The carotid body receptors respond to change in arterial pH, PCO_2 and PO_2 , with the aortic chemoreceptors responding only to pH and CO_2 levels. The carotid chemoreceptors exert a more potent influence over respiratory control than those in the aortic bodies. Peripheral chemoreceptors are the only receptors mediating changes in ventilation in response to hypoxia, and complement the central chemoreceptors in responding to alterations in CO_2 levels [149]. Feedback to the respiratory centres from the carotid body receptors is via the glossopharyngeal (ninth cranial) nerves, and from the aortic body receptors is via the vagus nerve (tenth cranial nerve) [86]. Due to the high blood flow received by the peripheral chemoreceptors, they are able to respond rapidly to changes in blood gas concentrations, although their overall contribution to the response to changes in pCO_2 is less than that from the central chemoreceptors [150].

1.3.3.2 Respiratory system afferents

Afferent feedback from receptors in the chest wall, respiratory muscles and lungs provide input to brainstem respiratory centres via the vagus nerve and thoracic and cervical nerve roots. The receptors involved in the respiratory system can be divided into four principal types: stretch receptors, juxta-pulmonary capillary ("J") receptors, irritant receptors, and proprioceptors [151].

Both slowly- and rapidly-adapting stretch receptors (SARs and RARs) are located within the airway walls, with greater concentrations in the larger airways [152]. SARs are larger, myelinated fibres located within airway smooth muscle [153]. Stimulation of SARs by changes in lung volume and transpulmonary pressure plays a role in determining respiratory timing. Increased activity of SARs during inspiration is associated with reductions in tidal volume and inspiratory time in animal subjects [154], and the degree of both tonic and phasic SAR activity during expiration has been shown to assist in determining expiratory duration in dogs [155]. In humans, however, pulmonary stretch receptors appear to exert no influence on respiratory depth or timing at tidal volumes below approximately one litre [156, 157], despite clear activity being present [158]. SAR sensitivity may also be increased in the presence of bronchoconstriction, airway obstruction, or reduced lung compliance. Increased SAR firing frequency has been demonstrated in the presence of bronchoconstriction, thought to be as a result of smooth muscle contraction stimulating SARs [159].

A deep inspiration (either passive or active) results in increased SAR activity, triggering the Hering-Breuer inflation reflex, whereby inhibitory impulses are sent via the vagus nerves to inspiratory respiratory centres in the brainstem, inhibiting further inspiratory muscle activity and prolonging expiratory time [151]. This reflex is particularly strong in infants, protecting against excessive lung distension due to the greater chest wall compliance [37], but is weak in

adults, being activated only at higher tidal volumes such as during exercise. An increase in SAR activity is also thought to be involved, *via* a similar pathway, in the reflex bronchodilation occurring with deep inspiration in healthy individuals through reduction of parasympathetic tone to the airway [153, 160].

RARs are small-diameter, myelinated fibres and are widely distributed within and below the epithelium of the lower respiratory tract, with higher concentrations at the bifurcations of the trachea and bronchi [161]. Unlike SARs, these receptors demonstrate sensitivity to both mechanical (increases in tidal volume, respiratory frequency, or inspiratory and expiratory flows) and chemical (including cigarette smoke, dust, pro-inflammatory chemicals such as histamine and prostaglandin) stimuli, and may also be referred to as irritant receptors [162]. Stimulation of RARs results in cough, reflex laryngo- and broncho-constriction, airway vasodilatation, mucous hypersecretion, shortening of expiratory time and hyperpnoea, as well as cardiovascular responses, including tachycardia and hypertension [162]. They are also implicated in the deep augmented 'sigh' breaths that are seen every five to twenty minutes; RAR sensitivity increases as lung compliance decreases during tidal breathing, until a central nervous threshold is reached and an augmented breath triggered, reversing the reduction in lung compliance [163].

J receptors are unmyelinated fibres that respond to chemical changes in the bronchial and pulmonary circulations, and hence are also referred to as bronchial and pulmonary C-fibre receptors [151]. These receptors are stimulated primarily by chemical irritants such as capsaicin, histamine, bradykinin and prostaglandins, but also respond to increases in interstitial fluid in the alveolar wall and increased pulmonary blood flow [164]. Stimulation of these receptors results in increases in respiratory rate, constriction of the larynx and bronchi, and increased upper airway mucous secretion, in addition to reflex hypotension and bradycardia and a prolonged depression of spinal reflexes [165]. There is considerable overlap between the stimuli that activate the J and irritant receptors and between the reflex responses to the stimulation of both groups of endings.

Proprioceptors are located in the Golgi tendon organs and muscle spindles of the respiratory muscles, as well as in the joints of the thoracic cage, and are sensitive to changes in muscle tension and length respectively. Activation of the mechanoreceptors within the costovertebral joints results in a reflex inhibition of inspiratory activity in response to rib elevation [166]. The diaphragm demonstrates a high ratio of Golgi tendon organs to muscle spindles, in contrast to the large number of muscle spindles in the intercostal muscles [167]. In combination, the sensory information projected to both brainstem respiratory centres as well as higher cortical areas provide information to facilitate modulation of respiratory drive, as well as to assist in accurate respiratory load perception.

1.3.4 Respiratory control in childhood

Maturation of respiratory control is relatively rapid and adult patterns of response to stimuli are seen at a relatively young age. While there are some developmental changes in respiratory control during infancy, these will be discussed here in brief only as the children studied in this thesis were older than the age in which these changes are observed.

The substantially greater metabolic rate in infants and young children results in a relatively limited respiratory reserve [168]. Reduced lung volume and lung compliance and an easily distorted chest wall characteristic of the infant chest, in combination with a less mature brainstem respiratory control centre, predispose the infant to irregular breathing. Maintenance of lung volumes above closing volume is facilitated by active contraction of inspiratory muscles and laryngeal adduction during expiration [48], as well as initiation of inspiration prior to reaching a relaxed lung volume [37]. As a result of the absence during sleep of behavioural influences on respiratory pattern, chemoreceptor-mediated respiratory drive is of greater importance in the infant [168]. Beyond the immediate neonatal period, chemoreceptor influence

on respiratory behaviour shows relatively little difference to that seen in adulthood [169]. Other factors influencing respiratory control in infants include their greater sensitivity to environmental stress, particularly heat and cold, due to immature mechanisms of temperature regulation [170].

1.4 Load: capacity balance of the respiratory muscles

The mechanical action of any muscle is dependent on the imposed load and its capacity [58]. The load on the healthy human respiratory system during resting breathing is small. Transdiaphragmatic pressures of approximately 5cmH₂O are required to produce an adequate tidal volume. The range of pathophysiological changes that occur in lung disease can substantially alter the balance between the load imposed on the respiratory muscles and their capacity. In obstructive lung disease increased airways resistance, hyperinflation and intrinsic PEEP place an additional load on the respiratory system, while the inspiratory muscle shortening that occurs as a result of hyperinflation reduces the capacity of the inspiratory muscles to generate force [33] (Figure 7). Neural respiratory drive (NRD) must therefore increase to compensate for this change in load-capacity balance and maintain adequate levels of ventilation for blood gas homeostasis.

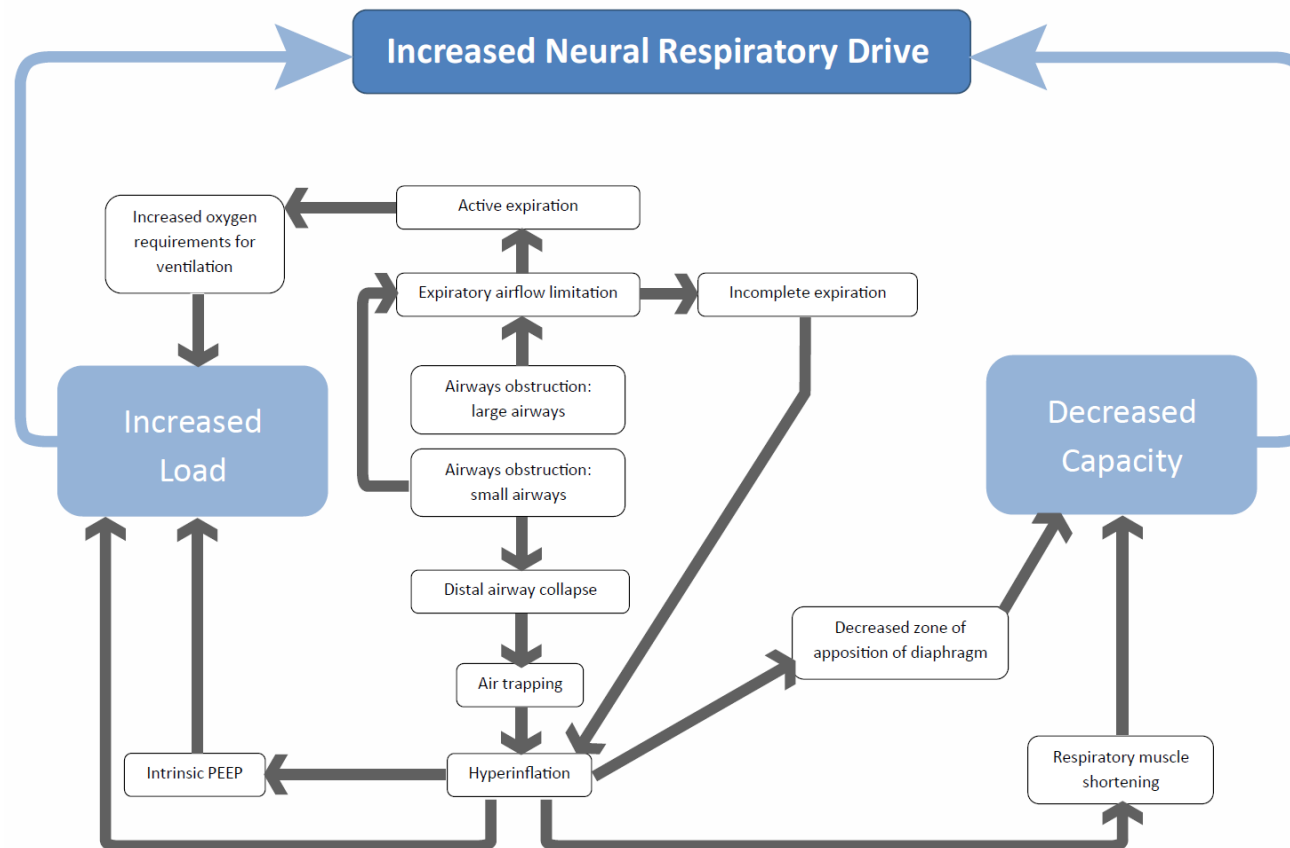


Figure 7 Influence of pathophysiological changes seen in obstructive lung disease on respiratory system load and capacity, and the relationship with neural respiratory drive.

1.4.1 Measurement of respiratory load

The load on the respiratory muscles can be quantified in a number of ways. Standard pulmonary function tests can be used to assess the individual components of the overall respiratory load imposed by respiratory disease. Forced expiratory techniques in spirometry measure the degree of expiratory airflow limitation, while lung hyperinflation can be assessed by measurement of functional residual capacity using body plethysmography or gas dilution techniques. These measures, however, only quantify a single aspect of disease pathology and correlate poorly with patient perception of disease.

Respiratory load can also be assessed by measuring the pressures generated by the respiratory muscles to produce ventilation. Intrathoracic (oesophageal (P_{oes})) and intra-abdominal (gastric (P_{gas})) pressures can be measured using balloon catheters. The pressure generated by the diaphragm, the transdiaphragmatic pressure (P_{di}), can then be calculated by subtracting P_{oes} from P_{gas} [171]. Mean or peak pressures generated during breathing can therefore be measured and used as an index of load on the respiratory system. The integral of pressure changes with respect to time, the pressure-time product, can be used as a measure of the work of breathing to quantify respiratory load. During tidal breathing in healthy individuals, the transduction of respiratory muscle pressure to respiratory flow and ventilation is highly efficient. In severe airways obstruction, however, gas trapping leads to lung hyperinflation and intrinsic PEEP. This positive resting intrathoracic pressure must be overcome before inspiratory flow will occur. The change in pressure from baseline must therefore be considered when using peak or integral of respiratory muscle pressure.

1.4.2 Measurement of respiratory muscle capacity

Muscle capacity or strength can be quantified simply and noninvasively by measuring the pressure at the mouth during a maximal volitional inspiratory effort against an occlusion ($P_{l,max}$ manoeuvre) or the maximum pressure in the nasopharynx during a maximal sniff (sniff nasal inspiratory pressure (SNIP)) [171]. The $P_{l,max}$ manoeuvre can be difficult to perform for some patients and as a result the values obtained can be submaximal and variable. As a sniff is a natural manoeuvre, the SNIP measurement technique is more easily understood and performed by patients, and provides a robust measure of global inspiratory muscle strength [171]. Both measures are quick, simple, relatively easy to perform and non-invasive and therefore can be performed in routine clinical practice. They do, however, rely on the subject being able to perform a maximal volitional effort. The validity of the SNIP may also be compromised in severe airways obstruction due to poor pressure transmission across the lung to the airway opening. In these instances, such measures may significantly underestimate true muscle strength.

More detailed measurements of respiratory muscle strength may be obtained through the use of oesophageal and gastric balloon catheters to estimate maximum intrathoracic, intra-abdominal and transdiaphragmatic pressures during volitional manoeuvres. In patients unable to reliably perform maximal voluntary efforts, the phrenic nerves can be stimulated using either magnetic or electrical stimulation techniques to generate a non-volitional diaphragm contraction, and the resulting respiratory pressures measured.

Invasive measures of inspiratory load and capacity are not without risk, and can be unpleasant for the patient. Such measurements are unsuitable for use in routine clinical practice, particularly with young children. Non-invasive pressure-based measures of respiratory muscle strength are affected by patient cooperation and motivation as well as airways obstruction. Alternative measures are therefore required to accurately

characterise the load imposed on the respiratory system relative to its capacity.

1.4.3 Neural respiratory drive

Neural respiratory drive (NRD) is the electrical output from the respiratory centre in the brainstem to the respiratory muscles [33]. NRD varies in proportion to the loads placed on the respiratory system and any changes in the capacity of the respiratory muscles to respond to these loads [172]. The ideal method for quantifying NRD would be to measure the total brainstem respiratory neural output; however, such approaches are not possible in human subjects. Pressure based measurements have been developed, but are subject to restrictions in their accuracy. Measuring the neural input to selected important respiratory muscles would appear to overcome such limitations.

1.4.3.1 Airway occlusion pressure

The pressure generated in the first 100 milliseconds of inspiration against an occluded airway ($P_{0.1}$) can be used as an index of respiratory drive. Unlike other measures of respiratory drive such as mean inspiratory flow (tidal volume divided by inspiratory time), $P_{0.1}$ is independent of lung mechanics and does not elicit a conscious response from the subject due to the brief duration of the manoeuvre [173]. $P_{0.1}$ is correlated with mean inspiratory flow in children [174]. To obtain an accurate measurement of $P_{0.1}$, the airway must be occluded at end expiration, at the point of zero flow. Although a high value of $P_{0.1}$ always indicates high respiratory drive, a low value can be difficult to interpret. Respiratory muscle weakness and fatigue, the lung volume at the point of measurement, and any phase lag between pressure and flow (such as may occur in the presence of airways obstruction) will influence $P_{0.1}$ [175]. Expressing $P_{0.1}$ as a function of $P_{l,max}$

has been used to overcome this [176]. The accuracy of $P_{0.1}$ is also affected by dynamic hyperinflation and intrinsic PEEP, as $P_{0.1}$ does not take account of the respiratory effort required to overcome PEEPi prior to generation of negative inspiratory pressure [177].

While the nature of this measure makes it applicable to a range of populations, including children [174] and mechanically ventilated patients [178], the effect of changes in lung volume on the pressure-generating capacity of the respiratory muscles results in measurements of $P_{0.1}$ being poorly representative of true respiratory drive in patients with altered end expiratory lung volumes [179]. Additional physiological measurements may be required in order to fully interpret the values obtained from measurement of $P_{0.1}$. Practically the requirement for the subject to breathe through a mouthpiece in order to obtain the measurement makes the technique challenging in the paediatric age group, as well as the need to average many breaths in order to obtain precise results.

1.4.3.2 Electromyography of the respiratory muscles

Electromyography (EMG) is the technique used to record myoelectric signals, the electrical manifestation of the neural excitation process required for muscular contraction [180]. EMG measurements can be performed using surface electrodes placed over the muscle of interest, needle electrodes inserted into the relevant muscle or, in the case of crural diaphragm EMG, catheter-mounted electrodes in the oesophagus. The EMG signal is representative of the electrical activity within the muscle [58] and represents motor unit recruitment. The magnitude of the EMG signal is therefore directly related to the force generated by the muscle during an isometric contraction. During an isotonic contraction when the muscle is also shortening, the EMG is related to both force generation and rate of change in muscle length [181, 182].

As the principal inspiratory muscle, the diaphragm is the clear choice for measurement of NRD and EMG of the diaphragm (EMG_{di}) has been shown to be sensitive to changes in respiratory load-capacity balance [172, 183]. EMG_{di} can be quantified using needle electrodes, though this technique carries a risk of pneumothorax and damage to internal organs and can be uncomfortable for the subject [184]. EMG_{di} can also be recorded transcutaneously using electrodes placed on the skin surface to measure the costal diaphragm EMG [185-187], or using an oesophageal electrode catheter to record crural diaphragm activity [172, 188, 189]. Transcutaneous measurement techniques are prone to contamination from overlying musculature, particularly postural muscle activity [190]. In the presence of airways obstruction, pronounced abdominal muscle recruitment may be present in an effort to increase expiratory flow [191], resulting in greater contamination of the surface EMG_{di} signal. Measurements employing oesophageal electrodes provide high-quality, uncontaminated signals but the technique is invasive and hence impractical as a routine clinical tool, particularly in the paediatric population. Other methods for quantification of NRD are therefore required.

1.4.3.3 EMG of the parasternal intercostal muscles

EMG of the parasternal intercostal muscles (EMG_{para}) has been used in a variety of laboratory and clinical studies, successfully demonstrating the ability of the measure to detect change in load in a number of patient populations. In addition, studies in dogs have provided insight into the relationship between the mechanical and electrical behaviour of the parasternal intercostal muscles under a range of conditions. The absence of overlying musculature results in the parasternal intercostal muscles being well suited to surface measurement of EMG, although needle techniques have also been used. The obligate nature of the parasternal intercostal muscles' recruitment, and their activation in tandem with the diaphragm [62, 77-80], indicates their suitability as an alternative method to quantify NRD.

The relationship between NRD measured using EMG_{di} and EMG_{para} has been examined and close agreement between the two measures observed [172, 192, 193]. It is noted, however, that patterns of activity of the two muscles are not identical and the two measures cannot be viewed as interchangeable. Parallel increases in EMG_{para} and crural EMG_{di} have been observed during incremental hypercapnic, inspiratory threshold loading, and incremental exercise to exhaustion though consistently higher levels of EMG_{di} were observed when compared to EMG_{para} [172, 192]. EMG_{para} and surface EMG_{di} of the costal diaphragm have also been shown to be closely related [194, 195], though a greater magnitude of increase in EMG_{para} than EMG_{di} activity was observed in these studies.

The reproducibility of EMG_{para} measurements is well established, having been examined in a range of populations. A study by Duiverman *et al* [187] examined the relationship between the increase in EMG_{para} recorded on two separate days in seven healthy subjects and seven patients with COPD undertaking a loaded breathing protocol, and reported a very strong correlation ($r=0.89$) in the COPD patients but a poorer relationship in healthy adults ($r=0.63$). An earlier study [185] from the same group had demonstrated strong agreement between EMG_{para} measures recorded in both healthy adults and pre-school children ($r=0.98$ in both populations). The agreement in school-age children, both with and without asthma, was less strong ($r=0.65$ in both groups), though Bland-Altman analysis showed minimal bias and there was no significant difference between the measures on two occasions. Reilly *et al* [172] and Murphy *et al* [196] assessed reproducibility of EMG_{para} in fifteen and ten healthy young adults respectively and reported values for inter-occasion coefficient of variation (CV) of 5-10%, with a strong correlation between the values obtained on the two occasions. Murphy reported slightly poorer agreement between measures obtained on two occasions in ten stable COPD patients, with a mean CV of 15%.

Measurements of EMG_{para} can be used to discriminate between healthy subjects and those with respiratory disease, with greater levels of EMG_{para} observed in patients with cystic fibrosis [172], asthma [197] and COPD [78, 198] in comparison to healthy subjects. Different methods have been used to allow comparison of levels of EMG_{para} activity between subjects: Gandevia *et al* [78] utilised needle EMG techniques and compared motor unit firing frequencies, whereas other work using surface EMG has expressed the EMG_{para} signal as a percentage of that obtained during a maximal inspiratory effort ($EMG_{para}\%max$) [172, 192, 197-199]. $EMG_{para}\%max$ has been shown to relate to disease severity as quantified by FEV_1 in both asthma [197] and cystic fibrosis [172]. Additionally, Reilly *et al* [172] demonstrated a positive relationship between $EMG_{para}\%max$ and end expiratory lung volume, and an inverse relationship with dynamic lung compliance. EMG_{para} activity increases with the development of hyperinflation in asthmatic subjects, with strong relationships having been demonstrated between changes in thoracic gas volume and EMG_{para} activity following bronchoconstriction [32] and bronchodilation [200]. The parasternal muscles demonstrate less mechanical disadvantage than the diaphragm in the presence of lung hyperinflation [201], and have greater mechanical efficiency at total lung capacity (TLC) compared to FRC [202]. It is suggested, therefore, that the parasternal intercostal muscles may be preferentially recruited under conditions of increased end expiratory lung volume when compared to the diaphragm.

The response of EMG_{para} to changes in respiratory load has been demonstrated under a number of circumstances. Animal studies have shown increases in EMG_{para} activity during hypercapnia and histamine-induced airway narrowing [203, 204]. A study by Duiverman *et al* [187] in adult human subjects examining diaphragm, parasternal intercostal and scalene muscle EMG showed different patterns of recruitment of the three muscle groups between COPD patients and healthy subjects. Greater increases in EMG_{para} were observed in the COPD patients compared to healthy controls, with both groups exhibiting increases in EMG_{para} activity

when inspiratory resistive loads were applied. Inspiratory activity of the parasternal intercostal muscles has also been shown to increase significantly above baseline values at the point of maximal histamine-induced bronchoconstriction in both pre-school children and those over the age of five years, using clinical signs and spirometry respectively as reference points [190, 195]. A subsequent study by the same group suggested an exponential relationship between changes in FEV_1 and EMG_{para} during a histamine challenge, with a return to baseline values following the administration of salbutamol [194]. The same authors also studied pre-school children hospitalised with acute viral induced wheeze, and demonstrated reductions in EMG_{para} with resolution of the illness. EMG activity was also shown to correlate with a clinical measure of illness severity in this cohort [186]. In these five studies, the logarithm of the EMG activity ratio (ratio of the recorded EMG signal to that obtained at baseline, $\log EMGAR$) was used. This method does not allow for comparison of levels of NRD between subjects, nor does it allow assessment of longitudinal change or quantification of disease severity from a single measurement, only of degree of change from an initial recording.

To overcome the limitations of EMGAR, other authors have expressed EMG_{para} as a percentage of that obtained during a maximal inspiratory manoeuvre ($EMG_{para}\%max$). Utilising this measure, Steier *et al* [197] demonstrated greater diurnal variability of $EMG_{para}\%max$ in asthmatic subjects compared to healthy individuals, reflecting the patterns of variability seen in standard measures of pulmonary function. During maximal incremental exercise, Reilly *et al* [172] showed $EMG_{di}\%max$ and $EMG_{para}\%max$ to be strongly correlated with patient reported breathlessness, both before and after the onset of neuromechanical dissociation (the point at which further increases in neural drive are no longer translated into increases in respiratory muscle pressure generation), indicating that measurement of respiratory muscle EMG is reflective of the subjective experience of respiratory disease. This is further supported by a study in COPD patients during an acute exacerbation [198], in whom

reductions in EMG_{para} correlated strongly with improvements in breathlessness and could discriminate between patients deemed to have improved clinically and those who deteriorated. In addition, EMG_{para} provided a potential index that could predict the likelihood of readmission within fourteen days, indicating the ability of the technique to track treatment response as well as to give prognostic information. A further study demonstrated a significant reduction in EMG_{para} from hospital admission to discharge in a cohort of adult CF patients hospitalised for an acute exacerbation of CF lung disease [199].

The existing literature therefore supports the use of EMG_{para} as a marker of load on the respiratory system. There has been relatively little work to date examining the applicability of this technique in paediatric populations. Of these studies, the method used for expressing the EMG_{para} signal ($\log EMGAR$) does not allow for the technique to be used as a measure of disease severity, nor does it permit inter-individual comparisons to be made. There is a need for studies examining the use of EMG_{para} in children with a range of respiratory diseases and to evaluate the response of EMG_{para} to common clinical interventions. The use of $EMG_{para}\%max$, as has been used in previous studies in adult subjects, also places limitations on the populations in which the technique can be used; the utility of the raw EMG_{para} signal has not yet been explored.

1.5 Summary

Childhood respiratory disease represents a substantial burden on healthcare systems worldwide. The availability of objective measures of pulmonary function facilitates accurate diagnosis and optimal management of lung disease. Existing measures have significant limitations in terms of both their ability to accurately represent the full range of pathophysiological changes occurring, as well as their applicability to all age groups. Measurement of neural respiratory drive via the parasternal intercostal

muscle electromyogram (EMG_{para}) represents a promising alternative for objective measurement of pulmonary function.

EMG_{para} is feasible in both healthy subjects and patients with a range of lung disease, and is responsive to change in respiratory load-capacity balance. Much of the work to date, however, has been conducted in adult populations and therefore further assessment and validation of this technique is required to determine its value and efficacy in paediatric patient groups. The more recent work undertaken in adult patients has utilised the normalised signal ($EMG_{para}\%max$) and while this approach has advantages over logarithmic transform of the ratio of a point measure of EMG_{para} to a baseline value ($\log EMGAR$), as it allows comparison between individuals and therefore provides a quantifiable measure of disease severity, it does require a maximal inspiratory effort. Volitional inspiratory and expiratory manoeuvres are the hallmark of many existing measures of pulmonary function and represent the major limitation of their applicability in younger age groups. Investigation of alternative methods for expressing the EMG_{para} signals is also therefore warranted.

1.6 Aims of thesis

The work contained within this thesis aimed to explore the feasibility and utility of EMG_{para} in the assessment of paediatric respiratory disease. The ability of the technique to detect changes in respiratory load has been explored. Changes in EMG_{para} have also been related to change in conventional measures of pulmonary function, allowing the performance of EMG_{para} to be considered in the context of current clinical practice. Studies have been conducted in healthy children to evaluate the effect of maturation, provide comparative data, and to allow investigation of technical considerations inherent in the measurement of EMG_{para}.

Study One aimed to investigate the response of EMG_{para} following bronchodilator administration in asthmatic children, and to explore the relationship with conventional measures of pulmonary function.

Study Two aimed to investigate the relationship between EMG_{para} and FEV₁ during incremental bronchoconstriction induced by methacholine challenge test.

Study Three aimed to explore in greater depth the pathophysiological changes occurring in response to methacholine challenge testing and the relative influences of these changes on EMG_{para}.

Study Four aimed to explore changes in EMG_{para} during a hospital admission for an acute respiratory exacerbation in children with cystic fibrosis, and the relationship of these changes with conventional lung function testing parameters.

Study Five aimed to examine levels of EMG_{para} in a large cohort of healthy children, including maturational changes in EMG_{para}, and addressed technical concerns regarding normalisation and reproducibility. The study

also aimed to compare EMG_{para} values from healthy subjects to those obtained in children with asthma and cystic fibrosis from Studies One and Four.

2. Chapter Two: Methods

2.1 Ethical approval

All studies contained within this thesis were granted ethical approval by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from parents or guardians of all paediatric participants and from the participants themselves in the case of adult subjects.

2.2 Anthropometry

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) with a resolution of 1mm (range 600-2100mm). Subjects were asked to remove footwear and stand upright with heels, back and head against the stadiometer. A 1kg weight was placed on the stadiometer carriage to improve accuracy.

Weight was measured using an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK) with a resolution of 50g (max weight: 300kg). Subjects were required to remove footwear and any heavy clothing and remain stationary on the scale until a steady reading to the nearest 0.05kg was obtained.

Accuracy of each device was checked daily, using a one-metre fixed bar for the stadiometer and validated five-kilogramme weights for the weighing scales.

Body mass index (BMI) was calculated by dividing the individual's weight in kilograms by the square of their height in metres. World Health Organisation Anthro and Anthro Plus software (World Health Organization,

Geneva, Switzerland) was used to calculate weight-, height- and BMI-for-age percentiles based on WHO Child Growth standards [205].

2.3 Pulmonary Function Testing

2.3.1 Spirometry

Spirometric measurements were obtained using a Jaeger Masterscreen PFT system (Cardinal Health Ltd, Basingstoke, UK). Flow was measured using a Lilly-type heated screen pneumotachograph and volume measurements obtained using a software-based integrator (JLAB software, version 4.0). Calibration of the system was performed daily in accordance with manufacturers' instructions, using a 3L syringe discharged six times at a range of flows, as recommended by the ATS/ERS standards [14].

Spirometry was performed in accordance with ATS/ERS criteria [14]. The correct technique was explained and demonstrated to each subject prior to commencement of testing. Subjects were seated upright with head and neck in a neutral or slightly extended position, fitted with a noseclip and asked to seal their lips tightly around a flanged mouthpiece. The subject was then asked to inspire rapidly and fully to TLC and then, following a brief pause of less than one second, to perform a maximal forceful exhalation and to maintain expiratory flow until no more air could be expelled. Subjects were encouraged to "blast", rather than "blow", the air from the lungs, and verbal encouragement was given throughout each manoeuvre to promote complete exhalation. Software-based visual incentives were provided for all paediatric subjects to encourage maximal efforts. Any traces showing evidence of incomplete expiration, cough, air leak or glottic closure were rejected. Repeated efforts were performed until three acceptable maximal manoeuvres were obtained. Acceptable repeatability was considered to have been obtained when the difference between the largest and next

largest manoeuvres was $\leq 0.150\text{L}$ or 5%. Testing was ceased if any evidence of spirometry-induced bronchoconstriction was observed.

2.2.2 Impulse oscillometry

The impulse oscillometry technique uses small oscillatory forces imposed on an individual's tidal breathing. Through examination of the resulting pressure-flow relationship at the airway opening it is possible to ascertain the mechanical response of the tissues of the respiratory system [206]. Due to the use of externally-generated signals, this technique demonstrates more consistent results than other tests of pulmonary function, in particular those that rely on maximal efforts from the subject [207]. The overall response of the respiratory system to the imposed signals is termed impedance (Z_{rs}), which is subsequently broken down into two components, each of which are then expressed in the frequency domain following Fast-Fourier Transformation (FFT). The first is respiratory resistance (R_{rs}), representing the flow-resistive properties of the total respiratory system. This can be split into the resistance occurring during inspiratory and expiratory phases, or can be expressed as a time-average of the entire respiratory cycle [206]. The second component is reactance (X_{rs}), which is the degree to which the externally-imposed pressure leads flow and is the sum of the elastic and inertive properties of the respiratory system [118]. When elastic properties of the respiratory system predominate, flow occurs prior to an increase in pressure and thus reactance is negative. When inertial forces are the major response to the pressure impulses, pressure occurs prior to the development of flow, and reactance is therefore positive. The point at which elastic and inertive components are equal results in zero X_{rs} , and is termed the resonant frequency.

The literature reports a number of changes in IOS parameters occurring as a result of the pathophysiological changes seen in obstructive lung disease. It is well recognised that resistance is elevated in airways obstruction, primarily during expiration [208]. IOS provides measurement of both

inspiratory and expiratory resistance. Expressing resistance in the frequency domain allows the frequency-dependence of resistance to be evaluated. While the healthy respiratory system exhibits essentially frequency-independent resistance to the imposed oscillations, airways obstruction results in increased Rrs at lower frequencies [206]. Frequency-dependence of Rrs, as expressed by the absolute difference or percentage change between Rrs at 5 and 15 or 20Hz, can also therefore be used as a marker of severity of airways obstruction. In addition to changes in resistance, the reactance curve undergoes a rightward shift, that is, the elastic properties predominate and flow leads pressure to a greater extent, causing an increase in the resonant frequency.

IOS was measured in accordance with ERS recommendations [207], using the Jaeger Masterscreen PFT system, operating JLAB software, version 4.0. The Jaeger system uses impulses of 45msec duration generated at 0.2-second intervals; the impulses are comprised of frequencies of between 1 and 35Hz. IOS measurements were undertaken prior to spirometry, to avoid any effects on bronchomotor tone resulting from the maximal respiratory efforts involved in spirometry. Subjects were seated with the head in a neutral or slightly extended position, with lips tightly sealed around the flanged mouthpiece and a noseclip *in situ*. The subject was instructed to breathe quietly at the FRC level. The volume-time trace displayed by the software was observed and recording was commenced once a regular tidal breathing pattern had been established. Recording was continued for 90 seconds. Verbal encouragement was given to ensure maintenance of a tight seal around the mouthpiece, regular tidal breathing and avoidance of tongue movement. While international guidelines recommend manual support of cheeks [207], it has been suggested that this is not necessary [208]. Adding manual cheek support was found to distract many subjects and result in difficulty maintaining steady tidal breathing, and was therefore not used. At least three recordings were undertaken and the volume-time and pressure-time traces of each examined for evidence of swallowing, coughing, air leak, glottal closure, irregular breathing or

improper noseclip seal. If any evidence of the above was seen, or if coherence (a value used to evaluate the extent to which the obtained values may have been influenced by factors other than the characteristics of the respiratory system, used as a quality-assurance measure [209]) at any frequency was below 0.6, then the affected portion of the recording was eliminated, if at least 30 seconds of uncontaminated recording could still be preserved, or a further measurement was performed. The mean of three recordings was reported.

2.3 Electromyography

2.3.1 Parasternal intercostal muscle electromyography

Participants' skin was prepared in accordance with international recommendations [210]. Skin was scrubbed with an abrasive gel (Nuprep, Weaver and Company, Aurora, Colorado, USA) to remove dead skin cells and excessive sebum in order to minimise electrode-skin impedance. Any remaining gel and sebum was then removed with an alcohol-impregnated wipe before self-adhesive silver-silver-chloride electrodes were applied (Kendall Arbo, Tyco Healthcare, Neustadt/Donau, Germany). Electrodes were applied in the second intercostal space bilaterally, directly adjacent to the lateral border of the sternum, with a reference electrode applied on the acromion process of the scapula. While existing literature reports placing electrodes 3cm lateral to the sternal edge [172, 185, 198], this was felt to be inappropriate for the age range included in the studies contained within this thesis. Data are not available regarding the dimensions of the parasternal intercostal muscles in children but 3cm constitutes a significant proportion of the overall rib length in younger children. For example, the length of the second and third ribs are reported to be 100mm and 125mm respectively in one year-old children, and 140mm and 180mm in six year-olds [211].

Placing the electrodes directly adjacent to the sternal edge was felt to represent the best option for standardisation.

Signals were amplified and band-pass filtered between 10 and 3,000Hz, with an additional analogue notch filter at 50Hz, to minimise mains frequency interference, using a PClab 3808 Biomedical Amplifier (Yinghui Medical Technology Co. Ltd, Guangzhou, China). EMG signals were acquired (Powerlab 16SP, ADInstruments, Sydney, Australia), and displayed on a laptop computer (MacBook Pro, Apple, Cupertino, California, USA) running LabChart software (Version 7.2, ADInstruments Pty, Colorado Springs, Colorado, USA) with analogue to digital sampling of 10kHz. This sample rate was chosen as it was the lowest sampling rate available that exceeded the Nyquist rate, which states that, in order to avoid aliasing and other signal distortion, the sampling rate must be greater than double the low-pass filter frequency [212]. Both amplifier and analogue-to-digital convertor were earthed to suitable points in the laboratory.

A post-acquisition 20Hz high-pass digital filter was applied using the LabChart software to assist in the removal of ECG artefact. Data were displayed both as raw EMG and as a rectified (root-mean-square (RMS)) trace, using a moving average window of 50ms [212].

The EMG from the parasternal intercostal muscles was recorded during resting breathing. Subjects were seated upright in a chair with their back supported, arms resting on armrests and feet flat on the floor. In the case of younger children, arms were rested on thighs and additional foot support was provided to minimise trunk postural activity. Subjects were instructed to remain still and not to speak throughout the recording period, with prompting given regularly for younger children. Age-appropriate films were played for all paediatric subjects. Three minutes of recording was allowed to achieve quasi homeostasis, thereafter a minimum of three minutes of uncontaminated EMG_{para} recording was obtained for all subjects. In some subjects this necessitated a recording period of no more than three minutes

but in some paediatric subjects longer recording periods were required due to movement, speech or laughter causing artefact. In order to obtain data regarding inter-minute variability of the measurement, recordings of up to fifteen minutes were undertaken, depending on the individual child's attention span and ability to remain still.

EMG_{para} recordings were undertaken prior to spirometry in all studies, with the exception of during methacholine challenge testing, in order to avoid alterations in NRD or airway calibre resulting from forced expiratory manoeuvres.

2.3.2 Diaphragm electromyography

Diaphragm EMG was recorded in adult subjects from the crural diaphragm using a multipair oesophageal catheter (Yinghui Medical Tech Ltd, Guangzhou, China), with an external diameter of 2mm. The electrode catheter consists of nine consecutive electrode coils, each 10mm in length and spaced at 0.5mm intervals, forming five electrode pairs (Figure 8) [183].

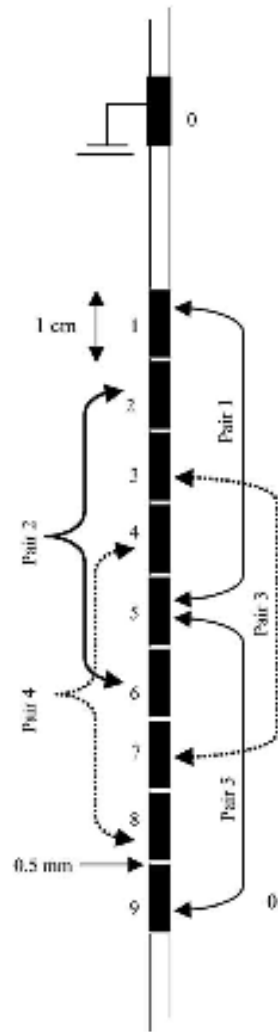


Figure 8 Diagram of diaphragm EMG electrode, showing electrode pairings.

The catheter was introduced nasally, following administration of lidocaine topical anaesthetic spray (Xylocaine, AstraZeneca UK Ltd, UK) into the nasal cavity and oropharynx. The catheter was lubricated with gel (KY Jelly, Johnson & Johnson, New Brunswick, NJ, USA), passed into the nasopharynx and swallowed into the oesophagus with small sips of water through a straw.

The catheter was positioned with coil five close to the crus of the diaphragm, based on the EMG signal obtained from each of the electrode pairs. Optimum positioning of the catheter was indicated by high EMG activity

from electrode pairs one and five (electrodes one & five and five & nine respectively), and low EMG from electrode pair three (electrodes three & seven) during tidal breathing [213]. Once the catheter was satisfactorily positioned, it was taped securely to the nose.

2.3.3 EMG interpretation

The EMG from the final minute of each recording was analysed (Figure 9).

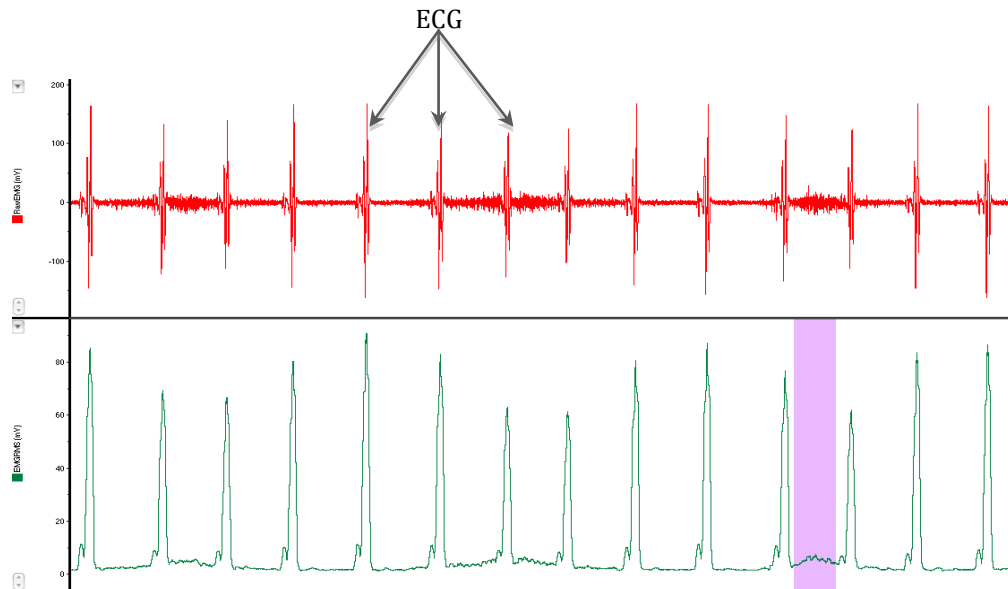


Figure 9 Section of raw EMG_{para} and root-mean-square (RMS) traces showing three breaths, with ECG also indicated. Highlighted portion contains peak EMG_{para} .

Periods of increased EMG activity characteristic of inspiration were identified. Inspiratory activity located between ECG complexes was identified and highlighted. The peak RMS EMG activity per breath was calculated using the acquisition software. For the purposes of data analysis, EMG was expressed in μV (EMG RMS per breath) and, where possible, as a

percentage of that obtained during a maximal respiratory manoeuvre ($EMG_{para}\%max$ or $EMG_{di}\%max$) obtained from an inspiration to TLC. Although previous studies utilising measurements of NRD have used a range of maximal respiratory efforts in order to normalise the resting EMG signal, including maximal inspiratory mouth pressures ($P_{I,max}$), maximal sniff, maximal voluntary ventilation and inspiration to TLC [172, 196, 214], such manoeuvres may be difficult in younger children. Laboratory studies with patients and healthy volunteers indicated that inspiration to TLC was the manoeuvre that could most consistently be performed by the widest age range of children without excessive contamination of the EMG_{para} signal from other muscle groups. During the adult, laboratory-based methacholine challenge study, $maxEMG_{para}$ and $maxEMG_{di}$ activity was obtained from inspiratory capacity manoeuvres.

During maximal inspiratory efforts, other muscles of the anterior chest wall may be recruited as accessory muscles of inspiration. The contraction of these muscles may contaminate the EMG_{para} signal, significantly affecting the magnitude of the $maxEMG_{para}$ signal and hence the accuracy of $EMG_{para}\%max$ as a standardised means of expressing the EMG_{para} signal. While needle EMG would have provided a method for identifying whether the electrical activity was originating from the parasternal intercostal muscles or elsewhere, this technique is unsuited to studies such as those contained within this thesis, nor indeed to routine clinical practice. Following discussion across the Respiratory Muscle Laboratories at King's College, St Thomas' and the Royal Brompton Hospitals, a consensus was reached regarding a qualitative method for determining whether the EMG_{para} signal recorded during a maximal inspiratory manoeuvre was considered to be contaminated; EMG interference patterns with very high amplitude or clearly visible individual spikes of EMG activity were rejected (Figure 10).

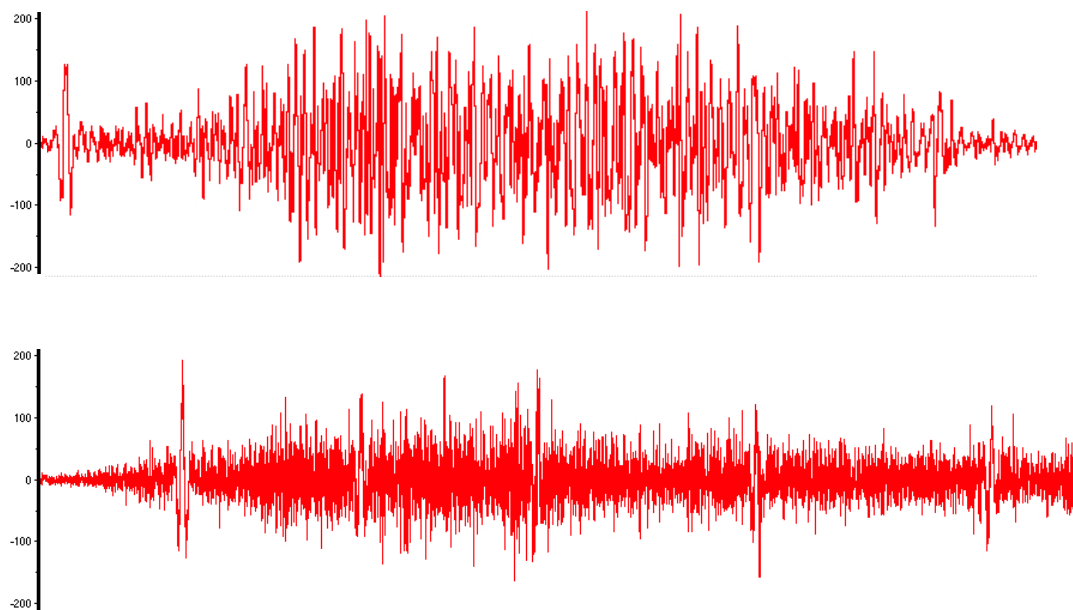


Figure 10 EMG_{para} recordings during maximal inspiratory efforts in two paediatric subjects. The upper trace demonstrates significant signal contamination, characterised by a much less dense pattern of EMG than the lower trace, which was deemed to contain EMG_{para} activity only.

2.3.3.1 Calculation of significant change in EMG_{para}

Although the relationship of EMG_{para} to other measures of pulmonary function has been studied [172, 185-187, 195, 198, 215, 216], the degree of change that can be defined as significant has not yet been described. Such data are available for other pulmonary function tests, and are the focus of much study. Early work to establish statistically significant changes in spirometric parameters [217-221] led to the now widely-recognised definition of 12% or greater increase in FEV_1 constituting a clinically important bronchodilator response [222]. Such “cut-offs” are based on the known variability of the measurement. By calculating the coefficient of variation (CV) of the measurement, and multiplying by 1.65, a cut-off value

above which only 5% of a normally-distributed population would be expected to lie can be defined [217]. Other authors have suggested the use of 95% confidence intervals to give a similar value [218-220].

Following the recent introduction of pulmonary function tests that can be used readily in younger children, such as impulse oscillometry and interrupter resistance, approaches to assess bronchodilator response in pre-school children have been considered. The use of 95% confidence intervals or two standard deviations from the mean of repeated measures have been suggested. It is, however, also important to evaluate the intra-subject, intra-occasion variability of the measure, and use these data to inform decisions regarding what should constitute a statistically significant change [223]. Individually derived cut-offs are clearly less practical for implementation into clinical practice but may be more accurate in assessing an individual patient.

Although previous work has suggested spirometric values to be more variable in diseased than in healthy populations [113, 217], many other physiological parameters have been shown to demonstrate reduced variability in diseased states [224]. Baseline variability of EMG_{para} measurements was therefore assessed in both healthy children and patient populations in this thesis. Intra-individual change was evaluated both in the context of the individual's own variability and that of the wider group. To evaluate bronchodilator response, baseline CV of the EMG_{para} was calculated. Only negative changes (a reduction in NRD) were considered significant. A reduction in NRD in excess of the CV multiplied by 1.65 was used as the threshold below which a significant change could be said to have occurred. The utility of using the patient's individual CV *versus* the group CV was also evaluated.

2.4 Methacholine challenge testing

Methacholine challenge testing was undertaken in accordance with ATS Guidelines [225]. Testing was performed in a well-ventilated room. Subjects were seated in a chair suitable for EMG recording, as described above. Baseline pulmonary function testing was performed using a portable electronic spirometer (KoKo spirometer, Ferraris Respiratory, Louisville, CO, USA). Repeated maximal efforts were performed until three acceptable FEV₁ values within 5% of one another were obtained.

The methacholine challenge was performed using a digidoser (KoKo USB Digidoser, Ferraris Respiratory, Louisville, CO, USA) and nebuliser (DeVilbiss 646 characterised nebuliser, Ferraris Respiratory, Louisville, CO, USA). The KoKo software utilises an automatic triggering system and delivers nebulised solution for 600 milliseconds during the first second of inspiration via the digidoser. The nebuliser had a certified output of between 1.019-1.134ml.min⁻¹. Use of a dosimeter and flow restrictor improve accuracy and repeatability of the dose delivered [225]. Subjects were required to wear a noseclip and inhale and exhale through the mouthpiece at a maximum flow of 0.5l.sec⁻¹ imposed by a flow restrictor. Each inspiratory and expiratory breath was also fixed at 5 seconds in duration using verbal prompts. Subjects were given verbal encouragement to maintain flow at the required rate and for sufficient duration. The five-breath dosimeter protocol was followed [225], with methacholine concentrations of 0.0625, 0.25, 1, 4, 8, 16 and 32mg.ml⁻¹ (Acetyl-B-Methyl Choline, Nova Laboratories, Wigston, Leicestershire, UK). A diluent (0.9% saline) step was also used.

A single technically acceptable FEV₁ manoeuvre was performed at both 30 and 90 seconds following inhalation of each solution, and consecutive inhalations were performed at five minute intervals. The lower of the two FEV₁ manoeuvres was selected. If the FEV₁ remained within 20% of the post-diluent value, the next highest concentration was administered. If a

20% or greater fall in FEV₁ was elicited, 400µg of salbutamol was given via a metered-dose inhaler and spacer (Pocket Chamber, nSpire Health, Inc, Longmont, CO, USA). Bronchodilator was not administered in individuals demonstrating a less than 20% fall in FEV₁, unless specifically requested by the subject due to a sensation of breathing discomfort or difficulty.

2.4.1 Calculation of PC20

PC20 is defined as the provocative concentration of methacholine inducing a 20% decrease in FEV₁ [226], and was calculated using the formula:

$$PC20 = \text{anti log} \left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{R_2 - R_1} \right]$$

Where:

C₁ = penultimate methacholine concentration

C₂ = final methacholine concentration

R₁ = percent fall in FEV₁ after C₁

R₂ = percent fall in FEV₁ after C₂

2.5 Measurement of respiratory flow and volume

In adult subjects, respiratory flow during tidal breathing was measured using a Lilly-type pneumotachograph with a range of 0-800l.min⁻¹ (Hans Rudolph model 4813, Hans Rudolph Inc, Kansas City, USA). The pressure drop across the screen was measured using a differential pressure transducer (MP45-16, Validyne, Northridge, USA). This signal was amplified by a carrier amplifier/demodulator (CD280, Validyne, Northridge, USA) and output to an analogue-to-digital converter (Powerlab 16SP, ADInstruments, Sydney, Australia). Signals were displayed on a laptop computer (MacBook

Pro, Apple, Cupertino, California, USA) running LabChart software (Version 7.2 ADInstruments Pty, Colorado Springs, Colorado, USA) with analogue to digital sampling of 100Hz. Flow was calibrated prior to each study using a calibrated rotameter. Volume was calculated *via* digital integration of the flow signal using LabChart software.

Linearity of the pneumotachograph-transducer-amplifier system was confirmed by applying a range of flows ($-200\text{L}\cdot\text{min}^{-1}$ – $200\text{L}\cdot\text{min}^{-1}$) to the pneumotachograph and plotting the amplified electrical output against actual flow delivered *via* the rotameter (Figure 11).

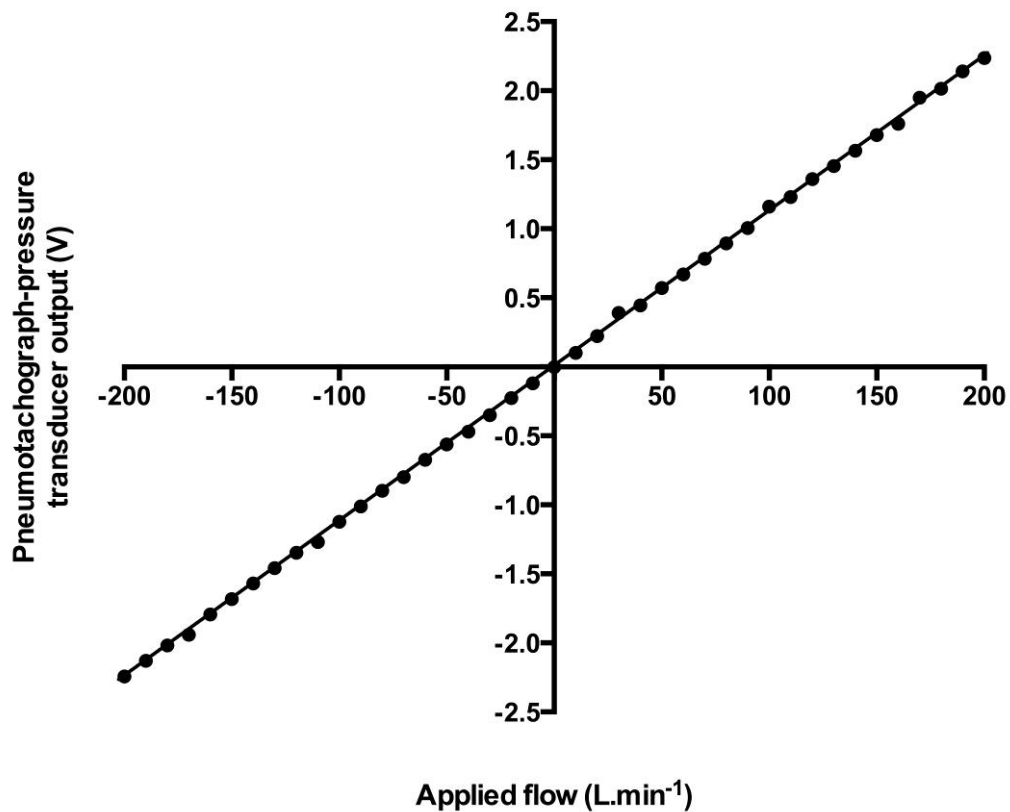


Figure 11 Linearity of the pneumotachograph-pressure transducer system.

The frequency response of the system was assessed using a balloon attached to the pneumotachograph via a three-way tap, which, while being allowed to

slowly deflate at a constant rate through the pneumotachograph, was then burst using a needle to provide an instantaneous cessation of flow. The time taken for the signal to decay from 90% to 10% of full-scale (t_{10-90}) was 8ms (0.008s, Figure 12).

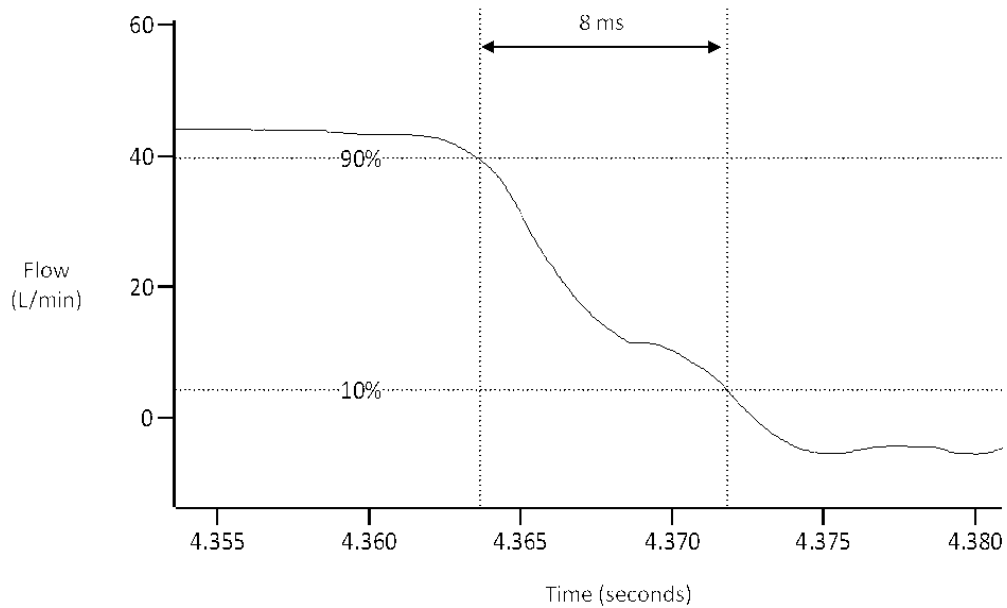


Figure 12 Frequency-response testing of the pneumotachograph-pressure transducer system.

The Fourier transformation of the t_{10-90} allows the frequency response of the system to be calculated [227]:

$$f_{3db} = 1/(3*(0.008)) = 42\text{Hz}$$

A frequency response of 42Hz is in accordance with ATS recommendations for equipment appropriate for the measurement of spontaneous respiratory flow [171].

Inspiratory capacity was obtained by asking the subject to perform a relaxed exhalation to FRC followed by a slow maximal inspiration. The manoeuvre was performed a minimum of three times and the mean of the inspired volumes recorded.

Linearity of the pneumotachograph-pressure transducer system and software-based digital integration for measuring inspired volume was assessed by passing known volumes of air (0.5L – 5.0L) through the pneumotachograph using a certified, adjustable 7 litre calibration syringe (Hans Rudolph Inc, Kansas City, USA). The applied volume was plotted against the recorded volume from the LabChart software (Figure 13). Linearity of the system was deemed to be acceptable.

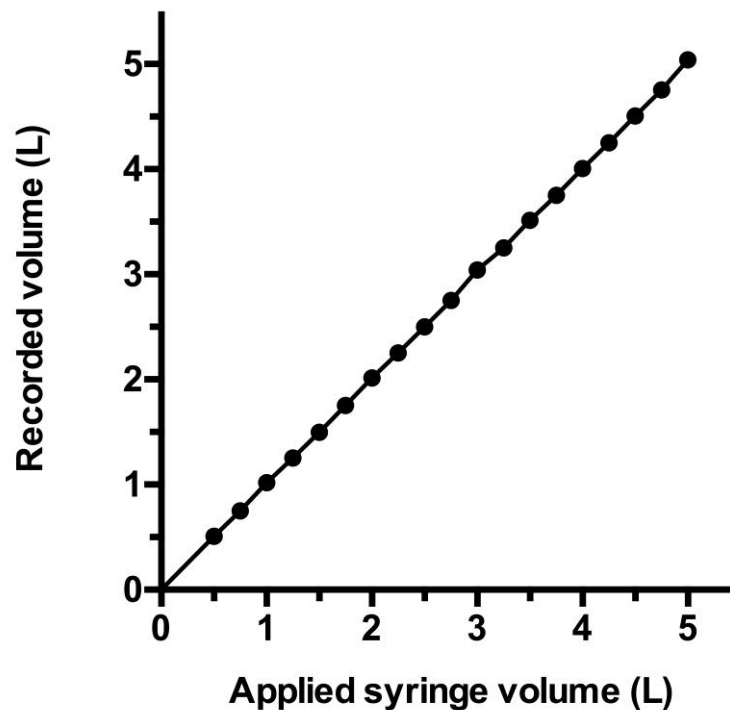


Figure 13 Linearity of the integrated volume output of the pneumotachograph-pressure transducer system.

2.6 Measurement of breathlessness

Subjects were asked to rate their breathlessness using the modified Borg scale (mBorg, Figure 14) [228]. This is a twelve-point categorical scale that uses descriptors to anchor responses, ranging from 0 (“no breathlessness at all”) to 10 (“maximum breathlessness”). The scale has been shown to be reliable and reproducible in subjects undergoing induced airways obstruction [229, 230].

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

Figure 14 Modified Borg (mBorg) breathlessness scale [231].

2.7 Statistical Analysis

All data with $n > 30$ were analysed for normality using the D'Agostino and Pearson omnibus test and if normally distributed expressed as mean (SD), otherwise as median (range). Where n was < 30 , or in the case of ordinal data such as the mBorg score, data were expressed as median (range) and non-parametric testing used. Paired t-tests were performed to assess the relationship between normally distributed within-subject variables, and Wilcoxon matched pairs tests for non-normally distributed data. Normally and non-normally distributed unmatched data were analysed using unpaired t-tests and Mann-Whitney tests respectively to assess for differences between groups. The relationship between variables was analysed using Pearson's correlation in normally distributed data and Spearman's rank correlation in non-normally distributed data. Linear and non-linear regression analyses were performed to assess the strength of anthropometric variables in predicting EMG_{para} values. Receiver-operator characteristic (ROC) analysis was used to assess the sensitivity, specificity, positive and negative predictive value of EMG_{para} to predict a significant change in FEV_1 . Data analysis was performed using Prism version 6.0 (GraphPad Software, Inc., San Diego, California, USA) software.

Power calculations were undertaken using G*Power 3.1.3 (Heinrich Heine Universität, Düsseldorf, Germany) [232].

3. Chapter Three: EMG_{para} to assess bronchodilator response in paediatric asthma

3.1 Introduction

Asthma is one of the most common chronic diseases of childhood, characterised by the presence of variable airflow obstruction, airway hyperresponsiveness, and airway inflammation, leading to symptoms including wheeze, chest tightness, cough, and difficulty in breathing [11]. Mucosal oedema, mucous hypersecretion and smooth muscle spasm contribute to expiratory airflow limitation and may also lead to small airway collapse and hyperinflation [22].

Although a clinical history can be sufficient in many cases to make a diagnosis, in children where a clinical history alone is inconclusive, objective testing is required [11]. A diagnosis of asthma in any age group is strongly supported by the reversibility of airways obstruction. In older children and adults, this is determined by assessing the change in spirometry induced by inhaled β_2 agonists. An increase in FEV₁ of 12% or greater is deemed a significant response [14]. It has been noted previously, however, that administration of a bronchodilator may induce changes in measures other than FEV₁ in patients with obstructive lung disease [233], and hence evaluating this single measure may not fully represent the full response to β_2 agonists. EMG_{para}, as a measure of overall respiratory load, may more accurately represent the range of pathophysiological changes. EMG_{para} has been shown to return to baseline levels when a bronchodilator is administered following airway challenge testing, though the specific relationship with spirometry following bronchodilator was not evaluated [194]. In addition, no objective measure is available to support the assessment of reversible airways obstruction in patients who are unable to reliably perform spirometry, including pre-school age children. The aims of

this study were, therefore, to assess bronchodilator-induced changes in EMG_{para} in asthmatic children, including those unable to complete conventional pulmonary function tests, and to investigate the relationship between the changes in the results of pulmonary function tests and EMG_{para} .

3.2 Subjects

Children from the paediatric chest and difficult asthma clinics at King's College Hospital were recruited. All children had a physician diagnosis of asthma or pre-school wheeze. Ethical approval for the study was granted by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from parents or guardians of all participants.

3.2 Methods

3.3.1 Equipment

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and weight with an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK).

EMG_{para} , IOS and spirometry were measured as described in Chapter Two. Briefly, EMG_{para} was recorded using surface electrodes placed over the second intercostal space. The signal was amplified (gain 1,000), band-pass filtered between 10 and 2,000Hz, and a 50Hz notch filter and a post-acquisition 20Hz digital high-pass filter applied. The signal was converted to root-mean-square (RMS) and mean peak RMS EMG_{para} per breath calculated. IOS was performed in accordance with ERS guidelines [207], with the mean of three reproducible recordings being reported. Spirometry was performed according to ATS/ERS recommendations [14], and the highest value of three reproducible efforts reported.

3.3.2 Protocol

Anthropometric and demographic data were recorded. Resting EMG_{para} was recorded for ten minutes with the child seated, at rest, and in children able to cooperate with the technique spirometry was performed. A subset of children also underwent impulse oscillometry, which was performed prior to spirometry to avoid any effects on bronchomotor tone resulting from the maximal respiratory efforts involved in spirometry. The bronchodilator salbutamol (400µg) was then administered via metered-dose inhaler and spacer. EMG_{para} recording was repeated fifteen minutes later, at a time point corresponding to peak bronchodilator activity, after which IOS and spirometry were repeated.

3.3.3 Statistical analysis

All statistical testing was performed using Prism version 6.0 (GraphPad Software Inc., San Diego, USA). On testing for normality, using the D'Agostino and Pearson omnibus test, no data were found to be normally distributed, hence all data were assessed using non-parametric statistical testing and expressed as median (range). Within-subject changes were assessed using the Wilcoxon matched pairs test. Differences between groups were assessed using the Mann-Whitney test. A p value <0.05 was taken as statistically significant. Relationships between variables were assessed using Spearman's correlation. Receiver-operator characteristic analysis was used to assess the sensitivity, specificity, positive and negative predictive value of EMG_{para} to predict a significant change in FEV₁.

3.4 Results

EMG_{para} recordings of acceptable quality (as detailed in Chapter Two) were obtained in 53 children (Table 1). Of those 53, technically acceptable spirometry was obtained in 33 children. IOS, EMG_{para} and spirometry were obtained in 12 children. Neither spirometry nor IOS were attempted in the 20 children of pre-school age.

3.4.1 Subject characteristics

	Spirometry group (n=33)	Pre-school group (n=20)
Age (years)	10.66 (3.67)	3.70 (0.65 – 5.12)
Sex (male: female)	15: 18	12: 8
Height (m)	1.448 (0.155)	0.976 (0.732 – 1.031)
Weight (kg)	40.14 (13.01)	15.03 (8.50 – 19.70)
BMI (kg m⁻²)	17.75 (13.35 - 27.25)	15.9 (13.6 – 18.6)
Ethnicity (n (%))	White: 13 (39.4%) Black: 15 (45.5%) Asian: 3 (9%) Other: 2 (6.1%)	White: 13 (65%) Black: 4 (20%) Other: 3 (15%)
Baseline FEV₁ (%predicted)	85.6 (22.7)	N/A

Table 1 Anthropometric and demographic characteristics of subjects for Study One. Data are expressed as mean (SD) or median (range).

3.4.2 Change in EMG_{para}

In the cohort as a whole, there was a significant ($p < 0.0001$) decrease in median (range) EMG_{para} following bronchodilator administration (8.36 (1.37 – 20.11) μ V to 6.30 (1.42 – 20.57) μ V), a mean (SD) decrease of -15.3 (19.1)% (Figure 15).

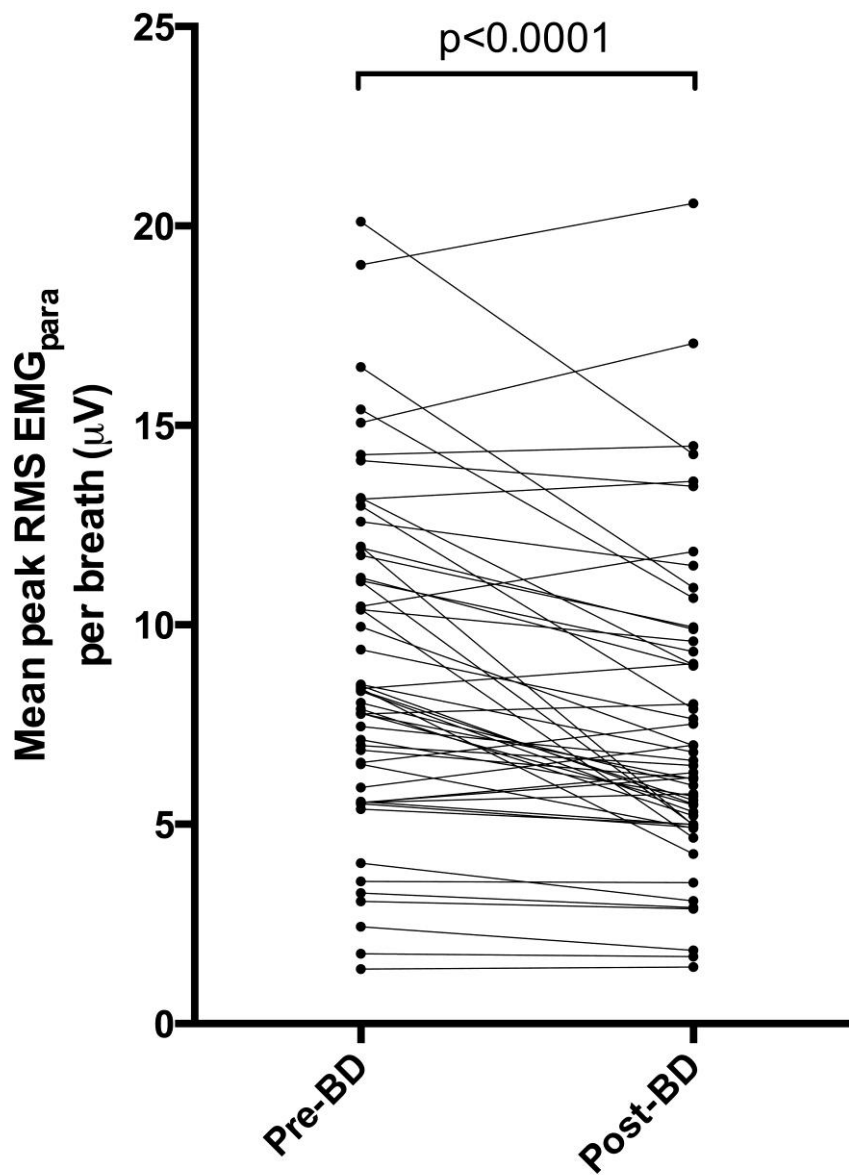


Figure 15 Decrease in EMG_{para} following bronchodilator (BD) administration in 53 children.

In the 33 school aged children who performed spirometry, a significant ($p<0.001$) decrease in median (range) EMG_{para} was observed (8.40 (1.37 – 20.11) μ V to 5.60 (1.42 – 20.57) μ V), -11.06 (-58.4 – 13.9)%. In the 20 pre-school children in whom spirometry was not performed, median (range) EMG_{para} also decreased significantly from 8.20 (5.57 – 16.46) μ V to 6.98 (4.99 – 17.06) μ V ($p<0.01$), -16.6 (-36.4 – 18.0)% (Figure 16).

There was no statistically significant difference in baseline EMG_{para} or the overall change following bronchodilator between the two age groups.

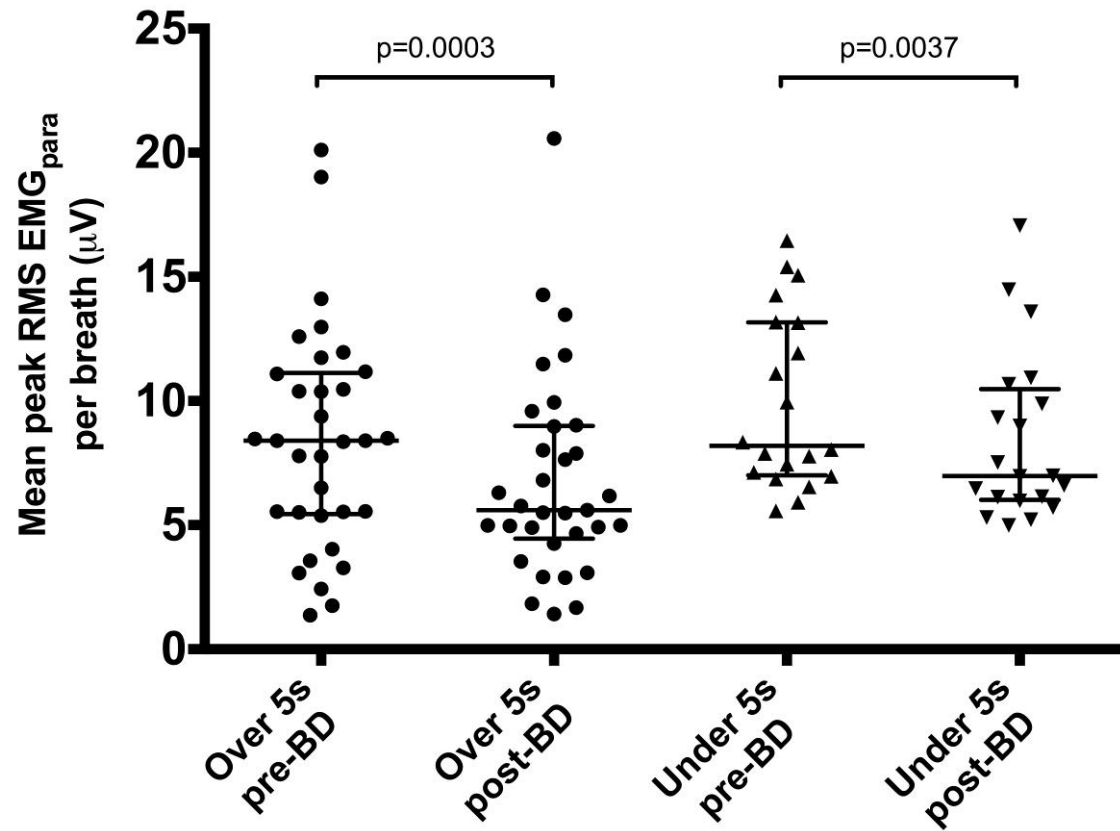


Figure 16 Decrease in EMG_{para} following bronchodilator (BD) administration in 33 school age children and 20 pre-school children. Lines denote median and inter-quartile range.

3.4.3 Statistical power

As no pre-existing data were available to support an *a priori* power calculation, retrospective power calculations were undertaken to assess the achieved power in detecting differences in EMG_{para} following bronchodilator. For the cohort as a whole, 99.8% power at the 5% level was achieved to detect the change in mean (SD) EMG_{para} described above. In the spirometry group (n=33), the power was 85.2% at the 5% level while in the no spirometry group (n=20), the achieved power was 65.8% at the 5% level.

3.4.4 Determining significant change in EMG_{para}

In 24 of the total cohort of patients, a sufficient duration of baseline (pre-bronchodilator) EMG_{para} recording was available to allow the calculation of the inter-minute coefficient of variation (CV) of peak RMS EMG_{para} per breath. This was used to determine a cut-off for the degree of change in EMG_{para} that would constitute a positive response to bronchodilator administration. Cut-off values were calculated for the group as a whole.

The group mean CV for the 24 children was 9.27%, which when multiplied by 1.65 (see Chapter Two) gave a cut-off value for change of 15.29%. Using this group mean derived threshold, 27 of the 53 patients (50.9%) showed a significant reduction in NRD following bronchodilator administration.

Median (range) baseline (pre-bronchodilator) EMG_{para} was significantly (p=0.017) higher in those children who demonstrated a reduction in EMG_{para} greater than the cut-off value compared to those who did not (9.38 (2.43 – 20.11)µV *versus* 5.98 (1.84 – 14.28)µV), possibly suggesting poorer asthma control.

3.4.5 Changes in pulmonary function measures

In the 33 school age children who underwent conventional measures of pulmonary function, significant changes were observed in all spirometry and impulse oscillometry parameters except R20Hz (Table 2).

No significant relationships were observed between changes in spirometric and oscillometric results.

	Pre-BD	Post-BD	p value
FEV ₁ (%predicted)	85.6 (22.7)	94.3 (22.4)	<0.0001
FVC (%predicted)	98.0 (38.2 – 126.0)	97.0 (46.0 – 137.0)	<0.0001
FEF ₂₅₋₇₅ /FVC	0.68 (0.32)	0.82 (0.33)	0.0006
Z5Hz (kPa(L.s ⁻¹) ⁻¹)	0.69 (0.26 – 0.89)	0.49 (0.29 – 0.66)	0.001
R5Hz (kPa(L.s ⁻¹) ⁻¹)	0.66 (0.27 – 0.80)	0.45 (0.25 – 0.64)	0.0005
R20Hz (kPa(L.s ⁻¹) ⁻¹)	0.37 (0.23 – 0.47)	0.33 (0.24 – 0.50)	<i>ns</i>
ΔR5-R20 (%)	37.6 (0.0 – 62.3)	28.2 (0.0 – 49.0)	0.001
RF	20.62 (8.04 – 31.97)	16.71 (7.47 – 20.71)	0.001
AX (kPa.L ⁻¹)	1.95 (0.18 – 4.93)	0.86 (0.10 – 1.72)	0.005

Table 2 Changes in pulmonary function following bronchodilator administration in asthmatic children (n=33 for FEV₁, FVC and FEF₂₅₋₇₅/FVC. n=12 for all other parameters). Data shown as mean (SD) or median (range).

3.4.6 Relationship with measures of pulmonary function

The median (range) percentage changes in EMG_{para} and FEV₁ (-11.06 (-58.4 – 13.9)% *versus* 9.1 (-4.7 – 36.1)%) were not significantly different.

No relationships were observed between percentage changes in EMG_{para} and any of the measures of pulmonary function. No correlation was observed between baseline EMG_{para} and any baseline measures of pulmonary function.

Of the 27 children in whom EMG_{para} exceeded the 15.29% group mean derived cut-off, 16 were over the age of five and had therefore also performed spirometry (lower segment of Figure 17). Of the sixteen, six (37.5%) demonstrated a 12% or greater change in FEV₁ (lower right quadrant of Figure 17). Seven children from the cohort of 33 demonstrated an increase in FEV₁ of greater than 12% without a significant decrease in EMG_{para} (upper right quadrant of Figure 17).

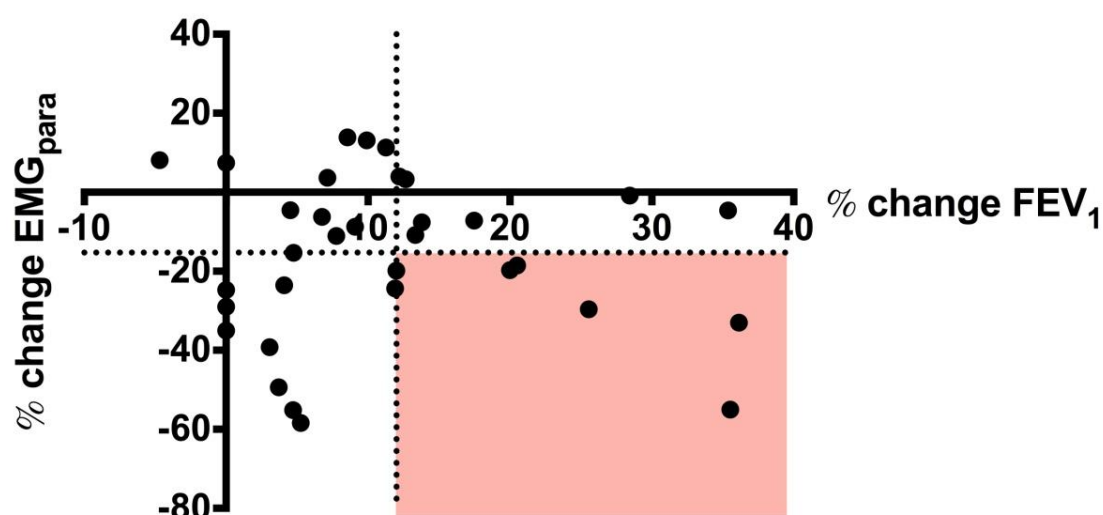


Figure 17 Relative changes in EMG_{para} and FEV₁ following bronchodilator administration in 33 school age children. Horizontal dotted line represents the group mean CV*1.65 (15.29%); vertical dotted line a 12% increase in FEV₁. Shaded area denotes individuals in whom both EMG_{para} and FEV₁ changed significantly.

Table 3 details the sensitivity, specificity, positive and negative predictive values of the 15.29% cut-off value for change in EMG_{para} in predicting a $\geq 12\%$ increase in FEV₁.

	$\Delta FEV_1 \geq 12\%$	$\Delta FEV_1 < 12\%$	
$\Delta EMG_{para} \geq 15.3\%$	6	10	<i>Positive predictive value: 37.5%</i>
$\Delta EMG_{para} < 15.3\%$	7	10	<i>Negative predictive value: 58.8%</i>
	<i>Sensitivity: 46.2%</i>	<i>Specificity: 50%</i>	

Table 3 Group data derived cut-off for EMG_{para} . Sensitivity, specificity, positive and negative predictive values for significant change in EMG_{para} to predict $\geq 12\%$ increase in FEV_1 , using the group-derived $CV \times 1.65$ of 15.3%. n=33.

Due to the poor predictive ability of the 15.3% cutoff, further analyses were performed to explore alternative cutoff values for significant change in EMG_{para} . A 16.94% decrease in EMG_{para} was found to offer the greatest ability to predict a $\geq 12\%$ increase in FEV_1 ; however, the values for sensitivity and specificity were 55.0% and 46.2% respectively and the area under the receiver-operator characteristic (ROC) plot was 0.5 (Figure 18).

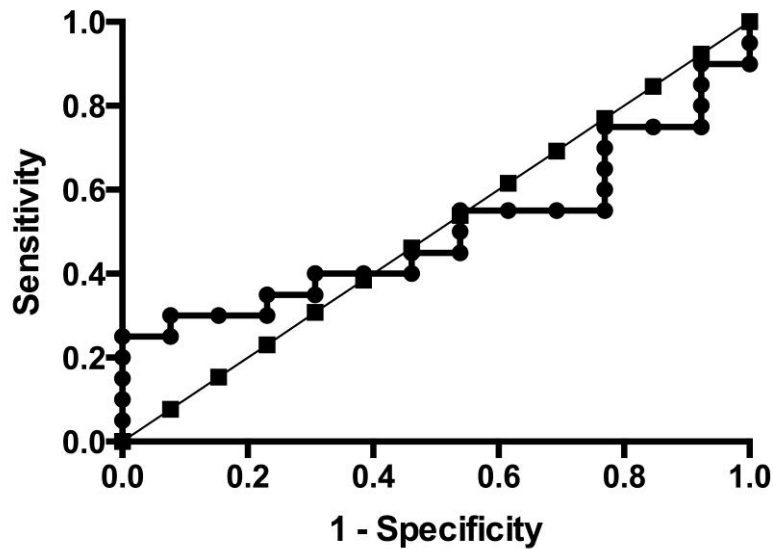


Figure 18 Receiver-operator characteristic curve using a value of -16.94% change in EMG_{para} to predict a $\geq 12\%$ increase in FEV₁. Square symbols indicate line of identity.

3.4.7 Determining significant change in EMG_{para} – individual subjects

A significant change in EMG_{para} was also calculated based on an individual subject's $CV \times 1.65$. Of the 24 patients in whom a sufficient duration of baseline (pre-bronchodilator) recording was available to calculate their individual CV, 10 (47.5%) showed a change in EMG_{para} following bronchodilator administration that exceeded their own personal cut-off value.

Seventeen of the 24 subjects had also performed spirometry. Three demonstrated an increase in FEV₁ greater than 12% as well as a decrease in EMG_{para} greater than their own cut-off. Four patients demonstrated an increase in FEV₁ of greater than 12% without a reduction in EMG_{para} in excess of their own $CV \times 1.65$. Five patients demonstrated a significant decrease in EMG_{para} without a concomitant significant increase in FEV₁. The remaining five showed significant changes in neither EMG_{para} nor FEV₁.

Table 4 details the sensitivity, specificity, positive and negative predictive values of subjects' own $CV \times 1.65$ as a cut-off value for change in EMG_{para} to predict a $\geq 12\%$ increase in FEV_1 .

	$\Delta FEV_1 \geq 12\%$	$\Delta FEV_1 < 12\%$	
$\Delta EMG_{para} \geq CV \times 1.65$	3	5	<i>Positive predictive value: 37.5%</i>
$\Delta EMG_{para} < CV \times 1.65$	4	5	<i>Negative predictive value: 55.6%</i>
	<i>Sensitivity: 42.9%</i>	<i>Specificity: 50%</i>	

Table 4 Individual data derived cut-off for EMG_{para} . Sensitivity, specificity, positive and negative predictive values for significant change in EMG_{para} to predict $\geq 12\%$ increase in FEV_1 , using subjects' own $CV \times 1.65$. $n=17$.

3.5 Discussion

The results of this study show that EMG_{para} is a feasible technique for use in asthmatic children, and demonstrated the ability of EMG_{para} to detect change in NRD following administration of a bronchodilator. Calculation of individual and group CVs allowed determination of a threshold for a statistically significant change in EMG_{para} from baseline values. Significant changes were observed in conventional measures of pulmonary function, supporting the premise that changes in respiratory load occurred following bronchodilator administration. The higher pre-bronchodilator NRD observed in those children demonstrating a significant change in EMG_{para} suggests that these children had greater levels of bronchoconstriction at baseline. However, no relationship was found between the relative changes seen in EMG_{para} and conventional measures of pulmonary function (spirometry and impulse oscillometry).

In children with asthma, the overall load on the respiratory system may be due to not only the changes in airway calibre, but also a result of lung hyperinflation and concomitant alterations in chest wall mechanics. The numerically greater, though not statistically significant, mean percentage change observed in EMG_{para} compared to that in FEV₁ following bronchodilator administration may be due to EMG_{para} reflecting the overall load on the respiratory system rather than a single component as with FEV₁. This may also explain the individuals in whom a significant change was observed in EMG_{para} in the absence of a significant FEV₁ change. Spirometry primarily measures airways obstruction occurring in the larger proximal airways. It has been suggested that 50-90% of the airways obstruction occurring in asthma may be present in distal airways [30]. Distal airway obstruction and collapse also contribute to hyperinflation and the development of intrinsic positive end-expiratory pressure (PEEPi). This would impose an additional threshold load on the respiratory muscles, which would not be measured using spirometry but would cause NRD to be

elevated. Conversely, reducing airway narrowing through administration of a bronchodilator may have reduced expiratory airway collapse and therefore air trapping, hyperinflation and PEEPi which will not be reflected in the FEV₁ but would result in a change in NRD, as shown by a decrease in EMG_{para}. Changes in end-expiratory lung volumes may have contributed to changes in NRD [172] and have been observed in asthmatic subjects following bronchodilator administration [234]. Static lung volumes were not measured in this study, which represents a limitation of the protocol.

In order to supplement the relatively limited information regarding overall respiratory system pathophysiology provided by FEV₁ alone, a number of other indices were examined. Mid-expiratory airflows (FEF₂₅₋₇₅) can be used to assess smaller airways function, and have been reported to change in asthmatics following bronchodilator administration [221] as occurred in this study. Such measurements, however, are affected by changes in vital capacity. The ratio of FEF₂₅₋₇₅ to FVC has been used to account for this [235]. As with FEF₂₅₋₇₅, FEF₂₅₋₇₅:FVC changed significantly following bronchodilation, but no correlation with EMG_{para} was observed.

Impulse oscillometry (IOS) was also performed in a subset of 12 subjects. This measurement was performed in a limited number of subjects only due to practical considerations – the addition of IOS lengthened the protocol, and as much of the testing was performed prior to a clinic appointment sufficient time was not available in many cases to perform IOS in addition to spirometry and EMG_{para}. A number of indices were examined, as recommended by a recent American Thoracic Society consensus publication [236]. Z5Hz is a composite measure of the resistance and reactance components that comprise the impedance of the respiratory system. Lower frequency components of the oscillometry signal have been shown to be discriminative between healthy and asthmatic children [207]. Measures of resistance at 5Hz and 20Hz reflect primarily the contributions of smaller and larger airways respectively to overall respiratory system resistance. The percentage difference between these two indices ($\Delta R5-R20$) assesses

the frequency-dependence of respiratory system resistance. In health, respiratory system resistance is essentially frequency-independent, but in airways obstruction, increases in smaller airways resistance lead to greater discrepancy between the Rrs values at lower and higher frequencies. $\Delta R5-R20$ hence provides a measure of smaller airways dysfunction. Reactance parameters (RF and AX) represent the interaction of elastic and inertial properties of the respiratory system and have been shown to demonstrate the greatest changes with disease and following treatment [118]. It has been suggested that composite measures (such as AX and $\Delta R5-R20$) including measurements at a number of frequencies offer an improved signal-to-noise ratio and thus are better able to detect clinically relevant changes [118].

It was anticipated that the FEF_{25-75}/FVC and IOS data could have provided further information regarding small airways function. The significant changes in FEF_{25-75}/FVC , R5 and $\Delta R5-R20$ indicate that an increase in smaller airways calibre did occur in this cohort; however, there was still no relationship with the relative changes in these parameters and EMG_{para} .

It is widely recognised that there is significant heterogeneity in the pathophysiological changes that occur in patients with asthma [237]. It is possible that airway obstruction was present in both the large and small airways in the patients in this study and may explain the absence of a correlation between flow- or pressure-based measures of pulmonary function (spirometry and IOS) and EMG_{para} . Similarly, for a given change in EMG_{para} the changes in larger and smaller airway calibre may have been different in each individual patient, resulting in an absence of relationships between EMG_{para} and spirometric or IOS variables. This premise is supported by the absence of significant relationships between oscillometric and spirometric parameters.

In addition to this, the fact that many of the subjects included in this study were receiving inhaled corticosteroid therapy (or other 'controller'

medication) and would therefore be expected to have a diminished or even absent response to β_2 agonists, may make the detection of relationships between variables more challenging. While the second aim of this study – to investigate the relationship between relative changes in EMG_{para} and conventional measures of pulmonary function – does not theoretically require significant changes in each parameter to be achieved, smaller changes may well lie within the natural variability of each measure; the signal to noise ratio may therefore not be sufficient to allow relationships to be detected.

Previous studies examining the use of EMG_{para} in adults with CF and COPD have demonstrated relationships between disease severity (measured by spirometry) and EMG_{para} [172, 196, 197]. It should be noted, however, that the patients in these studies had more severe lung disease, facilitating the detection of relationships between variables.

Recruiting patients likely to demonstrate a marked bronchodilator response would be difficult in this patient population as the majority of children studied were receiving inhaled corticosteroid therapy (or other ‘preventer’ medication) and would therefore be expected to have a diminished or even absent response to β_2 agonists. Steroid-naïve patients may have been a more appropriate population to study; however, many children referred to King’s College Hospital have an established diagnosis of asthma and are already receiving treatment. Asthma management in primary care settings is undertaken according to national and international guidelines, and referral to a secondary or tertiary centre is not recommended without a trial of inhaled steroids [11]. Recruitment of newly diagnosed asthmatic children not yet commenced on long-term treatment, although potentially presenting a greater logistical challenge and thus resulting in a smaller sample size, may have shown relationships between EMG_{para} and lung function.

While there were no significant correlations found between conventional measures of pulmonary function and EMG_{para}, it was anticipated that there

would have been a stronger agreement between the changes in FEV₁ and EMG_{para} when examined using a binary outcome (significant *versus* non-significant changes). In approximately half of patients (16 of 33 (48.5%)), EMG_{para} reflected the change in FEV₁. In the subjects in whom a significant change was observed in EMG_{para} but not in FEV₁, it could be hypothesised that the EMG_{para} reflected changes additional to those occurring in larger airways. The presence, however, of significant changes in FEV₁ in the absence of reciprocal changes in EMG_{para} is more challenging to explain.

An increase in FEV₁ in the absence of a fall in EMG_{para} may be explained in some patients by increased effort in the FEV₁ manoeuvre following bronchodilator. By evaluating the shape of the flow-volume loops and ensuring at least three reproducible FEV₁ efforts were produced, the likelihood of submaximal efforts was minimised [14]. Other authors have suggested, however, that submaximal efforts, both in skeletal muscle strength [238] and pulmonary function testing [219], may still be reproducible. It is conceivable that some subjects in the current study may have produced submaximal efforts prior to bronchodilator administration and, following salbutamol, a combination of the small improvement in pulmonary function together with a 'true' maximal effort produced a 12% or greater increase in FEV₁.

Acute administration of salbutamol has also been shown to increase resting metabolic rate and oxygen consumption in adult subjects [239]. An increase in oxygen consumption and CO₂ production would result in an increase in ventilation and hence NRD to maintain blood gas homeostasis. Increases in metabolism were, however, only observed in subjects who were salbutamol-naïve [239], not in those chronically exposed to the medication [240] and were only observed following dosages of inhaled salbutamol of 800µg or greater, double the dose used in the current study. It is possible that lower doses in children may result in similar effects on metabolism. Many of the children studied regularly used β₂ agonists, although a precise history of medication use was not taken. It is possible, therefore that some children

whose asthma was well controlled may not have been using inhaled salbutamol regularly. It is not possible to determine from the current data whether the reduction in EMG_{para} represented solely salbutamol-mediated bronchodilation reducing the load on the respiratory system or whether this was attenuated by a concomitant rise in NRD mediated by an increase in metabolism. It is feasible that this may explain why in some patients there was an increase in FEV_1 without an associated reduction in EMG_{para} .

Measurement of mean peak RMS EMG_{para} per breath does not take account of other factors such as inspiratory time or the rate of rise of muscle activity during inspiration. A decrease in total parasternal intercostal muscle activity (*i.e.* the area under the RMS EMG_{para} curve) may occur in the absence of a fall in peak EMG_{para} , and would not be seen by using the current method of analysis. Measurement of respiratory flow and timing (inspiratory, expiratory and total breath time) can be obtained using a pneumotachograph attached to a mouthpiece, but such techniques were found to be too distracting for younger subjects and resulted in marked alterations in the tidal breathing pattern. Technological limitations in EMG signal processing currently preclude the use of other analysis methods. Further developments in filtering algorithms should allow removal of ECG artefact and consequently more detailed analysis of the EMG_{para} signal.

Contamination of the EMG signal by cardiac artefact is a recognised limitation of the technique. Reliable methods for ECG removal that do not also remove a significant proportion of the EMG signal are not currently available. As a result the data from eleven children were excluded from analysis. Electrical interference from external sources resulted in a further four cases being excluded from analysis. External interference was encountered despite appropriate signal conditioning with electronic and digital filtering and grounding of equipment. In a further two cases a stable respiratory pattern that would allow analysis of one minute of EMG_{para} activity was not established. Further technological developments are likely to reduce the number of data sets that are rejected and improved filtering

algorithms may allow complete removal of the ECG signal. This study was also undertaken first and investigator inexperience when recording EMG_{para} may have resulted in a number of traces being unacceptable for analysis. For seventeen of 70 traces to be rejected could indicate that the technique may have limited utility, but it should be noted that such issues were not encountered in the studies contained within Chapters Four to Seven. The difficulties in obtaining optimal signals within this study do, however, highlight the need for experienced investigators when undertaking measurements of EMG_{para} using currently available systems.

The appropriateness of the use of the individual *versus* group CV to determine the threshold for a significant change cannot be determined from these data. The sensitivity and specificity of each method (group and individual CV*1.65) is very similar. Calculating a patient's individual CV from a baseline recording would not currently be possible in clinical practice due to the technological limitations of the technique and the current reliance on manual analysis, though further software advances may allow this in future. The sample size is small from which to develop a threshold for the group mean CV but the agreement with the CV from Chapter Seven (examining EMG_{para} in healthy children) indicates that this threshold may be appropriate and that a change in EMG_{para} of 15.3% could be viewed as significant.

3.5.1 Relationship with previous literature

There are few data currently available on the use of parasternal intercostal muscle EMG in children. The EMG_{para} response of paediatric subjects to bronchodilator administration has previously been assessed only following induced airways obstruction. Maarsingh *et al* demonstrated a return of EMG_{para} to baseline levels when salbutamol was administered to asthmatic children following a histamine challenge, in both children over the age of five years [194] and in pre-school children [195]. Maarsingh *et al* [194] did

not report the agreement between changes in FEV₁ and EMG_{para} following bronchodilator administration, although an exponential relationship between changes in FEV₁ and EMG_{para} was observed during the histamine challenge.

Reductions in EMG_{para}, recorded using fine-wire electrodes, have previously been shown in patients with severe COPD following inhalation of a combined β_2 agonist and corticosteroid (salmeterol fluticasone propionate) [241]. The subjects demonstrated minimal changes in FEV₁ and end-expiratory lung volumes, yet pronounced reductions in EMG_{para} activity. In keeping with the current results, these data suggested that relationships between changes in conventional measures of pulmonary function may not be seen. The authors speculated that this might have been due to a combination of improvements in respiratory mechanics and increases in skeletal muscle contractility, which enhanced the efficiency of respiratory muscle contraction. The same authors previously demonstrated increases in parasternal intercostal muscle contractility in dogs when high doses of intravenous salbutamol were administered [242]. Data obtained in a severe COPD cohort must, however, be used with caution when interpreting the data from this group of paediatric patients with relatively mild asthma, particularly in view of the methodological differences of using needle EMG *versus* surface techniques.

3.5.2 Relevance to clinical practice

While these data demonstrate that EMG_{para} changes in response to β_2 agonist administration in both pre-school and school-aged asthmatic children, suggesting that the technique may act as a marker of respiratory load in these populations, the lack of relationship with conventional measures of pulmonary function indicates that EMG_{para} cannot be viewed as a direct substitute for spirometry. In older children able to perform spirometry, the data from this study are unlikely to be sufficiently convincing to warrant the introduction of EMG_{para} in place of FEV₁ as a

determinant of reversibility of airways obstruction. The relationship between EMG_{para} and other aspects of pulmonary pathophysiology require further exploration in order to more fully understand the determinants of reductions in NRD following bronchodilator administration, which would facilitate translation into clinical practice.

The determination of a threshold for a statistically significant reduction in EMG_{para} activity, however, provides an important step towards the development of EMG_{para} as a clinical test. Although future studies will need to address the question of what constitutes a clinically significant change in EMG_{para}, the data presented from this study suggest that a reduction in EMG_{para} activity of greater than 15.3% constitutes a change that is unlikely to occur simply as a result of the natural variability of the measure. This may be more useful in wheezy pre-school children where other measures of reversibility of airways obstruction are lacking, and may provide objective evidence to support a clinical judgement of benefit from β_2 agonist.

3.6 Conclusions

In conclusion, these data demonstrate that EMG_{para} can detect change in NRD following administration of bronchodilator in asthmatic children. The magnitude of change observed was greater than that seen in FEV₁. There was poor agreement between the changes observed in EMG_{para} and conventional measures of pulmonary function. Using the coefficient of variation provided a method for determining a significant change in respiratory load, but there remained a number of children in whom the EMG_{para} did not change significantly despite an increase in FEV₁.

4. Chapter Four: EMG_{para} in assessing response to methacholine challenge in adults

4.1 Rationale

Data from studies using EMG_{para} in adult patients with cystic fibrosis [172] and using EMG_{di} in patients with COPD [189] have indicated a direct relationship between neural respiratory drive (NRD) and FEV₁ when measured on a single occasion. The relationship between dynamic changes in NRD and lung function is, however, unclear. Previous studies have shown no relationship between changes in FEV₁ and EMG_{para} [198, 199] in patients hospitalised with acute exacerbations of cystic fibrosis and COPD respectively. A study by Maarsingh *et al* [194] indicated that a linear relationship existed between changes in FEV₁ and the logarithm of the EMG_{para} activity ratio (EMG_{para} activity related to baseline, EMGAR) during a histamine challenge in asthmatic children. No such relationship has, however, been observed between FEV₁ and EMG_{para} in the data presented in Chapter Three. Bronchial hyperresponsiveness testing involves repeated inhalation of gradually increasing concentrations of a provocant agent resulting in controlled, incremental changes in lung function. Such a repeated-measures design allows relative changes in FEV₁ and EMG_{para} to be investigated without the potential confounding factors inherent in a longitudinal clinical study, though it is important to recognise that this may not be entirely representative of the spontaneous bronchoconstriction and hyperinflation occurring in asthma. The current study was undertaken to further elucidate the nature of the relationship between FEV₁ and EMG_{para} during incremental changes in airway calibre. As chemical challenge testing is not routinely undertaken clinically in children, the study was performed in adult subjects.

We hypothesised that:

- EMG_{para} would increase in a stepwise manner reflecting the decrement in lung function in response to the incremental dose of the chemical bronchoconstrictor agent.
- The magnitude of increase in EMG_{para} would be greater than the decrease in FEV_1 due to greater sensitivity of EMG_{para} to the range of pathophysiological changes occurring.

4.2 Subjects

Adult patients attending the Chest Unit of King's College Hospital following physician referral for methacholine challenge testing, and staff and students of King's College Hospital and King's College London were recruited. Ethical approval was granted by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from all participants.

4.3 Methods

4.3.1 Equipment

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and weight with an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK).

EMG_{para} was measured as described in Chapter Two (Methods). Briefly, EMG_{para} was recorded using surface electrodes placed over the second intercostal space. The signal was amplified (gain 1,000), band-pass filtered between 10 and 2,000Hz, and a 50Hz notch filter and a post-acquisition

20Hz digital high-pass filter applied. The signal was converted to root-mean-square (RMS) and mean peak RMS EMG_{para} per breath calculated.

The methacholine challenge test was performed using a portable electronic spirometer-digidoser system (KoKo USB spirometer and digidoser, Ferraris Respiratory, Louisville, CO, USA) and nebuliser (DeVilbiss 646 characterised nebuliser, Ferraris Respiratory, Louisville, CO, USA). A five-breath dosimeter protocol was followed [226], with the duration of both inspiration and expiration fixed at five seconds and inspiratory flow limited to 0.5L.sec⁻¹. A 0.9% saline diluent step was followed by methacholine concentrations of 0.0625, 0.25, 1, 4, 8, 16 and 32mg.ml⁻¹ (Acetyl-B-Methyl Choline, Nova Laboratories, Wigston, Leicestershire).

4.3.2 Protocol

EMG_{para} was recorded for three minutes prior to commencement of the challenge protocol to provide a measure of baseline NRD. Baseline measurements of FVC and FEV₁ were then performed. As per standard practice the first inhalation of the challenge test was saline, to screen for reaction to the diluent, which was followed by methacholine solutions of gradually increasing concentration (as stated above), administered at five-minute intervals. A single, technically acceptable FEV₁ manoeuvre was performed at both 30 and 90 seconds following each inhalation, with the lowest FEV₁ value recorded. Following the second FEV₁ manoeuvre, EMG_{para} was recorded for the remaining 3.5 minutes prior to the next methacholine dose, with the final minute of the recording used for analysis. The challenge test was terminated either when the subject demonstrated a 20% or greater fall in FEV₁, considered a positive response, or when the maximal dose of 32mg.ml⁻¹ methacholine was reached. In subjects demonstrating a positive response or reporting a sensation of respiratory discomfort, 400µg salbutamol was administered via a metered-dose inhaler and spacer. If salbutamol was administered, one further FEV₁ manoeuvre was performed

after 15 minutes to ensure the subject had regained their baseline level of pulmonary function. The changes in EMG_{para} and FEV_1 from those obtained following saline inhalation were calculated [226].

Changes in FEV_1 from the post-diluent stage were plotted against the log-transformed methacholine concentration. The provocative concentration of methacholine that induced a 20% fall in FEV_1 (PC20) was calculated using the following equation [226]:

$$PC20 = antilog \left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{R_2 - R_1} \right]$$

Where:

C_1 = penultimate methacholine concentration

C_2 = final methacholine concentration

R_1 = percent fall in FEV_1 after C_1

R_2 = percent fall in FEV_1 after C_2

Percentage changes in EMG_{para} were also plotted against log methacholine concentration. Linear interpolation of the EMG_{para} -log methacholine dose curve was used to calculate the percentage change in EMG_{para} at PC20.

4.3.3 Additional analyses

Area under the dose-response curve and the slope of the dose-response regression line have been used previously to evaluate bronchial hyperresponsiveness [243, 244]. These methods have the benefit of including the response at each stage of the challenge test, not only baseline and end of test. Area under the curve was calculated by plotting percentage change in FEV_1 or EMG_{para} against methacholine concentration. Due to the non-linear nature of the methacholine concentrations, doses were numbered to give a linear scale along the x-axis (with saline assigned zero,

0.0625mg.ml⁻¹ one, and so on). Linear regression was performed to determine the slopes of the dose-response lines of EMG_{para} and FEV₁ percentage changes in response to increasing methacholine concentrations, with the Wald-Wolfowitz runs test applied to assess for significant deviation from linearity.

4.3.4 Statistical analysis

Data were tested for normality using the D'Agostino and Pearson omnibus test, although where $n < 30$, data were always treated as non-normally distributed and non-parametric statistical testing was used. Within-subject changes were assessed using the paired t-test or Wilcoxon matched pairs test for normally and non-normally distributed data respectively. Differences between group medians of non-normally distributed data were assessed with the Mann-Whitney test. Unpaired t-tests were used to assess differences between group means in normally distributed data. Relationships between variables were assessed using Spearman's rank correlation, as no variables in which correlations were assessed fitted a Gaussian distribution. A p value < 0.05 was taken as statistically significant. When comparing the relative magnitudes of changes in FEV₁ and EMG_{para}, changes in FEV₁ were multiplied by minus one in order to give a positive value.

4.3.4.1 Sample size calculation

We hypothesised that the increase in EMG_{para} observed in a positive test would be greater than the 20% reduction in FEV₁ that defines a positive methacholine challenge test [226]. Assuming a baseline mean (SD) raw EMG_{para} of 4.8 (2.0) μ V as reported by Reilly *et al* [172], 25 subjects were

required to detect a 30% increase in EMG_{para} activity with 90% power at the 5% level.

4.4 Results

A total of 32 subjects were studied in order to obtain the minimum sample of 25 subjects in whom a significant response to methacholine was observed (defined by a 20% or greater decrease in FEV₁ [226]). The demographic and anthropometric characteristics are shown in Table 5. Subjects' diagnoses (reason for inclusion in the study) are shown in Table 6.

Age (years)	29.2 (22.0 – 50.8)
Sex (male: female)	12: 20
Height (m)	1.70 (1.51 – 1.90)
Weight (kg)	68.4 (51.6 – 105.9)
BMI (kg m⁻²)	24.4 (20.2 – 32.1)
Ethnicity (n (%))	White 26 (81.25%) Black: 1 (3.13%) Asian: 4 (12.5%) Other: 1 (3.13%)
Baseline FEV₁ (%predicted)	97.1 (80.0 – 122.3)

Table 5 Anthropometric and demographic characteristics of participants in Study Two. Data are expressed as median (range).

Responders (n=25)	Asthma (taking medication)	5 (20%)
	Self-reported asthma (no medication)	6 (24%)
	Self-reported atopy/allergies	14 (56%)
Non-responders (n=7)	Asthma (taking medication)	1 (14%)
	Self-reported atopy/allergies	2 (29%)
	Clinically suspected bronchial hyperresponsiveness	4 (57%)

Table 6 Diagnoses of subjects included in Study Two. Data are shown as n (%).

Both EMG_{para} and FEV_1 changed significantly from baseline to end of test in the whole cohort (Table 7, Figure 19) as well as the ‘responder’ subset (Table 7). Despite being below the threshold to be considered a positive response, the change in FEV_1 from baseline in the ‘non-responder’ group was also statistically significant. The corresponding change in EMG_{para} was not, however, statistically significant (Table 7).

		Baseline	End of test	p value	Percentage change
Entire cohort (n=32)	EMG _{para} (μ V)	5.02 (2.00 – 8.92)	5.86 (2.37 – 19.60)	<0.0001	47.0 (54.3)
	FEV ₁ (%pred)	97.10 (80.00 – 122.30)	70.95 (37.98 – 100.70)	<0.0001	-25.2 (12.4)
Responders (n=25)	EMG _{para} (μ V)	5.37 (2.25 – 8.92)	6.27 (3.37 – 19.60)	<0.0001	31.6 (-21.1 – 144.4)
	FEV ₁ (%pred)	96.00 (80.00 – 122.30)	67.80 (37.98 – 92.27)	<0.0001	-27.8 (-53.7 – -20.7)
Non-responders (n=7)	EMG _{para} (μ V)	3.54 (2.00 – 5.01)	4.49 (2.37 – 9.32)	ns	23.0 (-32.4 – 196.2)
	FEV ₁ (%pred)	98.46 (84.91 – 105.8)	91.43 (72.98 – 100.70)	0.031	-7.2 (-14.2 – -2.2)

Table 7 Changes in EMG_{para} and FEV₁ in entire cohort, and responder and non-responder subsets. Data expressed as mean (SD) or median (range).

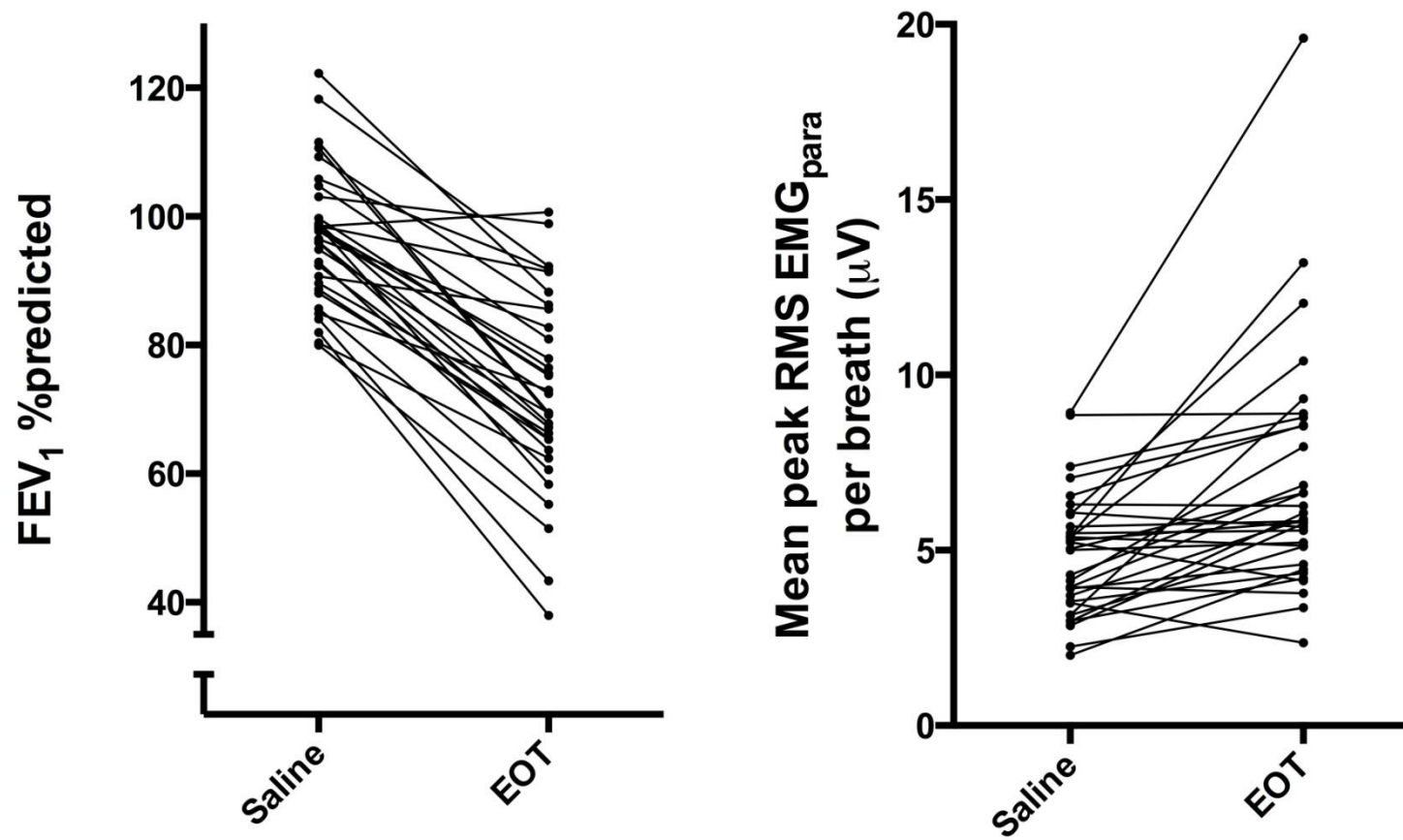


Figure 19 Decrease in FEV₁ and increase in EMG_{para} in 32 adults undergoing methacholine challenge test.

Although the median (range) percentage change in EMG_{para} was numerically greater than that observed in FEV_1 from baseline to end of test (EOT) this did not reach statistical significance for the cohort as a whole ($p=0.091$), the responder ($p=0.22$) or non-responder ($p=0.2188$) groups.

A one-sample t-test was used to compare the changes in EMG_{para} at the concentration of methacholine inducing a 20% fall in FEV_1 (PC20) to those in FEV_1 . The median (range) change in EMG_{para} (28.4 (-33.7 – 141.7) %) was not significantly different to the 20% change in FEV_1 ($p=0.2$). Similarly, there was no significant difference between the median (range) area under the curve (AUC) of FEV_1 (41.8 (19.3 – 71.2) arbitrary units (AU)) and EMG_{para} (29.35 (-228.8 – 289.1) AU) responses to methacholine ($p=0.7$).

Correlation analysis indicated there was no relationship between the baseline to EOT changes in EMG_{para} and FEV_1 in the cohort as a whole or in either the responder or non-responder subsets (Figure 20).

The median (range) slope of the individual subjects' relationships between EMG_{para} and methacholine dose number was significantly greater than that for FEV_1 against dose number in the entire cohort (7.13 (-4.36 – 35.70) *versus* 4.68 (-0.10 – 17.20), $p=0.0125$). This was also the case in the responder subset (11.17 (-0.86 – 35.70) *versus* 5.09 (2.64 – 17.18), $p=0.0451$), though not in the non-responder group. The greater slopes indicate that changes in EMG_{para} are greater and occur more rapidly than those in FEV_1 .

In the whole cohort, a weak correlation approaching statistical significance was seen between the individual slopes of FEV_1 and EMG_{para} against dose number ($r=0.337$, $p=0.0591$). This relationship was marginally stronger in the responder subset reaching statistical significance ($r=0.453$, $p=0.0229$).

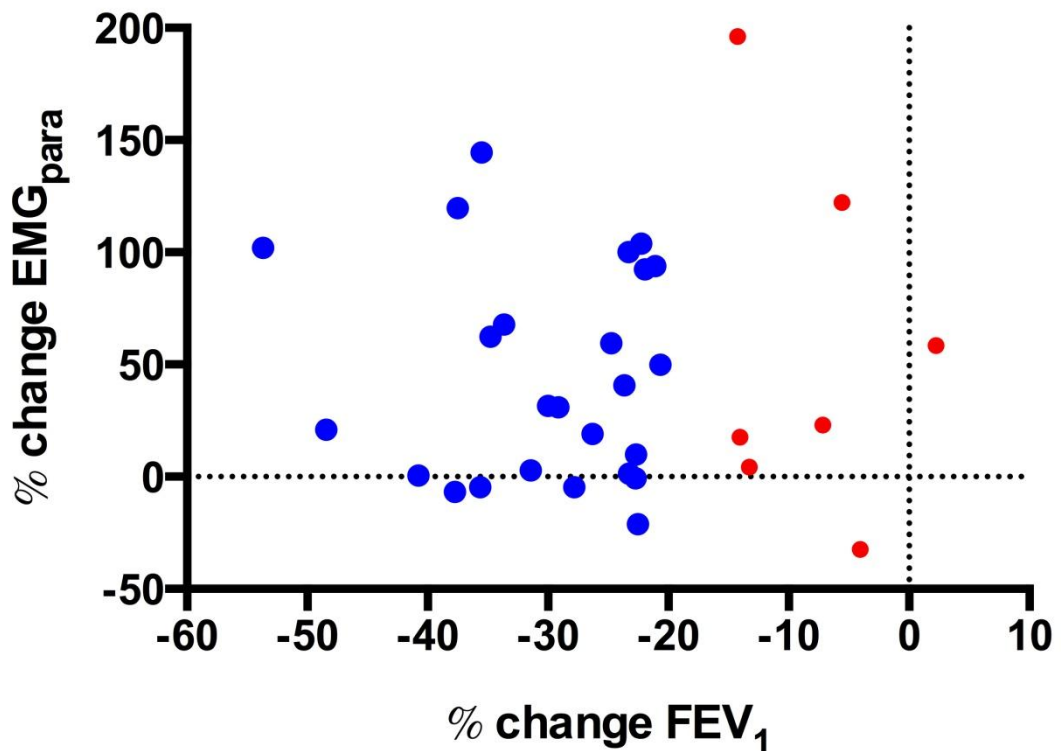


Figure 20 Baseline to end-of-test percentage changes in FEV₁ and EMG_{para} in the whole cohort of 32 adults undergoing methacholine challenge testing. Responders are shown in blue, non-responders in red.

4.4.1 Investigation of an alternative baseline

Twenty-one of 32 subjects (65.6%) demonstrated a reduction in EMG_{para} from the measure obtained following saline inhalation to that following the first methacholine dose. The fall in mean (SD) EMG_{para} for the whole cohort approached significance (4.86 (1.74) μ V *versus* 4.45 (1.72) μ V, $p=0.056$). This occurred despite a small but significant fall in mean (SD) FEV₁ % predicted (96.8 (10.4)% *versus* 94.1 (10.3)%, $p<0.0001$). Additional analyses were therefore performed to examine the relationship between EMG_{para} activity and FEV₁ using the first methacholine dose (0.0625mg.ml⁻¹) as the reference point. Although these analyses would not be acceptable in the context of a

clinical methacholine challenge test, controlling for the unexpected fall in EMG_{para} at the first methacholine stage allows more accurate analysis of the relationships between the changes in EMG_{para} and FEV_1 . The changes in each parameter from 0.0625mg.ml to EOT are shown in Table 8.

The fall in EMG_{para} activity from saline to 0.0625mg.ml⁻¹ may have been related to subject anxiety. Heart rate taken from the EMG_{para} trace was used to assess subject anxiety. However, no change in mean (SD) heart rate was observed between the diluent and first methacholine stage of the protocol (71.6 (10.9) to 72.2 (9.8) beats per minute, $p=0.996$).

		0.0625mg.ml ⁻¹ stage	End of test	p value	Percentage change
Entire cohort (n=32)	EMG _{para} (μV)	4.02 (2.12 – 7.91)	5.86 (2.37 – 19.60)	<0.0001	40.77 (-20.37 – 214.90)
	FEV ₁ (%pred)	94.72 (76.68 – 119.10)	70.95 (37.98 – 100.70)	<0.0001	-22.19 (-50.47 – 1.17)
Responders (n=25)	EMG _{para} (μV)	4.52 (2.18 – 7.91)	6.27 (3.37 – 19.60)	<0.0001	40.28 (1.36 – 214.90)
	FEV ₁ (%pred)	92.59 (76.68 – 119.10)	67.80 (37.98 – 92.27)	<0.0001	-24.33 (-50.47 – -15.24)
Non-responders (n=7)	EMG _{para} (μV)	3.26 (2.11 – 4.32)	4.49 (2.37 – 9.32)	0.156	41.26 (-20.37 – 196.2)
	FEV ₁ (%pred)	98.07 (80.70 – 104.0)	91.43 (72.98 – 100.70)	0.078	-6.77 (-15.77 – 1.17)

Table 8 Changes in EMG_{para} and FEV₁ in entire cohort, and responder and non-responder subsets from first methacholine dose (0.0625mg.ml⁻¹) to end of test. Data are expressed as median (range).

The median (range) percentage change in EMG_{para} was significantly greater than that observed in FEV₁ from the 0.0625mg.ml⁻¹ reference point to EOT in both the entire cohort (40.8 (-20.4 – 214.9)% vs -22.2 (-50.5 – 1.2)%, p<0.0001) and the responder group (40.3 (1.4 – 214.9) vs -24.3 (-15.2 – -50.5), p=0.0013) (Table 8). A significant relationship between the percentage changes in FEV₁ and EMG_{para} was observed in the responder subset (r=-0.421, p=0.036) (Figure 21).

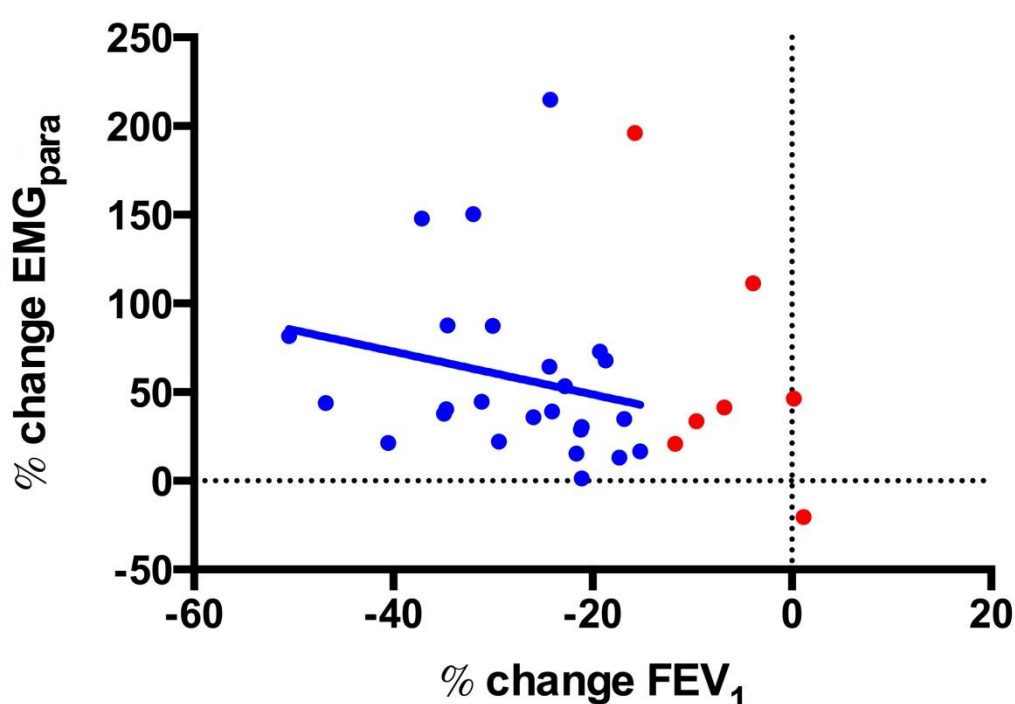


Figure 21 Graph showing changes in FEV₁ and EMG_{para} at end of test in 32 adults undergoing methacholine challenge, using 0.0625mg.ml⁻¹ as baseline. Responders shown in blue, non-responders in red.

In addition, when using 0.0625mg.ml⁻¹ as a reference point, the median (range) AUC for EMG_{para} (72.5 (-81.0 – 543.3) AU) was significantly greater than that for FEV₁ (25.6 (-4.9 – 62.9) AU, p=0.0007). AUC FEV₁ demonstrated a moderate but significant correlation with AUC EMG_{para}

($r=0.502$, $p=0.0034$). Similarly, the median (range) slope of the EMG_{para} against dose number regression was greater than that for FEV_1 (12.33 (-1.02 – 45.50) vs. 6.46 (2.76 – 25.20), $p=0.001$), and demonstrated a moderate but significant correlation ($r=0.487$, $p=0.0136$). These relationships indicate that incremental changes in FEV_1 and EMG_{para} are related. The greater overall change, AUC and slope for EMG_{para} than FEV_1 together indicate that EMG_{para} demonstrates greater and more rapid changes than FEV_1 .

4.4.2 Individual subject data

Individual plots of FEV_1 against EMG_{para} at each concentration of methacholine were constructed for the responder subset (fall in FEV_1 greater than 20%). The median (range) correlation coefficient of the individual subject relationships between FEV_1 and EMG_{para} was -0.57 (-1.00 – 0.18). Not all correlations reached statistical significance due to the small number of data points included.

If 0.0625mg.ml^{-1} was used as the reference concentration, the median (range) correlation coefficient increased to -0.80 (-1.0 – 0.0). Visual inspection of the plots led to each study subject being categorised into groups. For ease of visualisation, both EMG_{para} and FEV_1 for each subject were plotted on the same graph against methacholine dose.

Nine subjects demonstrated a clear relationship between FEV_1 and EMG_{para} , with correlation coefficients of -0.8 or greater in all cases (Figure 22 and Figure 23).

Six subjects demonstrated an increase in EMG_{para} prior to a decrease in FEV_1 (Figure 24).

Nine subjects showed no relationship between FEV_1 and EMG_{para} (Figure 25 and Figure 26).

One subject demonstrated a decrease in FEV_1 prior to any change in EMG_{para} (Figure 27).

Corresponding decrease in FEV₁ and increase in EMG_{para}

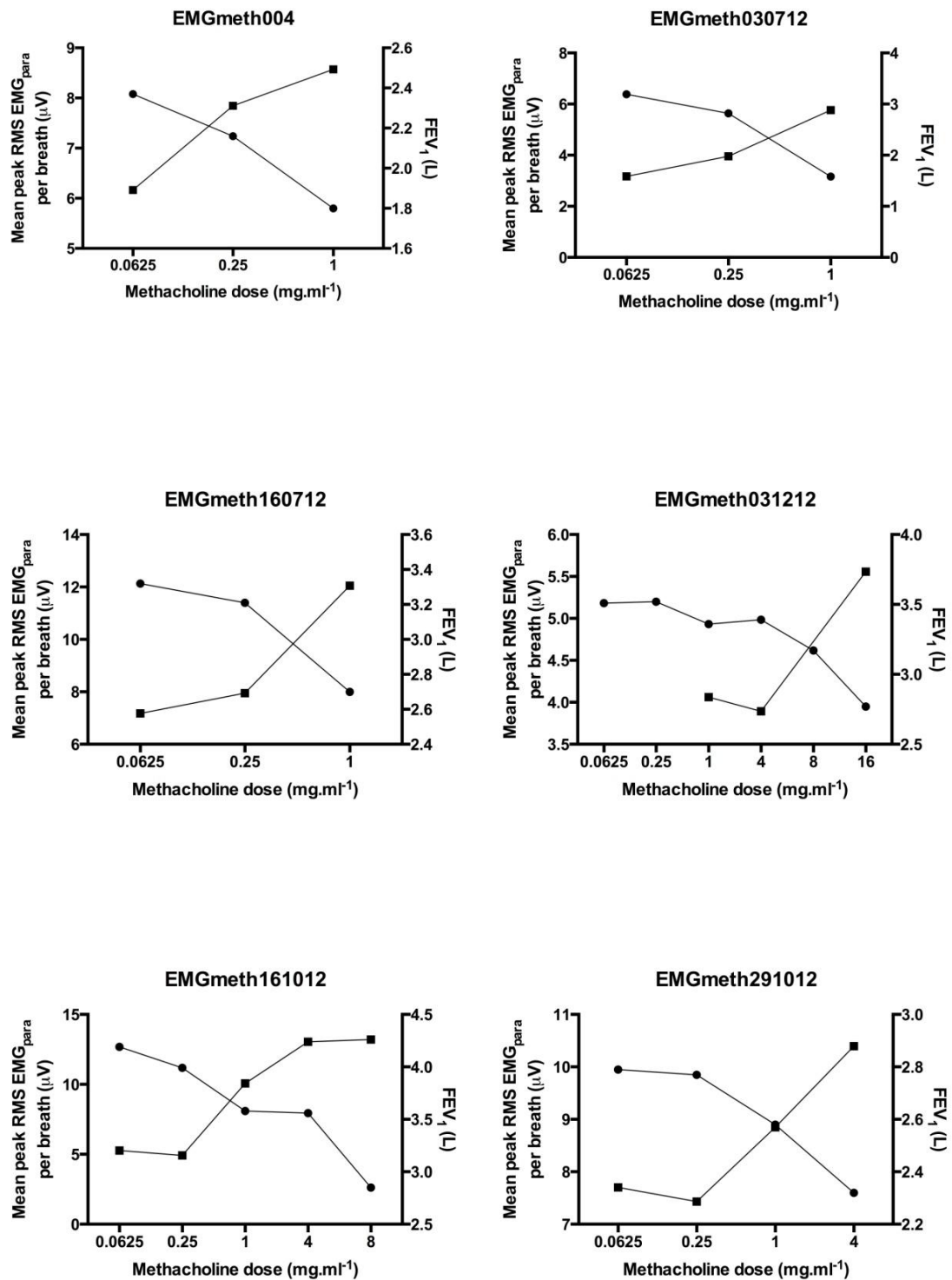


Figure 22 Six subjects demonstrating corresponding increases in EMG_{para} with decreases in FEV₁ while undergoing methacholine challenge. Square symbols represent EMG_{para}; circles represent FEV₁.

Corresponding decrease in FEV₁ and increase in EMG_{para}

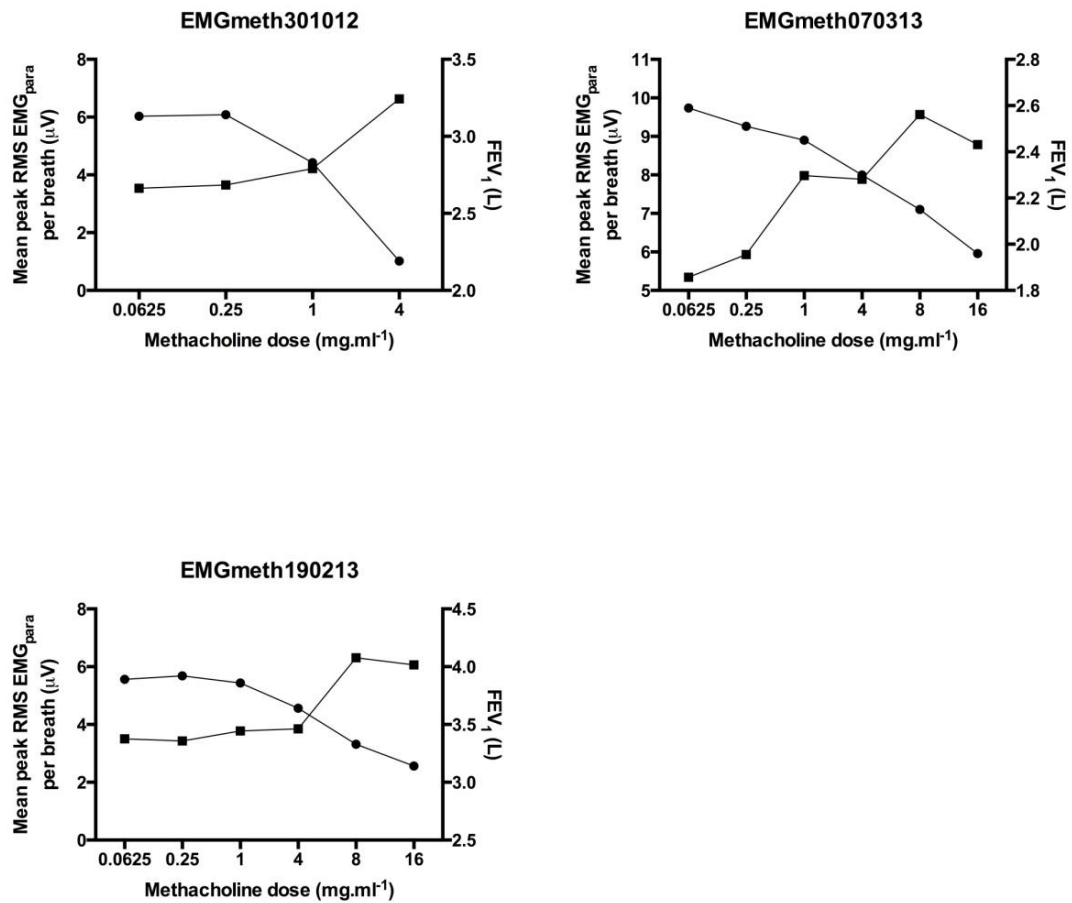


Figure 23 Three subjects demonstrating corresponding increases in EMG_{para} with decreases in FEV₁ while undergoing methacholine challenge.

Square symbols represent EMG_{para}; circles represent FEV₁.

Increase in EMG_{para} prior to decrease in FEV₁

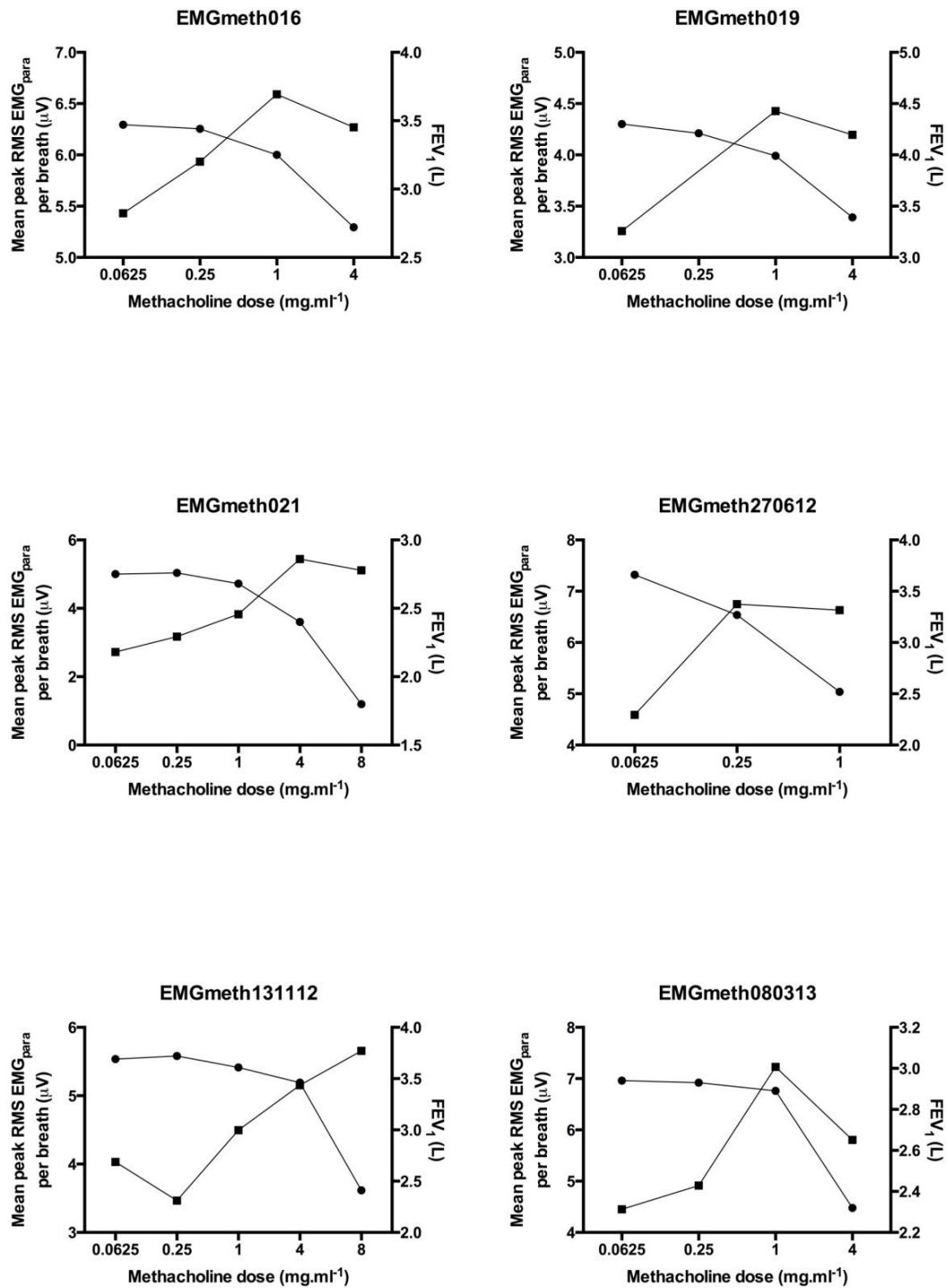


Figure 24 Six subjects demonstrating increases in EMG_{para} prior to reductions in FEV₁ while undergoing methacholine challenge.

Square symbols represent EMG_{para}; circles represent FEV₁.

No apparent relationship between EMG_{para} and FEV_1

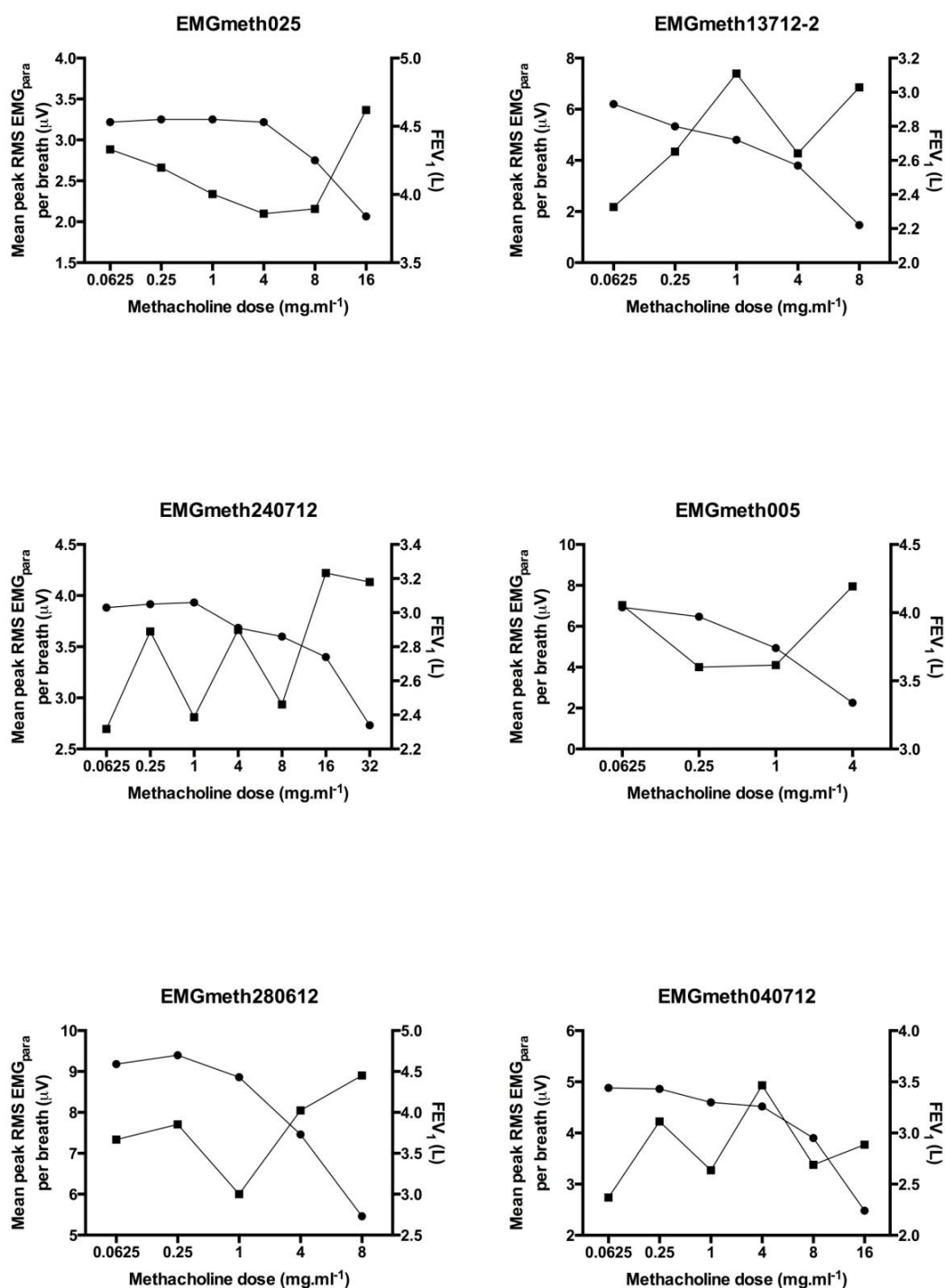


Figure 25 Six subjects with no apparent relationship between changes in EMG_{para} and FEV_1 during methacholine challenge.

Square symbols represent EMG_{para} ; circles represent FEV_1 .

No apparent relationship between EMG_{para} and FEV_1

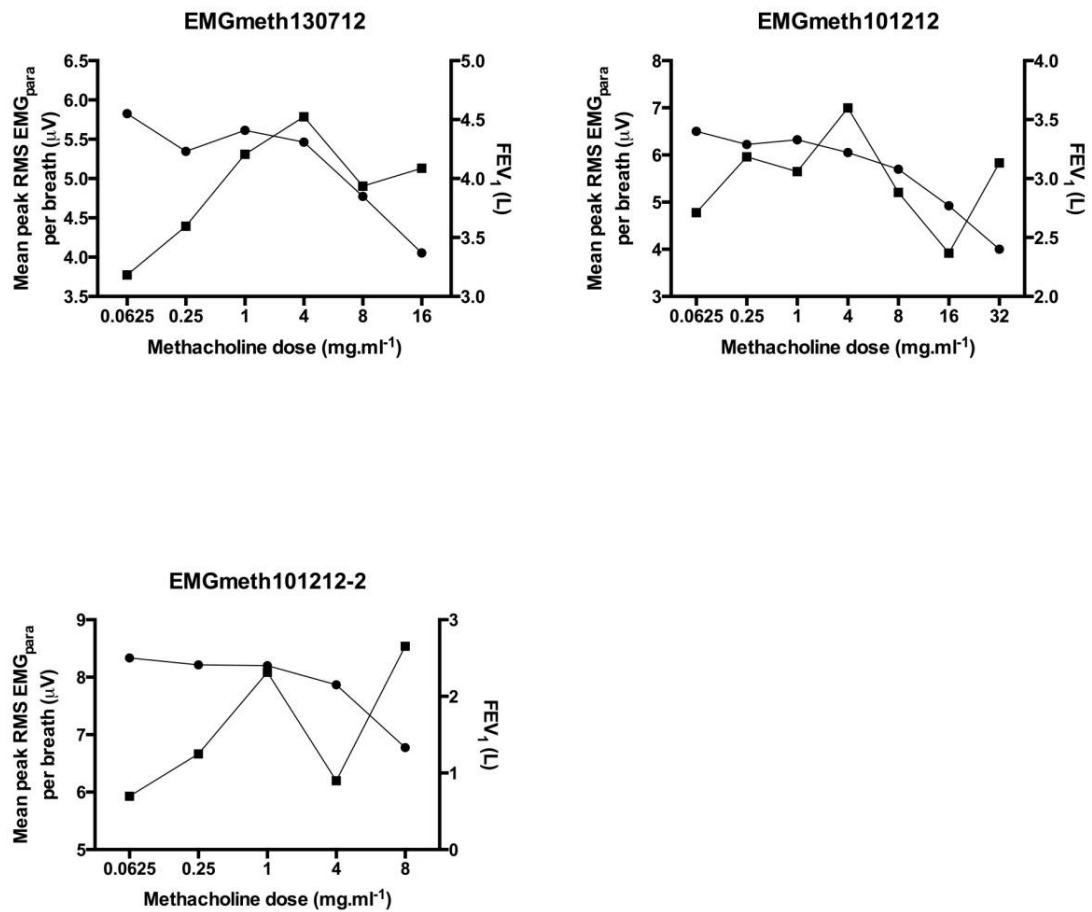


Figure 26 Three subjects with no apparent relationship between changes in EMG_{para} and FEV_1 during methacholine challenge.

Square symbols represent EMG_{para} ; circles represent FEV_1 .

**Decrease in FEV₁ prior
to increase in EMG_{para}**

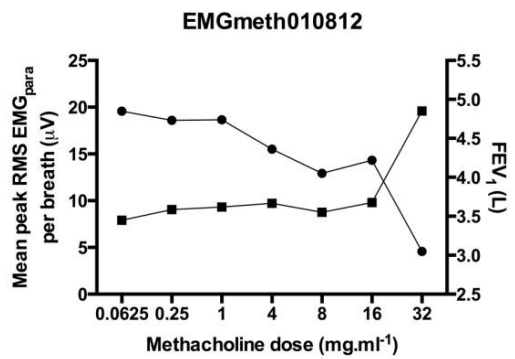


Figure 27 Single subject demonstrating a decrease in FEV₁ prior to increase in EMG_{para} during methacholine challenge.

Square symbols represent EMG_{para}; circles represent FEV₁.

4.5 Discussion

This study demonstrates that EMG_{para} can be used to detect an increase in respiratory load following chemically induced bronchoconstriction in adults. A relationship was observed between changes in FEV_1 and EMG_{para} between the first methacholine dose and end of test in the responder subset. The incremental bronchoconstriction induced in the current study offered an advantage over the protocol in Chapter Three, where only a single step change occurred, allowing a range of degrees of airways obstruction to be examined. The correlations observed between the FEV_1 -dose and EMG_{para} -dose regression lines and between the area under the FEV_1 and EMG_{para} dose-response curves further supports a relationship between EMG_{para} and measures of airway calibre. A wide range of pathophysiological changes occurs in response to induced airways obstruction as well as significant heterogeneity in individual responses to methacholine challenge testing [245, 246]. EMG_{para} cannot therefore be viewed as a substitute for spirometric measures of pulmonary function, but rather reflects the change in load on the respiratory system overall. In this context, the fact that the relationship between a measure of global respiratory load provided by EMG_{para} and a unidimensional measure of airway calibre (FEV_1) is relatively weak was perhaps unsurprising.

4.5.1 Critique of the method

Given the aim of this study to assess the agreement between dynamic changes in variables, the order in which measurements were performed and the time elapsing between each measurement represent limitations of the study protocol. Up to three minutes elapsed between measurement of FEV_1 and EMG_{para} . Studies have suggested that the spontaneous resolution of methacholine-induced-bronchoconstriction is slow, with recovery times of up to two hours reported [247-249]. This provides reassurance that the

action of the inhaled methacholine could be expected to be consistent during the time over which measurements were made.

The maximal respiratory manoeuvres required for spirometry and for delivery of methacholine may have potentially induced changes in bronchomotor tone of varying magnitude between asthmatic and non-asthmatic individuals [250, 251]. The use of the dosimeter technique involving maximal inspirations, in combination with the maximal inspiratory efforts required for spirometry, have been suggested to produce bronchodilation in subjects with mild airway hyperresponsiveness. The effect is reported to be much less pronounced in subjects with moderate or severe hyperresponsiveness [252, 253]. Asthmatic subjects also demonstrate more rapid airway re-narrowing following a deep inspiration [254], with degree of re-narrowing being inversely related to the extent of deep inspiration-mediated bronchodilation [255]. While some of the subjects in the current study had a formal diagnosis of asthma, others were healthy individuals with only mild airway hyperresponsiveness. A degree of heterogeneity in response to deep inspirations may have been expected and could have contributed to the variability of results, potentially affecting the relationship between EMG_{para} and FEV_1 .

To avoid the effect of this variability, a methacholine challenge with EMG_{para} as the sole outcome measure may have been desirable, though this method clearly does not allow comparison between EMG_{para} and other variables. The relatively poor between-day reproducibility of methacholine challenge tests (± 1.5 doubling doses in the absence of any additional confounding factors, such as exposure to sensitising agents) would not allow accurate comparison of changes in FEV_1 and EMG_{para} measured in isolation on different testing occasions. The use of tidal breathing techniques as outcome measures may be more desirable when examining the relationship of methacholine-induced changes in pulmonary function to changes in EMG_{para} . Plethysmographic measurement of airways resistance (R_{aw}) has been well documented as an outcome measure for airway challenge testing

[256, 257], but performing measurements of Raw at the time intervals recommended by international guidelines for methacholine challenge testing [226] requires the subject to remain sealed within the plethysmograph throughout the challenge test. In addition, the specialist equipment for conducting bronchial challenge tests within a plethysmograph was not available in our centre. Impulse oscillometry (IOS) represents an alternative method to measure airways resistance and as the measurements are obtained during tidal breathing technique, simultaneous measurement of EMG_{para} would be possible. Although IOS has been used to assess the response to bronchial challenge [258, 259], there is no consensus regarding the criteria for a significant response and hence the use of IOS as the sole outcome measure for a methacholine challenge test is not recommended [226].

The potential for factors unrelated to the respiratory system to influence the EMG_{para} signal is highlighted by the fall in EMG_{para} between the saline and 0.0625mg.ml⁻¹ steps, accompanied by a very small decrease in FEV₁. Anxiety or the awareness of or focus on breathing by the subject could have resulted in increases in the resting respiratory drive. No change in heart rate between the saline and first methacholine dose stages was observed however, which would suggest that subjects were not overtly anxious, although the use of heart rate to assess anxiety is relatively limited [260, 261]. While subjects were allowed a period of acclimatisation in the laboratory following the application of the EMG_{para} electrodes, including a baseline measurement prior to the administration of the saline diluent, this may have been insufficient to allow reproducible values to be obtained. The use of the 0.0625mg.ml⁻¹ dose as a reference stage for analysis in the current study allows the relationship between relative changes in FEV₁ and EMG_{para} to be more closely investigated. Such an approach would, however, be inappropriate for clinical use. A greater time for acclimatisation to the measurement, the introduction of a second saline step that could be used as the reference point, and real-time evaluation (even if only qualitative) of the

EMG_{para} signal to ensure stability, prior to obtaining recordings for analysis, should be considered for future studies.

4.5.2 Relationship of the current findings to previous literature

Sprykkelman *et al* [190] assessed diaphragm and intercostal surface EMG activity in children undergoing histamine challenge testing and demonstrated a significant increase in the ratio of the EMG amplitude to that obtained at baseline (EMG activity ratio, EMGAR) at the final dose of histamine. No increases were observed in EMG activity at lower histamine doses. The authors reported a correlation coefficient of 0.65 between logEMGAR and FEV₁ percentage change following the final histamine dose. Some subjects in this study did, however, demonstrate EMGAR values of less than one, indicating substantial decreases in respiratory muscle activity, as well as some individuals showing increases in intercostal and diaphragm EMG in the absence of changes in FEV₁, in agreement with the findings of the current study.

In later work from the same group, Maarsingh *et al* [194] showed an exponential relationship between change in EMG_{para} and change in FEV₁ during a histamine challenge in asthmatic children, with strong agreement between changes in EMG_{para} and FEV₁ (correlation coefficients of 0.79 to 0.92). In view of the much weaker correlations observed in the current study, the differences between the two studies have been considered in detail.

The studies by Maarsingh [194] and Sprykkelman [190] were undertaken using ECG signal gating, which allowed automated analysis. The technique removes the cardiac signal and fills the resulting space with an average of the EMG activity before and after the removed ECG artefact. The EMG was

expressed as “mean peak-bottom respiratory activity”, which is analogous to the mean peak RMS EMG_{para} value used in this thesis. Despite the removal of the ECG artefact, the method of data analysis is similar and it is therefore unlikely that this alternative processing methodology would lead to substantially different results.

The use of logEMGAR by Maarsingh *et al* [194] to describe changes in EMG_{para} activity prevents direct comparison with the current study, although relative change can be calculated for comparative purposes. A fall in FEV₁ of 30% resulted in a mean logEMGAR of approximately 0.5, equivalent to an increase in EMG_{para} of greater than 200%. A fall in FEV₁ of 45% was associated with a logEMGAR value of 2, representing a 100-fold increase in EMG_{para} activity. While the percentage changes indicated by the logEMGAR values at the lower levels of bronchoconstriction were similar to the EMG_{para} changes observed in the current study, those occurring at greater levels of bronchoconstriction were markedly larger than the current results. In order to obtain changes of these magnitudes, the end of test EMG_{para} activity obtained by Maarsingh *et al* [194] must either have been substantially greater than the values reported in previous studies during maximal inspiratory efforts [172, 197], or their baseline values were markedly smaller than the level of ambient electrical interference commonly encountered in most environments, even when using very high quality biomedical amplifiers. In the absence of raw values for EMG_{para} from the Maarsingh *et al* study, it is difficult to fully compare their data to that in the current study, but 100-fold increases in EMG_{para} is substantially larger than that observed in the present study and other work from this laboratory [172, 197].

Another consideration when comparing the study by Maarsingh *et al* [194] and the current work is the method of analysing the stepwise changes in FEV₁ and EMG_{para}. Maarsingh *et al*'s study expressed the change in EMG_{para} per 5% decrement in FEV₁, presumably through linear interpolation of the dose-response curve. The response to direct airway challenge tests is

known to be sigmoidal and since the upper plateau is rarely reached, in practice, the curve appears exponential [226]. The appropriateness, therefore, of calculating arbitrary 5% steps is unclear. Subjects may demonstrate falls in FEV₁ of greater than 5% between consecutive doses of the provocant agent, meaning that one stage of the challenge tests would, using this methodology, contribute more than one data point to the analysis. This may result in inappropriate biasing of the results in favour of a relationship.

Maarsingh and colleagues [194] comment that the relationship between logEMGAR and FEV₁ was linear between 5% and 25% fall in FEV₁ from baseline. Inspection of their graphs indicates that the relationship appears less strong above this point. It is not clear, however, whether the reported correlation coefficients of 0.79 and 0.92 on test days one and two respectively represent the 5%-25% range of FEV₁ changes only, or whether this is inclusive of all values. It is therefore difficult to ascertain to what extent the results of the current study would be expected to agree with those of Maarsingh *et al* [194], given that fourteen of the 32 subjects in the current study demonstrated a decrease in FEV₁ in excess of 25%. Nonetheless, the findings in the current study of relationships between incremental changes in EMG_{para} and FEV₁ (using slope and area under the curve analyses) concur with the findings of Maarsingh *et al* [194] that changes in EMG_{para} and FEV₁ are related, despite the relationships appearing less strong.

4.5.3 Rationale for results

When the first methacholine dose was used as the reference stage a significantly larger percentage change in EMG_{para} than FEV₁ was observed. These data are consistent with the results from Chapter Three and the suggestion that EMG_{para}, rather than just reflecting change in airway calibre as measured by FEV₁, also represents any other changes in pulmonary

function occurring as a result of methacholine inhalation. Lung hyperinflation and small airway narrowing may occur during methacholine challenge, as well as large airways changes. The larger AUC for EMG_{para} compared to FEV₁ further supports this hypothesis, and unlike using only the percentage change from baseline to EOT, which assumes a linear relationship between lung function and methacholine dose, takes into account the changes that occur at each stage of the challenge test. This was further supported by the greater median slope of the regression line of EMG_{para} against dose compared to the FEV₁ against dose plot.

Small airways dysfunction can lead to distal airways collapse and hyperinflation. Hyperinflation is frequently reported to occur in patients undergoing methacholine challenge testing [262-265]. It has been suggested that methacholine induces more extensive small airways changes than histamine [266], which may explain in part the stronger relationships between FEV₁ and EMG_{para} observed in previous studies [190, 194]. The degree of hyperinflation during exercise has been shown to correlate with increases in EMG_{para} in adult patients with cystic fibrosis [172]. The presence of lung hyperinflation in some of the subjects in the current study may have resulted in additional increases in EMG_{para} that consequently served to weaken the relationship between EMG_{para} and FEV₁. The increase in respiratory load associated with lung hyperinflation may also explain the substantial increases in EMG_{para} (up to 196%) observed in some subjects from the non-responder group in whom only modest changes in FEV₁ occurred.

Increased inspiratory flow and tidal volume have been reported in humans, as well as increased phrenic nerve activity in dogs, following methacholine inhalation, even in the presence of only mild to moderate levels of bronchoconstriction [267, 268]. Increases in respiratory rate and duty cycle in the absence of changes in tidal volumes or inspiratory flow have also been reported following methacholine inhalation [269]. Interestingly, these studies have been unable to show any relationship between the ventilatory

responses observed and the degree of airways obstruction [270]. Significant heterogeneity of individual responses to methacholine inhalation was also noted. Given that the magnitude of EMG activity is related to both the force generated by the muscle and the rate of development of tension within the muscle [182], changes in respiratory pattern will influence EMG_{para} . The lack of agreement between the findings of previous studies and the presence of inter-individual differences within these studies supports the premise that different phenotypes may exist and hence simple and consistent relationships between bronchoconstriction and peak EMG_{para} per breath may not always be observed.

Similar to the results of previous studies examining changes in ventilatory parameters following methacholine challenge [267-270], substantial inter-individual variability was found in the relationship between changes in FEV_1 and EMG_{para} in the current study. Assessing individual subjects' patterns of FEV_1 and EMG_{para} changes allowed a more qualitative assessment of the data. These can be considered in the context of previous data regarding pathophysiological changes following airway challenge testing.

As already discussed, a previous study has demonstrated a linear relationship between $\log EMGAR$ of the parasternal intercostal muscles and FEV_1 change induced by histamine challenge [194]. The group of subjects in the current study in whom a clear relationship was observed between EMG_{para} and FEV_1 may represent a similar cohort to the study by Maarsingh *et al* [194] in which individuals responded to the inhaled provocation with relatively parallel changes in EMG_{para} and FEV_1 . The magnitude of the EMG_{para} change (from $0.0625 \text{ mg.ml}^{-1}$) in this group compared to the FEV_1 change (median (range) 67.9 (1.36 – 150.5)% *versus* -24.1 (-50.5 – -16.8)%) suggests that large airways changes are unlikely to account solely for the subjects' changes in NRD, but the close agreement between the two parameters would imply that any other changes undetected by spirometry are at least occurring simultaneously to those affecting FEV_1 values.

While FEV₁ primarily measures the function of more proximal airways, it has been shown previously that asthmatic individuals can demonstrate a positive response to methacholine inhalation primarily driven by increases in peripheral airways resistance, as opposed to the more widely-reported pattern of large airways changes in response to the provocative agent [271, 272]. The individuals in whom EMG_{para} appears to increase ahead of substantial changes in FEV₁ may represent a group in whom changes in smaller airways, undetected by spirometry, could have occurred prior to the development of overt larger airways obstruction sufficient to cause a reduction in FEV₁. Work by Amirav *et al* [273] in anaesthetised pigs exposed to methacholine challenge testing demonstrated heterogeneous changes in small and large airways calibre, assessed by high-resolution computed tomography (HRCT) scan. The changes in airway diameter were related to respiratory drive, as measured by airway opening pressure, at moderate and severe levels of airway narrowing, but not when the degree of narrowing was mild.

In the absence of more detailed physiological measurements, the mechanisms underlying the patterns of change in the nine subjects in whom no relationship existed between EMG_{para} and FEV₁ remain difficult to explain. While some of the subjects in this group clearly demonstrate initial falls in EMG_{para}, which may be related to anxiety and/or hyperventilation as described above, others show a highly variable pattern of EMG_{para} activity over the course of the challenge test. Perception of bronchoconstriction during methacholine challenge has been reported to be much more variable in healthy subjects than asthmatics [274], which may contribute to the wide range of changes in EMG_{para} observed in the current study (including within the non-responder group). Measures of NRD relate closely to subjects' perception of breathlessness [172, 198], thus changes in EMG_{para} may have been influenced by perception of the respiratory sensation associated with airway obstruction rather than solely due to airway obstruction alone. It is also important to consider that attempting to relate changes in unidimensional measures such as FEV₁ to EMG_{para}, a global measure of

respiratory load that reflects the combined effects of a broad range of pulmonary variables was likely to be unsuccessful.

Also challenging to explain is the one subject in whom a fall in FEV₁ occurred prior to an increase in EMG_{para}. This subject, however, highlights an important consideration given the population investigated in the current study and the magnitude of changes induced by the study protocol. This subject (Figure 27) demonstrated the highest EMG_{para} value at baseline, despite the FEV₁ being greater than 110% predicted. While the decrease in FEV₁ from baseline to the penultimate dose of methacholine was relatively large in absolute terms (660ml), the corresponding %predicted FEV₁ following the penultimate dose was 95.7%, suggesting that the respiratory system was not heavily loaded, despite a relatively high EMG_{para} at baseline. The extent of any other changes in pulmonary function other than those in large airways was, however, unknown. The relatively high respiratory drive suggested a degree of hyperventilation at baseline, and indeed this subject demonstrated a fall in heart rate from 66 to 58 beats per minute from saline to 0.0625mg.ml⁻¹ stages, supporting the premise that anxiety may have been at least a contributing factor in the high EMG_{para} at baseline. The substantial decrease in FEV₁ induced by the final methacholine dose (to 69.2% predicted) was accompanied by a clear increase in EMG_{para} activity.

Inferences drawn from this single subject are limited in applicability to the wider cohort, but these data highlight that the levels of respiratory load to which the subjects in the current study were exposed were relatively low. The median (range) end of test FEV₁ %predicted was only 70.8 (38.0 – 100.7)%, and the 25th percentile value of 64.1% predicted indicated that few subjects experienced severe bronchoconstriction. Studies in patients with CF and COPD [172, 189] have demonstrated significant relationships between pulmonary function and NRD using both EMG_{di} and EMG_{para}. The patients in these studies had a range of lung disease severities, unlike the current cohort of subjects who reported no respiratory symptoms at baseline. Elucidating relationships between variables across relatively low

levels of respiratory load makes detection of such relationships more challenging. High dose methacholine challenge testing to induce more substantial changes in respiratory load in healthy individuals or recruiting subjects with poorer baseline lung function may have helped to overcome this problem.

Reilly *et al* examined changes in NRD both during exercise in healthy subjects and adults with cystic fibrosis [172] and also in healthy subjects undergoing hypercapnic or negative pressure threshold loading [192]. The studies demonstrated different recruitment patterns for the diaphragm and parasternal intercostal muscles; EMG_{para} and EMG_{di} increased in parallel during inspiratory threshold loading but greater increases in EMG_{di} than EMG_{para} were observed during both hypercapnic ventilation and incremental exercise. A different pattern of costal diaphragm and parasternal intercostal muscle EMG activity was also described by Maarsingh *et al* [194] during histamine-induced airflow limitation in children. $\log EMGAR$ for diaphragm and parasternal increased linearly from baseline until a fall in FEV_1 of 25% was reached; thereafter, greater variability in the pattern of increase in EMG_{para} activity was seen, with the increase in EMG_{para} being greater than that in EMG_{di} . This is in direct contrast to the results of Reilly *et al* [172, 192]. Sprickelman *et al* [190] also reported variability in patterns of diaphragm and parasternal intercostal muscle activity during histamine challenge, even suggesting that the relative contributions of each muscle group could change between consecutive doses of the challenge agent. The different results in these studies indicate that relative contributions of the diaphragm and the parasternal intercostal muscles to breathing may vary under different circumstances of imposed load on the respiratory system. The current study did not measure EMG_{di} , but the available data [172, 190, 192, 216] indicate such measures are not interchangeable.

4.5.4 Relevance to clinical practice

International guidelines for methacholine challenge testing recognise that the reliance of the test on spirometry limits the application of this test to subjects able to perform spirometry to an acceptable standard, and identify the need for alternative test end-points less dependent on subject effort [226]. While the lack of consistent relationships between changes in EMG_{para} and FEV_1 observed in the current study indicate that EMG_{para} cannot be viewed as a substitute for FEV_1 in bronchial challenge testing, the significant increase observed in EMG_{para} in this cohort suggested that the technique may be able to detect the increase in respiratory load, indicated by the fall in FEV_1 , induced by methacholine inhalation. There was also an indication that, in some populations at least, the technique may offer additional information over and above that obtained by spirometry alone. The substantial increases in EMG_{para} seen in some subjects in the 'non-responder' subset suggested the presence of changes in the respiratory load-capacity balance in the absence of significant changes in FEV_1 , which may indicate the potential of EMG_{para} to enhance diagnostic possibilities, though a greater understanding of the driving mechanisms underlying the increase in EMG_{para} in this group is required.

4.6 Conclusion

The results contained in this chapter demonstrate that EMG_{para} can be used to detect an increase in respiratory load following chemically induced airways obstruction in adults. After controlling for the increased level of EMG_{para} activity present at baseline, a larger change was observed in EMG_{para} compared to FEV_1 , supporting the results from Chapter Three. Using the same baseline (0.0625mg.ml^{-1}), a moderate relationship was observed between total change in FEV_1 and EMG_{para} . The lack of a relationship between end of test changes in the two variables, however, highlights the

complexity of the physiological changes that occur during methacholine inhalation. The following chapter attempts to examine in more details some of the physiological changes that occur during bronchial hyperresponsiveness testing.

5. Chapter Five: A detailed laboratory study of the relationships between conventional measures of pulmonary function and EMG_{para} following methacholine challenge

5.1 Rationale

Chapter Four demonstrated the ability of EMG_{para} to detect change in respiratory load following methacholine inhalation, but the weak relationship observed between changes in FEV₁ and EMG_{para} suggested that factors additional to proximal airways obstruction might have influenced the changes in EMG_{para}. Changes in end-expiratory lung volume (EELV) have been demonstrated following methacholine inhalation in previous studies [245, 263-265, 267], and lung hyperinflation is known to relate to changes in NRD [172]. Changes in small airways resistance would not be detected by FEV₁ but would influence NRD and have been shown to occur in response to methacholine challenge test [259]. The extent of such changes, and their relationship with changes in EMG_{para}, may help to explain the greater change in EMG_{para} than FEV₁ observed in Chapter Four, and warranted investigation.

A range of changes in different ventilatory parameters has been reported as occurring in response to methacholine challenge testing [267-269, 275]. While the known relationship between rate of change in muscle length and EMG activity suggests that changes in inspiratory flow will affect EMG_{para} even in the absence of specific changes in the respiratory load-capacity balance, a previous study [276] has demonstrated that there is little

influence on the magnitude of respiratory EMG until inspiratory flow reaches levels substantially in excess of those observed during tidal breathing. Increases in tidal volume, however, will increase EMG_{para} amplitude, and therefore by expressing EMG_{para} per breath relative to the corresponding tidal volume generated (termed neuroventilatory efficiency (NVE), and expressed in $ml \cdot \mu V^{-1}$) the potential confounding effects of changes in tidal volume can be controlled for.

Despite strong agreement between the two measures, differing magnitudes of change in EMG_{para} and EMG_{di} have previously been shown in response to inspiratory threshold loading [192], hypercapnic loading [192] and exercise [172] in adult subjects, and during histamine challenge in asthmatic children [194]. The relationship between EMG_{para} and crural EMG_{di} has not previously been investigated in an asthmatic model of airways obstruction. Alterations in the relative recruitment of the diaphragm and parasternal intercostal muscles may also assist in explaining the relatively weak relationship between changes in FEV_1 and EMG_{para} observed in Chapter Four.

In view of the strong relationships previously observed between breathlessness scores and measures of NRD in patients with COPD and cystic fibrosis [172, 198], despite poor agreement between dyspnoea and conventional measures of pulmonary function [33, 277], it was felt that the relationship between breathlessness and EMG_{para} warranted examination during induced airflow obstruction.

The aims of this study were, therefore:

- To quantify any changes in end-expiratory lung volume utilising measurements of inspiratory capacity and investigate the relationship with EMG_{para}
- To investigate the utility of neuroventilatory efficiency as an alternative measure of NRD

- To study the relationship between EMG_{para} and EMG_{di} as measures of NRD during methacholine-induced airflow obstruction
- To assess the relationship between NRD and subject perception of breathlessness during induced airways obstruction
- To evaluate the response of the distal airways (using impulse oscillometry) to methacholine inhalation, and investigate the relationship between changes in these measures and those in EMG_{para} .

We hypothesised that:

- The magnitude of change in EMG_{para} would be greater in those subjects demonstrating hyperinflation than in those without changes in end expiratory lung volume
- Both bronchoconstriction and hyperinflation would be associated with a reduction in NVE_{para}
- EMG_{para} would be closely related to subject-reported breathlessness
- Levels of NRD as measured by EMG_{para} and EMG_{di} would be closely related
- Both small and large airways would exhibit changes in resistance in response to methacholine challenge.

5.2 Subjects

Subjects were recruited from staff and students of King's College Hospital and King's College London, having already undergone Study Two (Chapter Four) and demonstrated a positive methacholine challenge test. Ethical approval was granted by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from all participants.

5.3 Methods

5.3.1 Equipment

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and weight with an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK).

EMG_{para} and EMG_{di} were measured as described in Chapter Two (Methods). Briefly, EMG_{para} was recorded using surface electrodes placed over the second intercostal space and EMG_{di} was measured from the crural diaphragm using a multipair oesophageal EMG catheter. The signal was amplified (gain 1,000), band-pass filtered between 10 and 2,000Hz, and a 50Hz notch filter and a post-acquisition 20Hz digital high-pass filter applied. The signal was converted to root-mean-square (RMS) and mean peak RMS EMG_{para} per breath calculated. Respiratory flow was measured using a pneumotachograph and pressure transducer system as described in Chapter Two.

Signals were acquired at 10kHz for EMG and at 100Hz for respiratory flow using a Powerlab 16SP analogue-to-digital convertor (ADInstruments, Sydney, Australia), and displayed on a laptop computer (MacBook Pro, Apple, Cupertino, California, USA) running LabChart software (Version 7.2 ADInstruments Pty, Colorado Springs, Colorado, USA). Tidal volumes and inspiratory capacity were obtained by digital integration of the flow signal.

The methacholine challenge test was performed as described in Chapters Two and Four using a portable electronic spirometer-digidoser system (KoKo USB spirometer and digidoser, Ferraris Respiratory, Louisville, CO, USA) and nebuliser (DeVilbiss 646 characterised nebuliser, Ferraris Respiratory, Louisville, CO, USA). A five-breath dosimeter protocol was followed [226], with the duration of both inspiration and expiration fixed at five seconds and inspiratory flow limited to 0.5L.sec⁻¹. A 0.9% saline diluent step was followed by methacholine concentrations of 0.0625, 0.25, 1, 4, 8, 16

and 32mg.ml⁻¹ (Acetyl-B-Methyl Choline, Nova Laboratories, Wigston, Leicestershire).

Impulse oscillometry was measured using the Jaeger Masterscreen PFT system, as described in Chapter Two. Subjects breathed steadily at the FRC level via a flanged mouthpiece for 90 seconds.

5.3.2 Protocol

All subjects underwent measurement of EMG_{para}, spirometry, inspiratory capacity, tidal flow and volume, and breathlessness using the modified Borg (mBorg) scale. Two subsets of participants underwent additional measurements: subjects for Part A undertook measurements of EMG_{di}, while subjects for Part B undertook impulse oscillometry.

All participants: Baseline measurement of EMG_{para} activity during 3 minutes of resting tidal breathing was recorded, with flow measured using a pneumotachograph attached to a flanged mouthpiece. Baseline inspiratory capacity (IC) and spirometry manoeuvres were then performed.

The methacholine challenge was then performed. The subjects inhaled saline, followed by incremental doses of methacholine (0.0625, 0.25, 1, 4, 8, 16 and 32mg.ml⁻¹), as described in Chapters Two and Four. A single technically acceptable forced expiratory manoeuvre, allowing measurement of both FEV₁ and FVC, was performed at both 30 and 90 seconds following each delivery, with the lowest value being recorded. Three technically acceptable IC manoeuvres were then performed, with the mean of these values being used for analysis. Following this, the subject was instructed to breathe in a relaxed manner for the remaining time prior to the next dose (approximately 2.5 minutes), and EMG_{para} was recorded simultaneously with tidal flow and volume. The final minute of this recording was used for analysis. Immediately prior to the next dose of methacholine, the subject

was asked to rate their breathlessness using the modified Borg scale (mBorg). The protocol was ceased when the subject demonstrated a 20% or greater fall in FEV₁, at which point 400µg salbutamol was administered via a metered-dose inhaler and spacer. Spirometry was repeated after 15 minutes to ensure return to pre-test values.

Changes in FEV₁ from the post-diluent stage were plotted against the log-transformed methacholine concentration. The provocative concentration of methacholine that induced a 20% fall in FEV₁ (PC20) was calculated using the following equation [226]:

$$PC20 = antilog \left[\log C_1 + \frac{(\log C_2 - \log C_1)(20 - R_1)}{R_2 - R_1} \right]$$

Where:

C₁ = penultimate methacholine concentration

C₂ = final methacholine concentration

R₁ = percent fall in FEV₁ after C₁

R₂ = percent fall in FEV₁ after C₂

Percentage changes in EMG_{para} and IC were also plotted against log methacholine concentration. Linear interpolation of the EMG_{para}-log methacholine dose curve was used to calculate the percentage change in EMG_{para} and IC at PC20.

Sub-study A: This study allowed examination of the relationship between EMG_{para} and EMG_{di} during induced bronchoconstriction, including any differences in relative recruitment patterns. It also allowed investigation of the relationship between EMG_{di} and conventional measures of pulmonary function (FEV₁ and IC) during a methacholine challenge test. Prior to commencement of the protocol, an oesophageal multipair electrode to measure EMG_{di} was inserted and positioned using the procedure described

in Chapter Three. There were no differences in study design to that described above; EMG_{di} was recorded simultaneously to EMG_{para} at all points.

Sub-study B: Impulse oscillometry was performed to examine changes in resistance of the distal airways during methacholine-induced bronchoconstriction. EMG_{para} and IOS were recorded simultaneously at baseline, followed by measurements of IC and spirometry. The protocol for the methacholine challenge was followed. The subject performed a forced expiratory manoeuvre at 30 and 90 seconds following the methacholine dose, then three IC manoeuvres. IOS measurements were then performed for 90 seconds, during which EMG_{para} was simultaneously recorded. Tidal volume measurements were extracted from the IOS software and used to calculate NVE_{para}. The subjects were asked to rate their breathlessness using the mBorg scale following the IOS/EMG_{para} measurement.

5.3.1 Additional analyses

Area under the dose-response curve and the slopes of the dose-response regression lines were calculated (as described previously (Chapter Four)) to assess incremental changes in measured variables at each stage of the methacholine challenge test. As previously, methacholine doses were numbered to give a linear scale along the x-axis, and plotted against percentage change in each variable.

5.3.2 Statistical analysis

Non-parametric statistics were applied as the sample size was <30. Within-subject changes were assessed using the Wilcoxon matched pairs test. Differences between group medians were assessed with the Mann-Whitney test. Relationships between variables were assessed using the Spearman's

rank correlation. A p value <0.05 was taken as statistically significant. When comparing the magnitude of changes in variables with opposite polarities (*e.g.* increases in EMG_{para} against decreases in FEV₁ or IC), variables expected to decrease were multiplied by minus one in order to give a positive value.

5.3.2.1 Sample size calculation

No formal sample size calculation was undertaken for this study; the subjects represented a convenience sample taken from those who demonstrated a positive response to methacholine challenge testing having undergone the study presented in Chapter Four.

5.4 Results

In total 20 subjects undertook this study. Ten subjects underwent the additional measurements of Part A and six subjects underwent Part B; the demographic and anthropometric characteristics of the twenty subjects are shown in Table 9. There was no overlap between the subjects for Parts A and B of the study. Subjects' diagnoses are shown in Table 10.

Changes in EMG_{para}, FEV₁, IC, NVE_{para} and mBorg from baseline to end of test in all 20 subjects are summarised in Table 11. IC is expressed as both raw values and %predicted due to the two thresholds that were investigated for determining a significant increase in resting end expiratory lung volume. All variables demonstrated statistically significant changes from baseline to end of test.

	Entire cohort (n=20)	Part A (n=10)	Part B (n=6)
Age (years)	29.00 (22.01 – 50.78)	29.23 (24.13 – 50.78)	31.57 (22.01 – 42.83)
Sex (male: female)	6: 14	4: 6	1: 5
Height (m)	1.71 (1.61 – 1.90)	1.71 (1.61 – 1.90)	1.70 (1.61 – 1.84)
Weight (kg)	68.36 (56.70 – 91.81)	71.85 (56.70 – 91.81)	64.37 (62.87 – 84.82)
BMI (kg m⁻²)	24.1 (20.2 – 32.1)	24.9 (20.2 – 32.1)	23.0 (21.3 – 26.5)
Ethnicity (n (%))	White: 19 (95%) Black: 1 (5%)	White: 10 (100%)	White: 5 (83.3%) Black: 1 (16.7%)
Baseline FEV₁ (%predicted)	96.9 (80.0 – 122.3)	97.9 (80.0 – 118.2)	93.2 (80.3 – 98.9)

Table 9 Demographic and anthropometric characteristics of 20 adults undergoing methacholine challenge. All data are shown as median (range).

Part A (n=14)	Asthma (taking medication)	3 (21%)
	Self-reported asthma (no medication)	4 (29%)
	Self-reported atopy/allergies	7 (50%)
Part B (n=6)	Asthma (taking medication)	1 (17%)
	Self-reported asthma (no medication)	2 (33%)
	Self-reported atopy/allergies	3 (50%)

Table 10 Diagnoses of subjects included in Parts A and B of Study Three.

Data are shown as n (%).

	Baseline	End of test	p value	Percentage change
EMG_{para} (μV)	5.37 (2.85 – 8.92)	6.63 (3.77 – 19.60)	0.0012	26.2 (-21.1 – 144.4)
FEV₁ (%predicted)	96.86 (80.00 – 122.30)	67.50 (37.98 – 92.27)	<0.0001	-28.9 (-53.7 – -21.1)
IC (L)	2.99 (2.14 – 4.49)	2.78 (1.36 – 4.18)	0.037	-7.1 (-44.7 – 8.7)
IC (%predicted)	91.9 (65.6 – 133.3)	84.9 (39.8 – 129.0)	0.0037	-7.1 (-44.7 – 8.7)
NVE_{para} (ml.μV⁻¹)	170.2 (84.5 – 403.8)	127.5 (60.9 – 246.6)	0.0064	-18.2 (-69.1 – 66.2)
mBorg	0.0 (0.0 – 1.0)	2.0 (0.0 – 4.0)	<0.0001	N/A

Table 11 Changes in EMG_{para}, FEV₁, IC, NVE_{para} and mBorg in 20 adults undergoing methacholine challenge. All data are shown as median (range).

No relationships were observed between changes in EMG_{para} or NVE_{para} and FEV_1 or IC. The changes in FEV_1 and IC were also unrelated ($r=0.323$, $p=0.614$).

Similarly to the results in Chapter Four, a small decrease was observed in median (range) EMG_{para} between saline inhalation (5.37 (2.85 – 8.92) μV) and the first methacholine dose (4.59 (2.18 – 7.91) μV), although this did not reach statistical significance ($p=0.096$). Changes in variables between the 0.0625mg.ml⁻¹ dose and end of test were therefore assessed (Table 12). All variables continued to demonstrate statistically significant changes. However, despite adjusting for the saline inhalation dose, no relationships were observed between the changes in EMG_{para} or NVE_{para} and FEV_1 or IC from 0.0625mg.ml⁻¹ to end of test.

	0.0625mg.ml⁻¹	End of test	p value	Percentage change
EMG_{para} (μV)	4.59 (2.18 – 7.91)	6.63 (3.77 – 19.60)	<0.0001	44.3 (1.36 – 214.9)
FEV₁ (%predicted)	94.7 (76.7 – 119.1)	67.50 (37.98 – 92.27)	<0.0001	-27.7 (-50.5 – -16.8)
IC (%predicted)	89.5 (65.3 – 141.1)	84.9 (39.8 – 129.0)	0.0037	-7.67 (-45.6 – 16.2)
NVE_{para} (ml.μV⁻¹)	170.0 (97.5 – 366.7)	127.5 (60.9 – 246.6)	0.0006	-27.5 (-67.8 – 46.5)
mBorg	0.0 (0.0 – 2.0)	2.0 (0.0 – 4.0)	<0.0001	N/A

Table 12 Changes in EMG_{para}, FEV₁, IC, NVE_{para} and mBorg in 20 adults undergoing methacholine challenge between first methacholine dose and end of test. All data are shown as median (range).

5.4.1 Hyperinflation

Two thresholds for determining significant lung hyperinflation were examined: a decrease in inspiratory capacity of $\geq 300\text{mL}$ or a decrease of 15% of predicted IC [245]. Using the 300ml threshold, 10 of the 20 subjects (50%) were classified as 'hyperinflators'. Using the threshold of a decrease of 15% predicted IC, five subjects (25%) were defined as hyperinflators. The relative changes in FEV_1 , EMG_{para} and NVE_{para} in the hyperinflator and non-hyperinflator groups using the two thresholds are shown in Table 13. No differences in FEV_1 , EMG_{para} or NVE_{para} were observed between hyperinflators and non-hyperinflators using either cut-off.

		Hyperinflators	Non-hyperinflators	p value
300ml threshold	ΔFEV_1 (%)	-32.5 (-53.7 – -21.1)	-23.3 (-48.5 – -21.2)	0.22
	$\Delta\text{EMG}_{\text{para}}$ (%)	25.3 (-6.86 – 144.4)	40.1 (-21.1 – 119.8)	0.78
	$\Delta\text{NVE}_{\text{para}}$ (%)	-25.3 (-69.1 – 66.2)	-16.3 (-53.2 – 46.2)	0.47
15% predicted threshold	ΔFEV_1 (%)	31.4 (-53.7 – -21.1)	-26.3 (-48.5 – -22.0)	0.573
	$\Delta\text{EMG}_{\text{para}}$ (%)	31.6 (-4.7 – 101.9)	20.9 (-21.1 – 144.4)	0.997
	$\Delta\text{NVE}_{\text{para}}$ (%)	-32.8 (-54.3 – 66.2)	-16.9 (-69.1 – 46.2)	0.691

Table 13 Changes in FEV₁, EMG_{para} and NVE_{para} in the hyperinflator and non-hyperinflator subgroups using two cutoff thresholds. All data are shown as median (range).

No significant relationships were seen between baseline to end of test changes in EMG_{para} or NVE_{para} and those in FEV_1 or IC in the hyperinflator subset. Changes in FEV_1 and IC were also unrelated ($r=-0.248$, $p=0.492$, Figure 28).

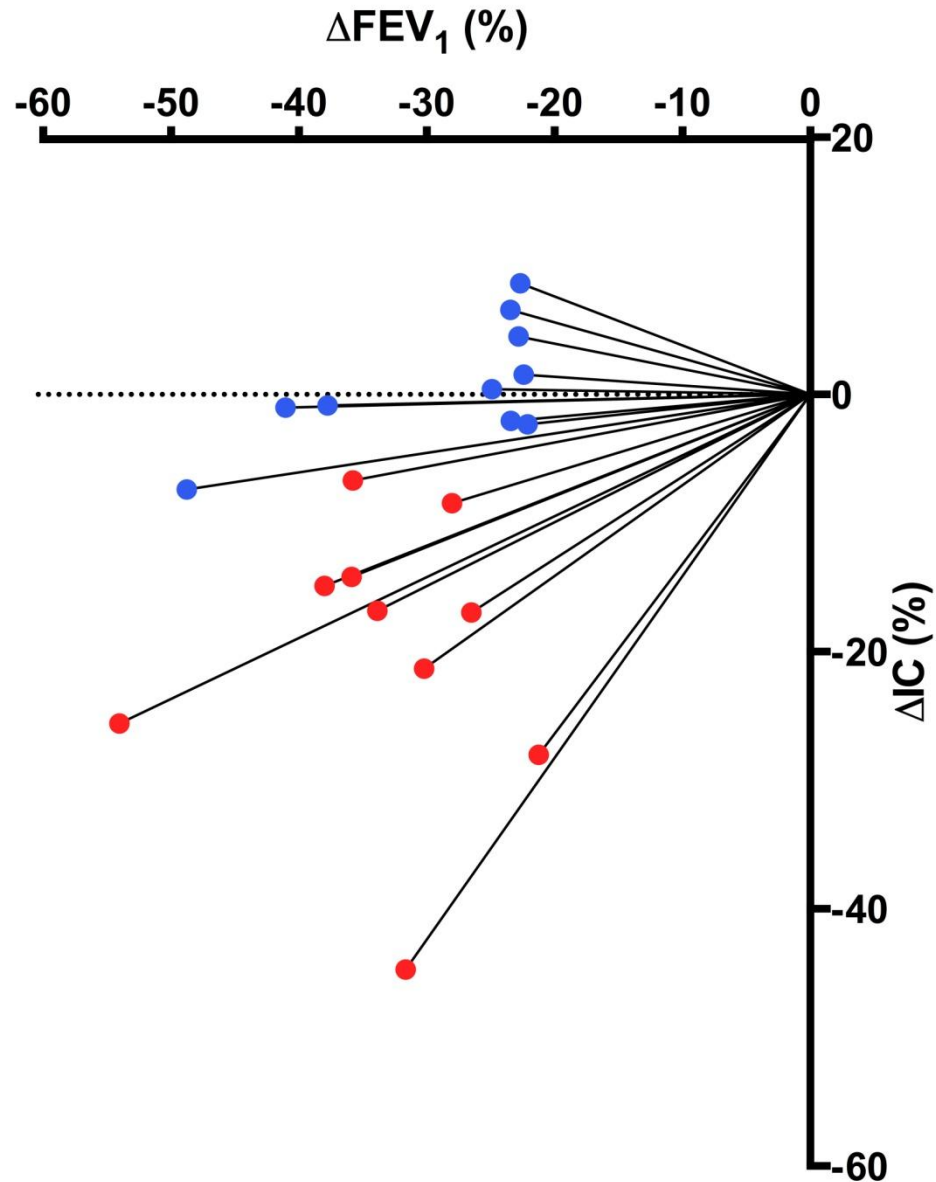


Figure 28 Graph showing baseline to end of test changes in FEV_1 and IC. Red symbols denote subjects demonstrating significant hyperinflation (change in IC of ≥ 300 ml); blue symbols represent those without significant changes in IC.

Percentage changes in EMG_{para} and IC at the concentration of methacholine inducing a 20% decrease in FEV_1 (PC20) were calculated through linear interpolation of the log dose-response curve for both parameters. There was no relationship between change in EMG_{para} and change in IC at PC20 in either the whole cohort ($r=0.092$, $p=0.701$) or the hyperinflator subset, using either the 300ml ($r=0.115$, $p=0.759$) or 15% predicted ($r=-0.10$, $p=0.95$) threshold values for significant hyperinflation.

5.4.1.1 Area under the curve and linear regression analysis

As described above, area under the curve analysis was performed to assess the incremental changes in measured variables. A significant relationship was observed between AUC EMG_{para} and AUC FEV_1 when using saline ($r=0.546$, $p=0.013$) or the $0.0625\text{mg}\cdot\text{ml}^{-1}$ methacholine dose ($r=0.577$, $p=0.008$) as the baseline value. A significant relationship between AUC NVE_{para} and AUC FEV_1 was also observed ($r=0.592$, $p=0.006$) when using the $0.0625\text{mg}\cdot\text{ml}^{-1}$ methacholine dose as the baseline. No relationships were observed between AUC IC and AUC EMG_{para} or AUC NVE_{para} , either in the cohort as a whole or the hyperinflator subset (using either threshold value for hyperinflation).

The median (range) slopes of the EMG_{para} -dose and FEV_1 -dose lines were 9.18 (-0.86 – 35.70) and 4.97 (2.73 – 17.18) respectively. The difference between slopes did not reach statistical significance ($p=0.083$), however, a significant relationship was observed between the slopes ($r=0.462$, $p=0.0405$). No relationship was seen between EMG_{para} -dose slope and IC-dose slope, either in the cohort as a whole or in the hyperinflator subgroup (using either the 300ml or 15% predicted thresholds). NVE_{para} -dose slope also did not demonstrate a significant relationship with either IC-dose or FEV_1 -dose slope.

5.4.2 Breathlessness

mBorg score increased significantly from baseline to end of test (Table 11), though the magnitude of the median (range) increase (2.0 (0.0 – 4.0)) was relatively small. There was no relationship between change in mBorg and EMG_{para} or NVE_{para} from baseline to end of test, either when using saline or the 0.0625mg.ml^{-1} dose as the reference stage. A weak relationship was observed between change in mBorg and that in IC ($r=-0.488$, $p=0.034$). There was no relationship between change in mBorg and change in FEV_1 .

Correlation coefficients for the relationships between EMG_{para} and mBorg and between NVE_{para} and mBorg were calculated for each subject. The median (range) correlation coefficient for individual subjects' relationships between EMG_{para} and mBorg was 0.70 (0.00 – 1.00). The median (range) correlation coefficient for individual subjects' relationships between NVE_{para} and mBorg was -0.70 (-0.926 – 0.949).

No significant relationships were observed between changes in mBorg and those in EMG_{di} or NVE_{di} from baseline to end of test. The median (range) correlation coefficients for individual relationships between EMG_{di} and mBorg and NVE_{di} and mBorg were 0.625 (-1.00 – 1.00) and -0.618 (-0.890 – 0.800) respectively.

5.4.3 Part A: Diaphragm EMG

In the ten subjects undergoing additional measurement of EMG_{di} , significant changes were observed in all parameters except NVE_{di} (Table 14).

EMG_{para} was related to EMG_{di} both at baseline ($r=0.649$, $p=0.049$) and end of test ($r=0.770$, $p=0.013$). The percentage changes in EMG_{di} and EMG_{para} were, however, not related ($r=0.188$, $p=0.607$). There was no significant difference between the median (range) percentage changes observed in EMG_{di} and EMG_{para} ($p=0.322$).

To assess any relative changes in muscle recruitment and neural respiratory drive to each muscle, the ratio of EMG_{di} to EMG_{para} activity was calculated. No significant difference between median (range) EMG_{di} to EMG_{para} activity ratio was observed between baseline (3.44 (1.77 – 7.84)) and end of test (3.75 (1.99 – 5.97), $p=0.432$).

Due to the varying number of data points between subjects, individual correlation coefficients for the relationship between EMG_{para} and EMG_{di} were calculated for each subject and the median (range) correlation coefficient determined (0.30 (-0.70 – 1.00)). No individual subject's relationship reached statistical significance.

The percentage changes in EMG_{di} did not correlate with the changes in FEV_1 ($r=-0.042$, $p=0.918$) or mBorg ($r=-0.356$, $p=0.269$). There was no significant relationship between percentage changes in EMG_{di} and IC in the group of ten subjects ($r=0.564$, $p=0.096$) or those in this subset demonstrating hyperinflation ($n=7$, $r=-0.143$, $p=0.783$). Due to the small numbers, comparison between the hyperinflator and non-hyperinflator subgroups was not performed.

When using the 0.0625mg.ml^{-1} dose as the reference stage, median (range) raw EMG_{di} was 18.19 ($8.40 - 30.91$) μV and increased significantly ($p=0.0059$) by 93.27 ($-33.03 - 437.10$)% to the end of test.

As seen with EMG_{para} , percentage changes in EMG_{di} from 0.0625mg.ml^{-1} to end of test were unrelated to changes in FEV_1 ($r=-0.164$, $p=0.657$), IC ($r=0.261$, $p=0.47$) or mBorg ($r=-0.194$, $p=0.518$).

	Baseline	End of test	p value	Percentage change
EMG_{para} (μV)	5.37 (2.85 – 8.91)	7.29 (3.77 – 19.60)	0.027	93.11 (-6.86 – 144.40)
EMG_{di} (μV)	19.42 (7.31 – 42.32)	33.77 (13.51 – 51.56)	0.027	61.73 (-41.85 – 517.20)
FEV₁ (%predicted)	97.86 (80.00 – 118.20)	68.18 (37.98 – 92.27)	0.002	-32.76 (-53.67 – -21.08)
IC (%predicted)	112.10 (75.11 – 133.30)	88.47 (59.10 – 121.60)	0.0098	-11.32 (-28.01 – 6.59)
NVE_{para} (ml.μV⁻¹)	208.00 (107.70 – 403.8)	130.7 (64.74 – 246.6)	0.027	-33.26 (-69.10 – 66.21)
NVE_{di} (ml.μV⁻¹)	48.38 (24.69 – 145.70)	32.94 (23.83 – 65.05)	0.0645	-38.11 (-79.04 – 103.80)
mBorg	0.0 (0.0 – 1.0)	3.0 (0.5 – 4.0)	0.002	N/A

Table 14 Changes in EMG_{para}, EMG_{di}, FEV₁, IC, NVE_{para}, NVE_{di} and mBorg from baseline to end of test in ten subjects undergoing Part A of the study. Data are expressed as median (range).

5.4.4 Part B: Impulse Oscillometry

IOS was measured to evaluate changes in small airways resistance occurring in response to methacholine inhalation. Significant changes were observed in EMG_{para} and FEV_1 in the six subjects who undertook the study (Table 15) between baseline and end of test. The fall in NVE_{para} did not reach statistical significance. The decrease in IC was not significant. Only two subjects demonstrated hyperinflation using the 300ml threshold, and only one if the value of 15% predicted was used. Differences in IOS parameters between hyperinflators and non-hyperinflators were not explored due to the small numbers.

Significant changes were seen in measures of small airway function (R5Hz, Diff R5-R20, and AX) using impulse oscillometry. The change in R20Hz did not reach significance (Table 15). The percentage increase in R5Hz was significantly greater than that in R20Hz ($p=0.0313$). No relationships were observed between percentage changes in EMG_{para} or NVE_{para} and any IOS parameters.

No relationships were seen in the dose-response slopes or area under the dose-response curves between EMG_{para} or NVE_{para} and the IOS parameters using either saline or 0.0625mg.ml^{-1} doses as reference points.

	Baseline	End of test	p value	Percentage change
EMG_{para} (μV)	5.58 (2.97 – 7.38)	5.95 (5.56 – 8.79)	0.0313	14.44 (1.41 – 103.9)
FEV₁ (%predicted)	93.20 (80.32 – 98.87)	66.57 (43.32 – 75.57)	0.0313	-24.79 (-48.45 – -22.28)
IC (%predicted)	81.36 (72.07 – 97.32)	75.07 (39.84 – 98.70)	0.2188	-4.72 (-44.72 – 4.53)
NVE_{para} (ml.μV⁻¹)	142.3 (86.68 – 397.0)	119.7 (60.90 – 211.9)	0.0625	-16.23 (-53.23 – 5.75)
R5Hz (kPa(L.s⁻¹)⁻¹)	0.40 (0.26 – 0.48)	0.61 (0.37 – 0.70)	0.0313	44.23 (6.67 – 75.68)
R20Hz (kPa(L.s⁻¹)⁻¹)	0.29 (0.16 – 0.39)	0.35 (0.20 – 0.41)	0.0625	21.76 (0.00 – 46.43)
Diff R5-R20 (kPa(L.s⁻¹)⁻¹)	0.10 (0.07 – 0.19)	0.26 (0.08 – 0.38)	0.0313	118.30 (14.29 – 200.00)
AX (kPa.L⁻¹)	0.44 (0.12 – 1.17)	3.28 (0.39 – 4.30)	0.0313	473.00 (105.30 – 866.70)

Table 15 Changes in EMG_{para}, FEV₁, IC, NVE and IOS parameters from baseline to end of test in six subjects undergoing Part B of the study. All data are presented as median (range).

5.5 Discussion

This study has highlighted the complex pathophysiological processes that occur during methacholine-induced bronchoconstriction, as well as the complexity in the relationships between measures of neural respiratory drive and conventional measures of pulmonary function. The study has demonstrated that significant hyperinflation did occur in a proportion of subjects following methacholine challenge test, but this did not have a simple additive effect on NRD over and above the effect of bronchoconstriction. Significant increases in small airways resistance were demonstrated. Agreement between NRD measured using EMG_{di} and EMG_{para} was demonstrated, although no relationship demonstrated between the relative magnitudes of increase in the activity of each muscle group. No relationships were observed between EMG_{para} or EMG_{di} and breathlessness as quantified by the mBorg score.

5.5.1 Critique of the method

The current study adopted the protocol for standard methacholine challenge testing as used clinically but included additional outcome measures to quantify and attempt to understand any other pathophysiological changes that may have occurred with induced bronchoconstriction. There are some potential weaknesses in the chosen study design.

Other studies that have used methacholine challenge testing to induce bronchoconstriction in healthy subjects have used high-dose protocols (maximum methacholine dose of 256mg.ml⁻¹) [278, 279], terminating at greater degrees of bronchoconstriction (decrease in FEV₁ of 50%) [245, 280, 281]. Such study designs result in a greater number of data points, facilitating the analysis of within-subject relationships between variables. High concentrations of methacholine were not available for use in the

current study and international guidelines were used for methacholine challenge testing, although these guidelines are designed to inform clinical, rather than research practice. The current study included some subjects with a diagnosis of asthma, who responded at relatively low concentrations of methacholine. The protocol involved the administration of quadrupling doses of methacholine at the lower concentrations (0.0625mg.ml^{-1} , 0.25mg.ml^{-1} , 1mg.ml^{-1} , 4mg.ml^{-1}), with doubling doses used at higher concentrations (8mg.ml^{-1} , 16mg.ml^{-1} , 32mg.ml^{-1}) for safety reasons. Had doubling doses been used at the lower concentrations, a greater number of data points could have been obtained, enhancing the statistical power of the within-subject analyses.

The outcome measure selected in the current study for quantification of breathlessness may have been insufficiently sensitive, partly explaining the lack of relationship between changes in mBorg score and EMG_{para} . The changes in mBorg in this study (median (range) change 2.0 (0.0 – 4.0)) are relatively small, and the ordinal nature of the scale may reduce sensitivity. The use of a visual analogue score may have allowed small changes in breathlessness to be detected, as such scales are used over a wider range than the mBorg, with higher end of test values reported [282, 283]. It is also unclear whether “breathlessness” is truly the predominant symptom experienced by subjects during methacholine challenge test. Symptom-specific scores such as those measuring “chest tightness” demonstrate greater changes during methacholine challenge testing than breathlessness scores alone [265]. The use of visual analogue scales to quantify “breathing discomfort” is also reported in the literature and has been shown to respond sensitively to methacholine-induced bronchoconstriction [275].

Using inspiratory capacity (IC) as a measure of end expiratory lung volume may also have limitations. IC has been shown to demonstrate acceptable reproducibility, with intra-class repeatability coefficients of greater than 0.7 reported [284, 285]. Similar relationships between IC and RV/TLC ratio and both EMG_{para} and EMG_{di} have also been shown [172], suggesting IC to be an

acceptable measure for use in studies of this nature. Nonetheless, it depends on the subject's ability to breathe in a relaxed manner at functional residual capacity (FRC), as well as their motivation to inspire fully to TLC [286]. Appropriate verbal prompting and encouragement was given to ensure both of these criteria were met, though greater accuracy may have been achieved using plethysmographic measurements of lung volumes. As discussed previously in Chapter Four, body plethysmography would also have allowed the measurement of airways resistance. Specialist equipment is also required, however, to conduct methacholine challenge testing within the plethysmograph while maintaining the recommended intervals between inhalations [226].

The IC manoeuvres that were performed to examine changes in end-expiratory lung volume (EELV) may also have had a direct effect on bronchomotor tone. Subjects performed a minimum of five maximal inspirations that may have resulted in alterations in airway calibre prior to measurement of resting EMG_{para} . The magnitude of this effect would potentially be greater in healthy than asthmatic subjects, with lesser degrees of deep breath-related bronchodilation and greater, more rapid airway re-narrowing occurring in asthmatic subjects [250-255]. The heterogeneity of the deep breath responses may have acted as a confounding factor when attempting to assess relationships between variables. Although the inclusion of subjects with both asymptomatic airway hyperresponsiveness or a formal diagnosis of asthma enhanced recruitment for the current study, narrower inclusion criteria for future studies may help reduce this potential source of variability.

Although recommended [207], performing three IOS measurements and reporting the mean value would have prevented adherence with guidelines regarding the timeframe between consecutive doses of methacholine [226]. Several baseline measurements were performed to ensure that consistent values were obtained prior to the methacholine challenge. In addition, all subjects undergoing Part B of this study were experienced in pulmonary

function testing and recognised the importance of steady tidal breathing during IOS. It was unlikely, therefore, that any variation in IOS values was due to poor technique, but rather represented true changes in airway calibre. The subset of participants undergoing IOS was, however, small, with limited power to discern relationships between variables.

5.5.2 Relationship of the current findings to previous literature

The results of the current study indicate the heterogeneous nature of pathophysiological changes that occur in obstructive airways disease. The lack of relationship between percentage changes from baseline to end of test in FEV₁ and IC indicates that hyperinflation can occur independently of the degree of large airways obstruction. This is in agreement with previous studies that have demonstrated a lack of relationship between severity of bronchoconstriction and extent of change in end expiratory lung volumes [263, 287]. It was not possible therefore to determine the relative influence of changes in lung volume and/or airway calibre on EMG_{para} activity.

Lavorini *et al* [275] examined ventilatory responses to methacholine challenge and described consistent increases in EMG_{para} in all subjects, regardless of whether minute ventilation increased or not. Similarly, changes in NVE_{para} were observed in the current study, in that NVE_{para} was reduced in some subjects whereas in other subjects it was maintained. Although Lavorini *et al* [275] stated that EMG_{para} activity was not observed at baseline, differences in EMG_{para} signal acquisition methodology prevent further direct comparison between the magnitudes of EMG_{para} change between studies.

Previous data have demonstrated strong agreement between the activity of the diaphragm and parasternal intercostal muscles [77, 79, 82, 172, 192]. The agreement between EMG_{para} and EMG_{di} activity in the current study was

poor when examined using individual subject correlation coefficients (median (range) r value 0.30 (-0.70 – 1.00)). It is possible, however, that these correlation coefficients were of little value in evaluating the relationship between diaphragm and parasternal intercostal activity as the number of data points available to perform correlation analysis was low for most subjects and individual relationships did not reach statistical significance (median (range) p value 0.653 (0.058 – 1.00)). Reilly *et al* [172] evaluated change in EMG_{para} and EMG_{di} activity measured continuously during incremental exercise to exhaustion, by dividing total exercise time into deciles. Similarly, the same author investigated the effects of inspiratory threshold loading and hypercapnia on EMG_{di} and EMG_{para} [192] and exposed all subjects to the same number of incremental loads. Both study designs allowed all subjects' data to be included in the overall analysis. The nature of the current study does not, however, allow this, resulting in variable numbers of data points per subject at discrete time points. Safety considerations precluded exposing all subjects to the same number of methacholine doses, as this would result in subjects who responded at lower doses being exposed to further, potentially dangerous, bronchoconstriction. Good agreement was, however, observed between EMG_{di} and EMG_{para} measured both at baseline and end of test.

Previous data have also demonstrated close agreement between changes in dyspnoea and changes in NRD (quantified by $EMG_{para}\%max$ or $EMG_{di}\%max$) [172, 198]. The current study demonstrated a wide range of within-subject relationships between mBorg and EMG_{para} (median (range) correlation coefficient 0.70 (0.00 – 1.00)), and a similar median but wider range of intra-subject agreement between mBorg and EMG_{di} (0.625 (-1.00 – 1.00)). As above, these results should also be interpreted with caution due to the small number of data points available for each subject. The lack of significant relationships between baseline to end of test changes in mBorg and EMG_{para} or EMG_{di} was surprising in view of previous findings of strong relationships between these variables [172, 196], although the magnitude of

changes in mBorg and the ordinal nature of the scale may not provide sufficient sensitivity to detect such a relationship (if one exists).

5.5.3 Rationale for results

We hypothesised that subjects demonstrating hyperinflation in response to MCT would show greater changes in EMG_{para} than those without changes in end expiratory lung volume. This did not occur and no significant differences in EMG_{para} or NVE_{para} were observed between the hyperinflator and non-hyperinflator groups. The heterogeneity of changes in FEV_1 and IC across subjects prevented detection of any specific influence of hyperinflation on levels of NRD over and above that caused by bronchoconstriction. Changes in airway calibre and end expiratory lung volume occurred to different extents within individual subjects as demonstrated by the lack of relationship between baseline to end of test percentage changes in FEV_1 and IC ($r=0.248$, $p=0.492$). The presence of lung hyperinflation does not appear to represent a phenotype in whom more severe responses to methacholine occur, as the range of percentage changes in FEV_1 was similar between the hyperinflator and non-hyperinflator subgroups.

Changes in EMG_{para} and IC at the concentration of methacholine inducing a 20% decrease in FEV_1 (PC20) were calculated as a means of examining the specific influence of hyperinflation. Using this method to standardise the contribution of bronchoconstriction to EMG_{para} did not, however, identify any relationship between changes in IC and EMG_{para} . The median change in IC at PC20 was small (-2.96%) and may therefore have been unlikely to exert a significant influence on EMG_{para} . Previous data from Reilly *et al* [172] demonstrated a relationship between baseline IC and both resting EMG_{para} and resting EMG_{di} in adults with cystic fibrosis; the strength of the relationship was similar to that with FEV_1 . Hyperinflation and gas trapping

are known to progress with worsening CF lung disease [26, 288, 289], as does FEV₁ [290]; these may both represent severity of CF lung disease. The simultaneous nature of these changes, however, indicated that IC could not definitively be said to exert an independent influence on NRD in the study by Reilly *et al.* It is also notable that the degree of hyperinflation observed in the patients with cystic fibrosis (mean (SD) IC 1.9 (0.5)L) was substantially greater than that in the current study (median (range) end of test IC 2.78 (1.36 – 4.18)L). Data regarding the isolated effect of hyperinflation on respiratory muscle activity in humans are not currently available.

Studies in dogs have suggested that the length-tension relationship of the parasternal intercostal muscles is improved at higher lung volumes, and that they are able to generate greater force per unit change in length at TLC than at FRC [202]. The reduced compliance of the ribcage at higher lung volumes, however, means that translation of electrical activity into rib motion and lung volume change becomes less efficient [291, 292], potentially offsetting these improvements in length-tension relationships. The ability of the diaphragm to generate force is significantly impaired at higher lung volumes as a result of substantial flattening of the domes of the hemidiaphragms and muscle shortening [293], requiring greater neural drive to effect the same change in lung volume. The relative change in length of the parasternal intercostal muscles at higher lung volumes is less than that of the diaphragm [73], suggesting that increases in EMG_{para} may be expected to be less pronounced than those in EMG_{di} in response to hyperinflation. In the current study, no significant difference was found in the magnitude of increase in EMG_{di} and EMG_{para}, neither was there any significant change in the ratio of EMG_{di} to EMG_{para}. The magnitude of hyperinflation (median decrease in IC in ten subjects: 11.32%) may have been insufficient to cause the diaphragm to become sufficiently shortened and any change in the relative neural drive to the diaphragm and parasternal intercostal muscles.

Hyperventilation with associated reductions in carbon dioxide partial pressures have been observed during methacholine challenge testing in asthmatic and non-asthmatic individuals [270, 275, 294]. The systemic effects of methacholine have been shown to be minimal at the doses used in the current study [295], hence any increases in ventilation were more likely to be mediated by a psychogenic mechanism rather than physiological demand. This increase in NRD and ventilation acts as a confounding variable when attempting to assess the effect of increases in respiratory load on NRD. While measurement of end tidal CO_2 concentrations could have provided a method for determining hyperventilation by the subjects, the use of NVE_{para} as a measure of NRD was felt to offer an advantage in the assessment of respiratory load.

An increase in tidal volume in the absence of any change in respiratory load would require greater activation of the respiratory muscles and hence a higher peak EMG_{para} would be observed. As previously discussed in Chapter Three, measuring the area under the rectified EMG_{para} curve may provide a more sensitive measure of overall respiratory load, this was not possible due to technological constraints associated with ECG artefact removal. Representing NRD as the ventilatory return for a given neural output (NVE) allowed the effect of changes in respiratory pattern to be differentiated from alterations in respiratory load. Any increases in tidal volume, while increasing EMG, would result in minimal changes in NVE. Substantial effects would only be expected to occur at much higher lung volume when, due to elastic recoil, the chest wall is more difficult to expand. The utility of this measure has previously been investigated in assessing readiness for extubation [296] and in titrating respiratory support [297] in critical care patient populations, as well as in assessing responses to increased respiratory load in healthy subjects [192].

Under conditions of hypercapnia-induced hyperpnoea, significant increases in EMG_{para} and EMG_{di} have been observed, with minimal change in NVE_{para} or NVE_{di} [192]. In contrast, at high levels of inspiratory threshold loading

(>30% of maximum inspiratory pressures), NVE_{para} decreased significantly. NVE_{di} , however, was relatively unaffected by the increasing load, possibly as a consequence of volitional changes in lung volume to adopt a more preferential position on the length-tension curve or as a result of increased recruitment of other ribcage musculature (including greater activation of the parasternal intercostal muscles).

Changes in tidal volume have previously been documented following MCT, although substantial heterogeneity of ventilatory responses was observed [267-270, 275]. To minimise the confounding effect of these potentially varied responses, NVE was felt to offer an improved method for assessment of NRD. When considered in the context of other reported data, however, it appears that the levels of respiratory load imposed may have been insufficient to induce substantial effects on NVE_{para} or NVE_{di} . For example, in mechanically ventilated adults with a range of pathologies, NVE_{di} during a spontaneous breathing trial was shown to be a sensitive and specific predictor of extubation outcome (area under the receiver-operator characteristic curve of 0.816) [296]. The optimal NVE_{di} threshold for predicting extubation failure was $25.0 \text{ ml} \cdot \mu\text{V}^{-1}$, which is substantially less than the end of test NVE_{di} in the current study (median (range) NVE_{di} 32.94 ($23.83 - 65.05$) $\text{ml} \cdot \mu\text{V}^{-1}$). The use of NVE to determine the effectiveness of respiratory muscle activation may have greater utility in very unwell patient populations, where the magnitude of the respiratory load-capacity imbalance may precipitate respiratory failure. NVE may, under such circumstances, indicate that the increasing NRD may not be resulting in appropriate increases in ventilation. However, in a healthy population such as in the current study, the capacity of the respiratory system to respond to (mild to moderate) increases in load is likely to be sufficient and the use of NVE as an outcome measure may not offer any advantage above measurement of EMG_{di} or EMG_{para} alone. No relationships were observed between NVE_{para} or NVE_{di} and conventional measures of pulmonary function, and the magnitudes of change observed in EMG and NVE for both the parasternal intercostal muscles and diaphragm were also similar,

suggesting little advantage being gained through the use of NVE as a measure of NRD.

Changes in small airways resistance were demonstrated in the subset of individuals undergoing Part B of the study. The percentage changes in R5Hz, diff R5-R20, AX, IOS measures of small airways function, were greater than that in R20Hz as well as in FEV₁, both measures predominantly of larger airway calibre. Although no direct relationship was observed between changes in EMG_{para} and those in the IOS measures of small airways function, the data from this small cohort suggest that methacholine challenge testing is associated with increases in resistance in small distal airways. The heterogeneity of the changes observed in both small and large airways resistance and end expiratory lung volumes seen in both in this and previous studies [263, 266, 287] make interpretation of the relative contributions of each to the overall change in NRD difficult. Nonetheless, the significantly greater increases in small airways resistance compared to that in large airways resistance suggests that distal airways obstruction may contribute to the increases in EMG_{para} occurring during methacholine challenge.

5.6 Conclusions

This study has demonstrated the complexity of the interactions between the pathophysiological changes occurring in induced bronchoconstriction. A clear additive effect of hyperinflation in addition to bronchoconstriction on the changes in NRD has not been observed; at the degrees of change seen in this study, the lack of additional increase in NRD in the hyperinflator group may suggest that bronchoconstriction exerts a stronger influence on NRD than changes in end expiratory lung volume. Nonetheless, significant changes in small airways resistance, large airways calibre and end expiratory lung volume have all been observed and it is likely that all of

these changes affect EMG_{para} . The extent to which each influences NRD cannot be elucidated from the current study. Weaknesses in the study design limit the ability of this study to clarify the relationship between EMG_{para} and EMG_{di} , as well as the relationship of EMG_{para} with breathlessness, during induced bronchoconstriction.

6. Chapter Six: EMG_{para} in paediatric cystic fibrosis

6.1 Introduction

Cystic fibrosis (CF) is the UK's most common life-limiting inherited condition, with one in every 2,500 live births affected [298]. Pulmonary disease is the most common manifestation of CF, with 80-85% of mortality attributed to severe lung disease [299, 300]. Repeated endobronchial infection and concomitant inflammation in the small airways lead to progressive pulmonary destruction and eventual respiratory failure [301, 302]. While severe lung disease is relatively uncommon in the paediatric setting, maintaining lung function during childhood is known to be associated with improved pulmonary function, health status and longer survival in adulthood [300]. Aggressive treatment of infections and exacerbations in the first few years of life are particularly associated with improved outcomes [103].

Although there is no standardised definition of what constitutes an exacerbation of CF lung disease, symptoms may include increased cough, breathlessness, malaise, low-grade fever, decreased appetite or weight, changes in sputum colour or quantity, deterioration in lung function, and new findings on chest auscultation [300, 303, 304]. These may be rapid in onset or more insidious and hospitalisation for intravenous antibiotics is indicated to prevent further disease progression [103, 305, 306]. In addition to clinician assessment, spirometry (predominantly FEV₁) is currently the standard assessment technique used during an acute exacerbation to assess changes in lung function and the effects of treatments [301, 302, 307, 308]. The volitional nature of spirometry, however, results in difficulty obtaining these measures in younger or acutely unwell children [308, 309], and makes assessment of treatment efficacy difficult in these populations. Objective measures suitable for assessment of pulmonary

function in acutely unwell and/or younger children with CF are lacking. EMG_{para} may offer a non-volitional option for monitoring pulmonary function in this population.

The aims of this study were to assess the feasibility of undertaking regular measurements of EMG_{para} in a population of children hospitalised for treatment of an exacerbation of CF lung disease, to investigate the changes observed in NRD, as measured by EMG_{para}, over the course of the admission, and to explore the relationship between EMG_{para} and conventional measures of pulmonary function. We hypothesised that EMG_{para} would gradually decrease during the treatment of an exacerbation of CF lung disease. We further hypothesised that the changes in EMG_{para} would be greater than those in FEV₁.

6.2 Subjects

Patients were recruited from the paediatric respiratory ward at King's College Hospital NHS Foundation Trust. Any child with a diagnosis of cystic fibrosis admitted for intravenous antibiotics (for respiratory reasons) was eligible for inclusion. Ethical approval for the study was granted by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from parents or guardians of all participants.

6.3 Methods

6.3.1 Equipment

Height was measured using a wall-mounted stadiometer (Harpenden, Holtain Ltd, Crymych, UK) and weight with an electronic medical scale (HR Person Scale, Marsden Ltd, Henley on Thames, UK).

EMG_{para} was measured as described in Chapter Two (Methods). Briefly, EMG_{para} was recorded using surface electrodes placed over the second intercostal space. The signal was amplified (gain 1,000), band-pass filtered between 10 and 2,000Hz, and a 50Hz notch filter and a post-acquisition 20Hz digital high-pass filter applied. The signal was converted to root-mean-square (RMS) and mean peak RMS EMG_{para} per breath calculated.

IOS and spirometry were measured as described in Chapter Two, using Jaeger Masterscreen IOS and PFT systems respectively, running JLAB software version 4.0 (Cardinal Health, Basingstoke, UK). IOS was performed in accordance with ERS guidelines [207], with the mean of three reproducible recordings being reported. Spirometry was performed according to ATS/ERS recommendations [14], and the highest value of three reproducible efforts reported.

6.3.2 Protocol

Patients were studied at the same time on each day of admission. Anthropometric and demographic data were collected on the day of admission and weekly thereafter. EMG_{para}, IOS and spirometry were recorded daily during the admission. Briefly, resting EMG_{para} was recorded for ten minutes, with the final stable minute used to calculate mean peak RMS EMG_{para} per breath. MaxEMG_{para} was recorded in those subjects able to reliably perform inspiration to total lung capacity (TLC). IOS followed by spirometry were performed after the EMG_{para} recording, in subjects able to comply with such measurements. As the study was conducted during an infective exacerbation where treatment burden is high and patients may experience a number of undesirable symptoms such as nausea and fatigue, patients and their parents had the option to decline any or all of the proposed measurements on any given day. If daily spirometry

measurements were declined, weekly lung function testing was completed (as per standard clinical care) and these data used for the study.

6.3.3 Additional analyses

Relationships between daily changes of individual parameters within patients were examined using area under the curve (AUC) analysis and by comparing the slopes of the regression lines. Such analyses have been used to assess incremental changes in pulmonary function during airway challenge testing [244, 310]. Daily percentage changes were plotted against number of days since admission and both the area under the day-response curve calculated and linear regression analysis performed. Regression lines were assessed for deviation from linearity using the Wald-Wolfowitz runs test.

6.3.4 Statistical analysis

Non-parametric statistics were used due to the sample size consisting of fewer than 30 subjects. A p value <0.05 was taken as statistically significant. Admission to discharge changes in all measured variables were assessed using the Wilcoxon matched pairs test. The Friedman test was used to assess repeated measures within subjects over the first five measurement points (as this was equal to the minimum number of data points available in the subject with the shortest length of stay, and allowed assessment of acute changes in CF lung disease status), with Dunn's test to correct for multiple comparisons. Spearman's rank correlation was used to assess relationships between variables. Changes in EMG_{para} , being generally negative, were multiplied by minus one for the comparison of whether the magnitude of change in EMG_{para} was greater than that in FEV_1 , using the Wilcoxon matched pairs test. AUC and regression line slopes were also compared for

each variable using the Wilcoxon matched pairs test and Spearman's rank correlation.

6.3.4.1 Sample size calculation

No formal sample size calculation for this study was performed as data are not available regarding EMG_{para} activity in children with CF. The data from the study by Reilly *et al* [199] in adults with CF were unsuitable to inform the sample size for this study due to the progressive nature of the condition, and it was not felt appropriate to extrapolate from data obtained in an asthmatic population. Subjects were therefore recruited on a convenience basis, according to inpatient admissions during the recruitment period and dependent on the availability of the researcher to undertake daily measurements.

6.4 Results

23 children were recruited for the study, from whom twenty complete data sets were obtained. One child declined daily measurements due to the severity of their clinical condition, a second withdrew consent after testing on the first day having reported discomfort during electrode removal and the third was withdrawn due to lack of researcher availability. The median (range) length of stay was 10 (5 – 18) days. The demographic and anthropometric data (as measured on admission) of the twenty participants are shown in Table 16.

	Entire cohort (n=20)	Subjects performing spirometry (n=18)	Subjects performing IOS (n=16)
Age (years)	12.20 (1.91 – 15.77)	12.41 (4.93 – 15.77)	12.84 (7.21 – 15.77)
Sex (male: female)	5 : 15	5 : 13	4 : 12
Height (m)	1.484 (78.30 – 172.20)	1.506 (1.101– 172.2)	1.545 (1.176 – 172.2)
Weight (kg)	36.00 (10.96 – 56.70)	39.20 (19.00 – 56.70)	41.35 (22.10 – 56.70)
BMI (kg m⁻²)	18.1 (14.8 – 20.7)	17.7 (14.8 – 20.7)	18.2 (14.8 – 20.7)
Ethnicity (n (%))	White: 20 (100%)	White: 20 (100%)	White: 20 (100%)

Table 16 Demographic and anthropometric characteristics of twenty children with CF undergoing measurements of EMG_{para} during an infective exacerbation. All data are shown as median (range).

EMG_{para} was successfully recorded on median (range) 88.8 (64.3 – 100.0)% of admission days. This was significantly greater than the median (range) percentage of days on which spirometry and IOS were obtained: 79.3 (0.0 – 100.0)%, $p=0.012$ and 77.5 (0.0 – 100.0)%, $p=0.003$ respectively. When the patients in whom spirometry and IOS were not attempted or not possible (due to age or technique) were excluded, measures of IOS and spirometry were still obtained on significantly fewer days (IOS 81.7 (23.5 – 100.0)%, $p=0.047$, spirometry 81.7 (38.9 – 100.0)%, $p=0.047$) than EMG_{para} (85.7 (64.3 – 100.0)%).

6.4.1 Changes in EMG_{para}

Median (range) EMG_{para} decreased in the cohort as a whole from 7.13 (3.19 – 13.18) μ V on admission to 5.60 (2.62 – 13.45) μ V at discharge, a median (range) percentage change of -14.67 (-54.57 – 29.56)% ($p=0.033$).

Repeated measures testing of EMG_{para} on the first five measurements indicated a significant change ($p=0.015$) in median (range) EMG_{para} from day one (7.13 (3.19 – 13.18) μ V) to day four (6.45 (2.63 – 13.41) μ V) only.

Satisfactory maxEMG_{para} values were obtained at admission and discharge in 16 participants. The change in EMG_{para}%max was not significant (11.65 (4.10 – 31.29)% to 10.29 (5.86 – 38.88)%, $p=0.632$, Table 17). The median (range) within-individual, between-day coefficient of variation of the EMG_{para}%max signal was, however, 29.2 (17.8 – 51.1)%, in contrast to 16.8 (8.0 – 32.3)% for the raw EMG_{para} signal ($p<0.0001$). The lack of a statistically significant change in EMG_{para}%max was most likely a consequence of variability in the maxEMG_{para} signal. Correlation analysis indicated that the variation of the EMG_{para}%max signal was strongly related to that of the maxEMG_{para} signal ($r=0.678$, $p=0.002$), but not to that of the raw EMG_{para} signal ($r=0.179$, $p=0.478$). There was no significant difference ($p=0.868$) between median (range) maxEMG_{para} values in all subjects on the first five days on which measurements were obtained, indicating that the lack of significant difference in EMG_{para}%max was unlikely to be due to a consistent change in maxEMG_{para} with clinical improvement.

	EMG_{para}	p value (comparison to day 1)	maxEMG_{para}	p value (comparison to day 1)	EMG_{para}%max	p value (comparison to day 1)
Day 1	7.13 (3.19 – 13.18)	N/A	56.92 (25.79 – 131.10)	N/A	11.65 (4.10 – 31.29)	N/A
Day 2	6.09 (3.67 – 15.67)	0.143	46.40 (24.36 – 90.32)	0.154	12.25 (5.61 – 39.20)	>0.999
Day 3	6.67 (3.32 – 12.53)	>0.999	51.76 (15.27 – 97.23)	0.578	11.25 (6.41 – 62.54)	>0.999
Day 4	6.45 (2.63 – 13.41)	0.015	54.70 (21.78 – 150.70)	>0.999	10.34 (4.71 – 26.60)	>0.999
Day 5	6.44 (2.95 – 13.32)	0.646	52.40 (20.26 – 119.30)	>0.999	10.78 (6.12 – 32.47)	>0.999

Table 17 Changes in EMG_{para} and maxEMG_{para} from day of admission to days two to five. Data are expressed as median (range).

6.4.2 Changes in pulmonary function

18 children were able to complete spirometry, while 16 were able to complete IOS. Significant increases were seen in FEV₁ and FVC, while changes in R5Hz approached significance (Table 18).

	Admission	Discharge	p value
FEV ₁ (%predicted)	66.9 (36.6 – 102.2)	87.0 (38.6 – 109.7)	0.0117
FVC (%predicted)	84.9 (40.8 – 109.0)	96.1 (44.2 – 112.8)	0.0005
Z5Hz (kPa(L.s ⁻¹) ⁻¹)	0.55 (0.28 – 0.96)	0.48 (0.28 – 0.92)	0.075
R5Hz (kPa(L.s ⁻¹) ⁻¹)	0.51 (0.26 – 0.87)	0.46 (0.27 – 0.81)	0.051
R20Hz (kPa(L.s ⁻¹) ⁻¹)	0.33 (0.18 – 0.60)	0.34 (0.19 – 0.42)	0.072
ΔR5-R20 (%)	32.63 (4.92 – 52.49)	33.06 (9.77 – 57.95)	0.756
RF	17.29 (10.38 – 22.47)	15.99 (10.00 – 19.94)	0.1297
AX (kPa.L ⁻¹)	1.01 (0.26 – 3.79)	0.81 (0.18 – 3.52)	0.14

Table 18 Changes in pulmonary function from admission to discharge in children with cystic fibrosis. n=18 for FEV₁ and FVC. n=16 for all other parameters. Data are shown as median (range).

6.4.3 Measurements of EMG_{para} and pulmonary function

In the 18 children in whom spirometry was measured, the median (range) percentage change in EMG_{para} (-16.68 (-54.57 – 29.56)%) was significantly greater than that in FEV₁ (10.33 (-22.09 – 84.81)%, $p=0.0008$). The median (range) percentage changes in EMG_{para} (-10.28 (-34.26 – 66.17)%) and FEV₁ (8.33 (-11.86 – 32.14)%) from the first to the fourth measurement were not significantly different ($p=0.702$).

Plots showing individual subjects' daily changes in EMG_{para} and FEV₁ are shown in Figure 29 to 32.

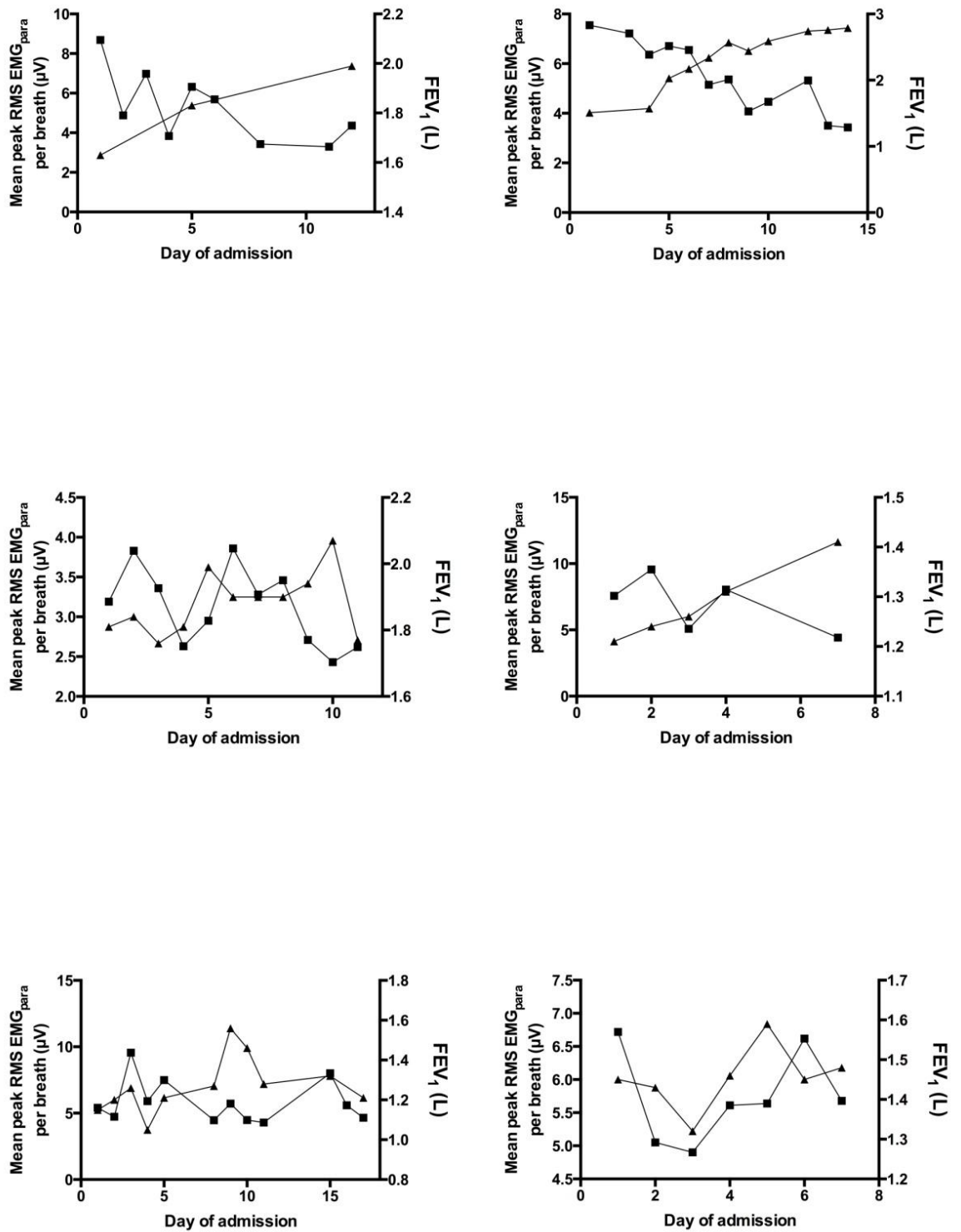


Figure 29 Daily changes in EMG_{para} and FEV₁ in subjects 1 to 6 from Study Four. Squares denote EMG_{para}; triangles denote FEV₁.

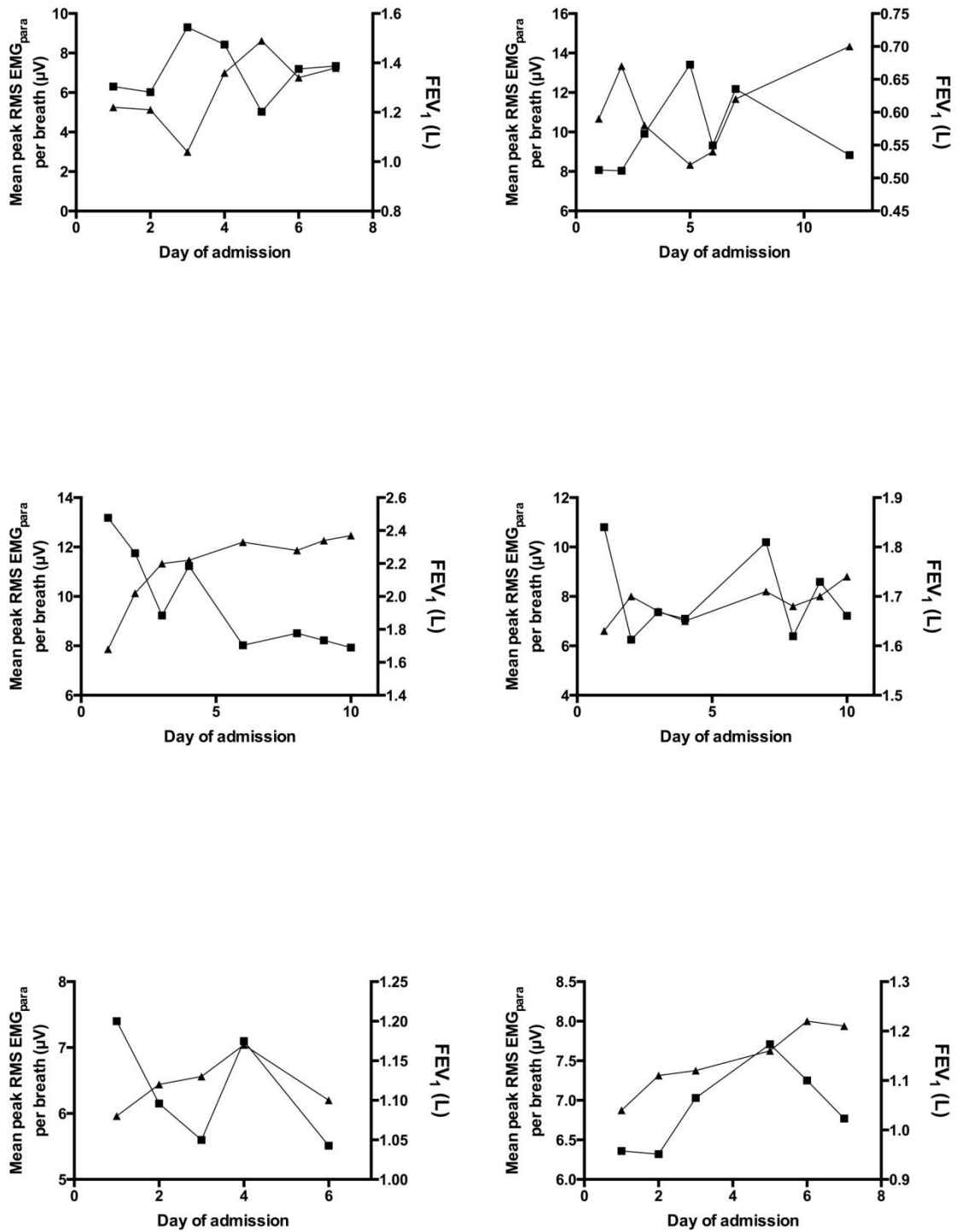


Figure 30 Daily changes in EMG_{para} and FEV_1 in Subjects 7 to 12 from Study Four. Squares denote EMG_{para} ; triangles denote FEV_1 .

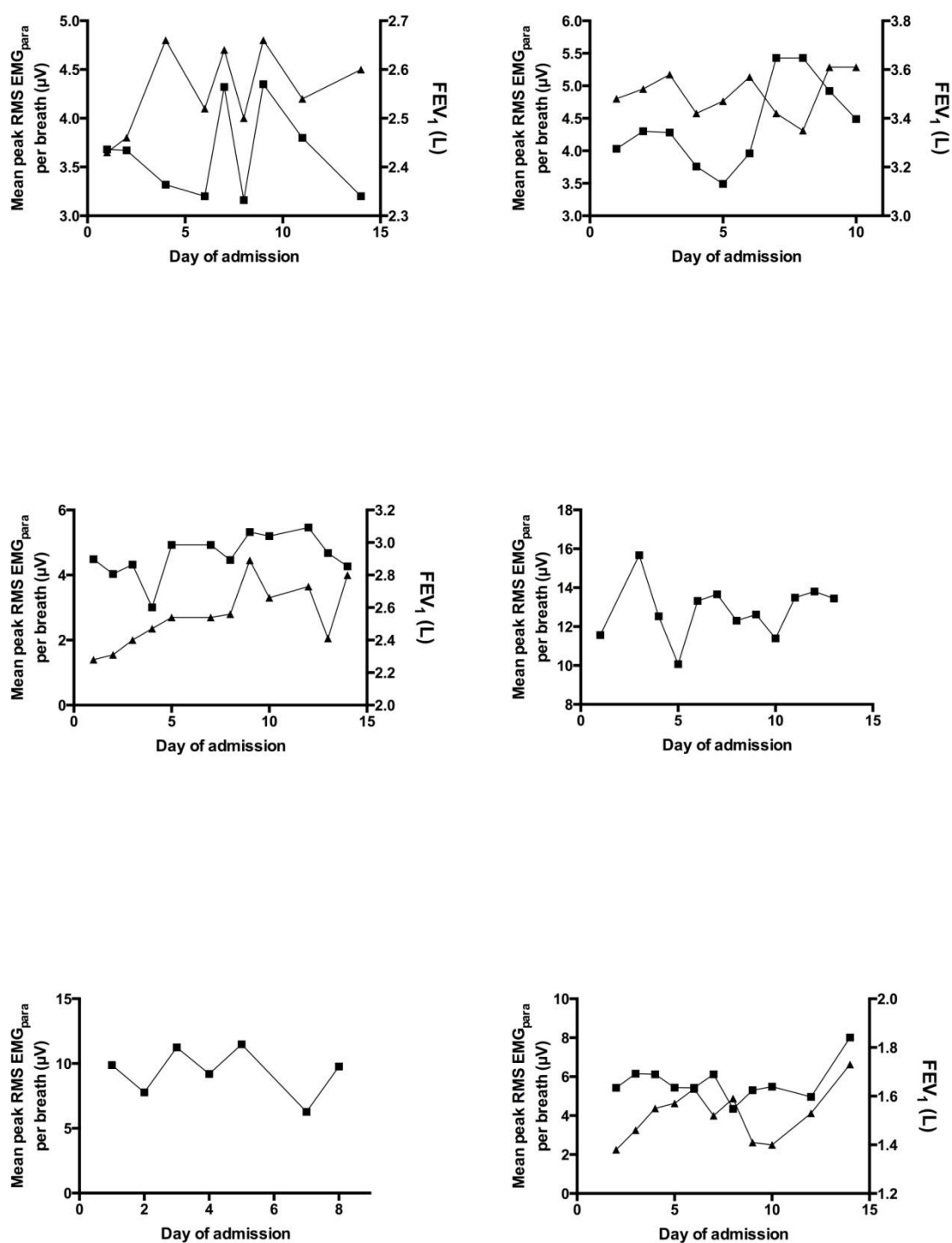


Figure 31 Daily changes in EMG_{para} and FEV_1 in Subjects 13 to 18 from Study Four. Squares denote EMG_{para} ; triangles denote FEV_1 .

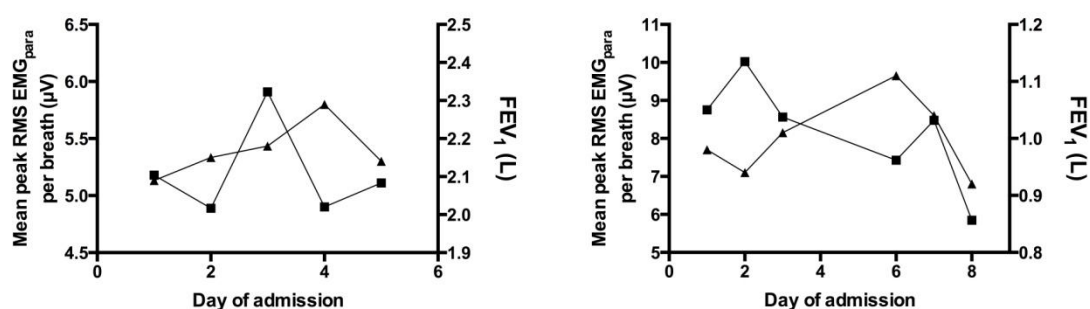


Figure 32 Daily changes in EMG_{para} and FEV₁ in Subjects 19 and 20 from Study Four. Squares denote EMG_{para}; triangles denote FEV₁.

No relationships were observed between percentage changes in EMG_{para} and those in any other measure of pulmonary function, either from admission to discharge or acutely between the first and fourth measurements. The relationship between EMG_{para} at admission and FEV₁ at admission approached significance ($r=-0.445$, $p=0.064$, Figure 33). The change in EMG_{para} was related to FEV₁ on admission ($r=0.490$, $p=0.039$, Figure 34), with a greater fall in EMG_{para} being observed in subjects with lower FEV₁ %predicted on admission. The length of stay was inversely related to severity of lung function impairment on admission, as indicated by FEV₁ %predicted ($r=-0.47$, $p=0.039$). No relationship was seen between change in EMG_{para} and length of stay ($r=-0.135$, $p=0.57$).

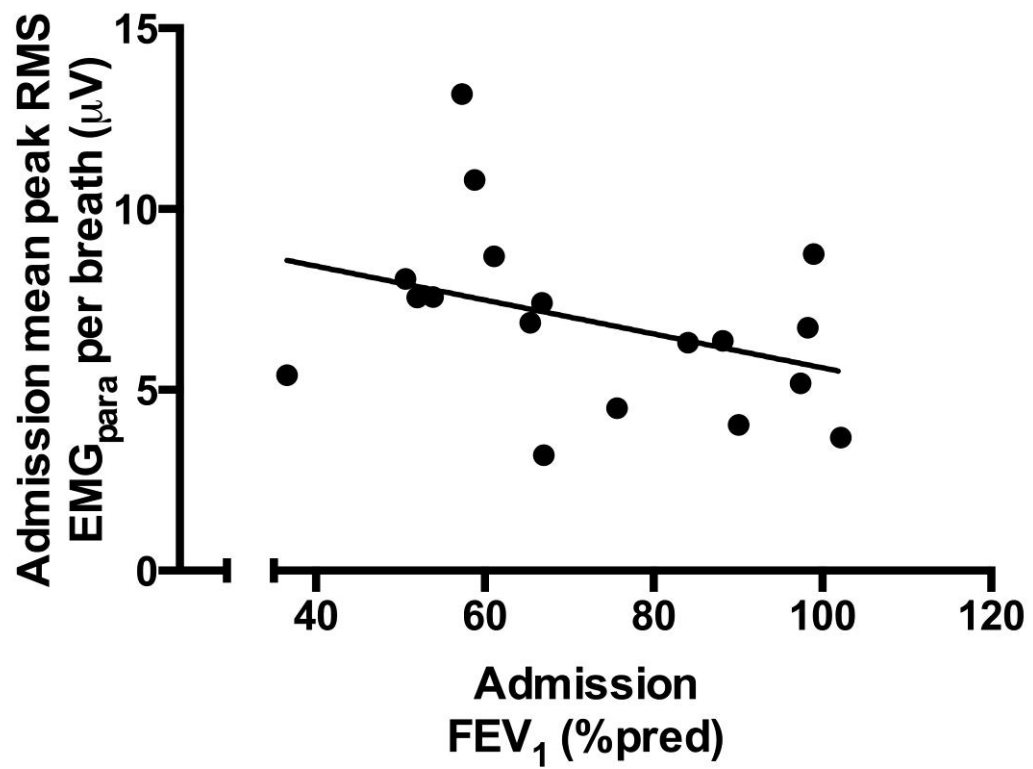


Figure 33 Relationship between FEV₁ and EMG_{para} on admission in 18 children with cystic fibrosis. $r=-0.445$, $p=0.064$.

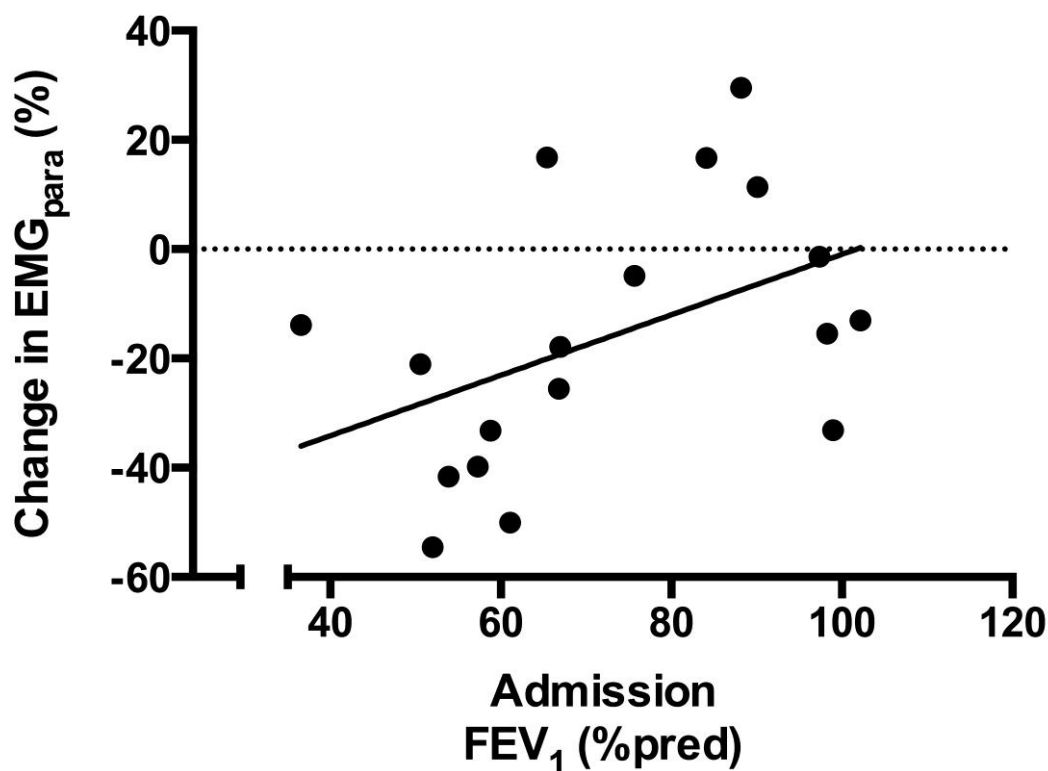


Figure 34 Relationship between FEV₁ on admission and change in EMG_{para} from admission to discharge in 18 children with cystic fibrosis. $r=0.490$, $p=0.039$.

There was no significant difference in the median (range) AUC for EMG_{para} and FEV₁ (30.02 (-504.1 – 349.3) AU vs. 60.9 (1.0 – 627.5) AU, $p=0.167$). There was no significant relationship observed between AUC EMG_{para} and AUC for any spirometric parameters. The only IOS parameter exhibiting a significant relationship with AUC EMG_{para} was the AUC for frequency dependence of resistance ($\Delta R5-20$, $r=0.547$, $p=0.031$).

To examine the nature of the progressive change in different measures, the slopes of the changes in individual parameters per day of admission were calculated. No relationship was observed between the slopes for EMG_{para} and FEV₁ or FVC. The only IOS parameter demonstrating a significant relationship with EMG_{para} was X5Hz ($r=0.741$, $p=0.0015$). There was no

significant difference in the median (range) slopes of the regression lines for EMG_{para} and FEV_1 (0.447 (-3.293 - 7.997) vs. 1.067 (0.131 - 7.071), $p=0.495$), indicating that improvements in disease status were not seen earlier when using EMG_{para} compared to FEV_1 .

6.5 Discussion

This study has demonstrated the feasibility of obtaining EMG_{para} measurements in children hospitalised for an infective exacerbation of CF lung disease. EMG_{para} fell during the resolution of the acute respiratory episode, suggesting a reduction in respiratory load, as indicated by the increase in FEV₁. Changes in EMG_{para} were not, however, related to changes in spirometry and IOS. The study also demonstrated that EMG_{para} as a measure of disease severity is applicable both to a wider age range and on a greater percentage of admission days than conventional measures of pulmonary function (spirometry and IOS).

6.5.1 Critique of method

The feasibility of obtaining measurements on each day of admission was limited by a number of factors, including patients' willingness to co-operate, clinical procedures, deterioration in clinical condition, and planned home leave. The greater acceptability of EMG_{para} compared to conventional pulmonary function measures, particularly spirometry, resulted in EMG_{para} data being recorded on some days in the absence of other measures of pulmonary function. When the relationships between daily changes in EMG_{para} and other measures of pulmonary function were analysed, only days on which both values were available were used, resulting in some data points being excluded. While for the majority of subjects, this only resulted in a relatively small number of missing or unused data points, one subject only performed spirometry, IOS and EMG_{para} measurements on four of seventeen admission days (23.5%). These missing data points resulted in reduced statistical power with which to evaluate relationships between daily changes in the different outcome measures. The short length of stay in some subjects (due to these subjects being discharged to complete the

course of intravenous antibiotics at their local hospital or at home) also reduced the number of data points.

Maximal manoeuvres were attempted in all subjects to allow the EMG_{para} signal to be expressed as a percentage of maximum ($EMG_{para}\%max$), as in previous studies [172, 192, 197-199]. Only 16 subjects were able or willing to perform these manoeuvres at both admission and discharge, highlighting the limitations of using this method for expressing EMG_{para} . While it has been suggested that the use of $EMG_{para}\%max$ allows more accurate inter-individual comparison [172, 189, 192, 197, 198, 311], the data in Chapter Seven suggest that the raw signal might be equally useful. Data are not available regarding the day-to-day variability of the raw EMG_{para} signal in clinically stable children with CF, which would be of benefit in interpreting the significance of the changes observed in the current study and would allow the determination of a threshold for a significant change in EMG_{para} .

Using $EMG_{para}\%max$ may also potentially minimise the influence of slight variations in electrode placement [172, 192, 197, 198] that underlies the poor reproducibility commonly reported when using surface EMG measurements. This was felt to be an important consideration in the current study, given that repeated measurements were obtained over many days. The significantly greater between-day CV in $EMG_{para}\%max$ when compared to the raw EMG_{para} signal in this cohort, as well as the strong relationship between the variability of the $EMG_{para}\%max$ and $maxEMG_{para}$ values, suggests that it is the variability of the maximum EMG_{para} signal that reduces the utility of $EMG_{para}\%max$. As a result of undertaking airway clearance techniques involving deep inspirations, children with CF are familiar with performing volitional inspiratory efforts from a relatively young age [304]. It was, therefore, felt that most subjects in this study would be likely to be able to reliably perform inspirations to TLC. Despite technically acceptable $maxEMG_{para}$ signals being obtained in sixteen of the twenty subjects, the between-day variability of these measures suggests that there may have been either contamination of the signal from other chest

wall musculature, influences on signal amplitude by the speed at which the inhalation was performed, or that submaximal efforts may have been performed on some occasions. It is not possible to ascertain whether changes in the subjects' clinical condition influenced this variability without knowing the variability of maxEMG_{para} values in clinically stable children with CF, though no consistent change in maxEMG_{para} was seen on repeated measures testing.

In addition to the variability potentially introduced by variations in electrode placement, the longitudinal study design also allows the results to be influenced by a variety of other factors. Individual patients' measurements were performed at the same time of day and were planned around scheduled clinical interventions. Timetabling of all planned activities, including time for schoolwork, clinical interventions and meals, is standard clinical practice at King's College Hospital for all inpatients with CF. Where possible, the interval between study measurements and treatments such as nebulised mucolytics, bronchodilators or antibiotics and airway clearance activities (including exercise) was standardised. Patients and/or their parents engaged well in ensuring the interval between measurements and clinical treatment was maintained, hence minimising their influence on the results.

No additional data regarding patients' symptoms or clinician assessment of clinical status were obtained in the current study. Many of the available patient- or parent-reported outcome measures (PROs) validated for use in children with CF have a recall period too long to be sensitive to acute changes in disease status, such as those occurring during treatment for an infective exacerbation. There is also no single PRO suitable for use across the age spectrum included in this study [312]. Similarly, although some dyspnoea scales have been shown to demonstrate responsiveness to clinical change in children with CF [313-315], none of these were suitable for use in the younger children included in the current study. Future studies could include a simple visual analogue scale or global rating of change

questionnaire to ascertain daily change in patient- or parent-perceived health status, as well as clinician opinion of clinical improvement or deterioration. Inclusion of such a measure would enable the relationship to EMG_{para} to be assessed, particularly in view of the strong relationships previously described between measures of NRD and patient perception of breathlessness [172, 189].

6.5.2 Discussion of findings

The significant decrease in EMG_{para} from admission to discharge suggests that the technique can detect change in respiratory load with clinical improvement, though no correlation with the change in other measures of lung function was observed. These data are in agreement with data from Reilly *et al* [199], who found a significant decrease in EMG_{para} activity from admission to discharge in adults hospitalised with an acute exacerbation of CF lung disease. The adult CF patients showed much greater impairment of lung function (mean (SD) FEV₁ %predicted 41 (17)%) compared to the children in the current study (median (range) 66.9 (36.6 – 102.2%). The improvement in FEV₁ in the adult patients was also much greater (mean (SD) percentage change 39 (30)% vs. median (range) percentage increase of 10.3 (-7.1 – 84.8)% in the current cohort). This greater change in FEV₁ was mirrored by a greater fall in EMG_{para} in the adult patients (mean (SD) change in EMG_{para} -38 (19)%) compared to the current study (median (range) change -14.7 (-54.6 – 29.6)%). Such differences were to be expected due to the progressive nature of CF lung disease and the increasing severity of acute exacerbations with worsening of pulmonary function. The results from the current study further support this with the relationship between FEV₁ on admission and the magnitude of the significant decrease in EMG_{para}, suggesting greatest change in those with more severe lung disease. In agreement with the current study, however, Reilly *et al* found no relationship between the percentage changes in FEV₁ and EMG_{para}.

A similar study by Murphy *et al* [198] in patients hospitalised with an acute exacerbation of COPD, demonstrated a significant fall in $EMG_{para}\%max$ but no relationship between changes in spirometric parameters and those in $EMG_{para}\%max$. The authors did not comment specifically on this lack of agreement. Maarsingh *et al* [186] assessed change in respiratory muscle EMG activity in infants and toddlers undergoing inpatient hospital treatment for acute wheezing illnesses and demonstrated a significant fall in both EMG_{para} and surface EMG_{di} . These changes correlated with falls in the Clinical Asthma Score, a validated scale for assessing severity of respiratory disease in pre-school children [316]. Due to the age of the children included, the authors did not compare changes in EMG activity to any other objective measures of pulmonary function. Nonetheless, the results of the current study can be said to be broadly in agreement with the results obtained by Murphy *et al* [198] and Maarsingh *et al* [186], despite the studies having been conducted in very different clinical populations.

Patients with CF have higher resting energy expenditure than healthy individuals due to the presence of chronic inflammation and infection [317], which increase further with an acute exacerbation of CF lung disease. Amin *et al* demonstrated reductions in resting energy expenditure in patients with CF following commencement of mucolytic therapy [318]. This reduction in metabolic demand is also likely to result in reduced respiratory drive, reflected in EMG_{para} changes but not in spirometry or oscillometry, and is a potential confounder when using this technique in acute CF lung disease, and may have influenced the lack of relationship between EMG_{para} in this and previous studies in acutely unwell populations.

Patients with a range of lung disease severities (as quantified by FEV_1), ranging from significant impairment (below 40% predicted) to greater than 100% predicted were included in this study. This further supports the applicability of the technique in all disease severities. That the measure can be obtained in hospitalised children as young as 23 months of age also indicates that the technique may have potential for use as a clinical tool to

monitor clinical improvement in children in whom objective measures of pulmonary function are currently lacking. The withdrawal by one subject due to the severity of their clinical condition was not due to poor acceptability of EMG_{para} to patients with severe disease, but moreover that the additional measures against which the EMG_{para} values were to be compared were too arduous for the patient to perform.

The trend towards a relationship between EMG_{para} and FEV₁ on admission indicates that EMG_{para} may have potential as a marker of disease severity, as has been suggested in previous studies in adult CF and asthma patients [172, 197]. Although FEV₁ is currently used as the primary indicator of lung disease severity in individuals with CF, small airways obstruction and hyperinflation also contribute significantly to the severity of CF lung disease and are not detected by FEV₁. EMG_{para} is likely to be sensitive to such changes, which will influence the relationship between EMG_{para} and FEV₁. The predominance of small airways disease in younger children may explain the weaker relationship observed in the current study when compared to previous data [172].

The population included in this study was heterogeneous, with a range of ages and lung disease severities. While this offers an advantage in terms of demonstrating the applicability of EMG_{para} in a range of clinical circumstances, the range of disease presentations resulted in varied treatment regimes and differing response patterns. Individual CF patients may be infected or colonised with a number of different pathogens, and with varying bacterial loads [300], which may lead to different degrees of involvement of airways throughout the bronchial tree. The pathogens may also demonstrate different susceptibility to antibiotic treatment, with resultant variations in reversibility of lung function impairment [319]. No record was kept of the organisms cultured on bronchoalveolar lavage or sputum sample in the subjects included in the current study, nor of medications administered. It was felt unlikely that any meaningful analysis using these data could have been conducted in a cohort of this size.

Heterogeneous changes in pulmonary function measures have previously been demonstrated with resolution of acute exacerbations of CF [320], suggesting that relationships between unidimensional measures of pulmonary function (such as FEV₁ and measures of airways resistance) and EMG_{para}, a composite measure of respiratory load-capacity balance, may not have been found. This heterogeneity may also contribute to the wide range of changes observed in EMG_{para} (-54.57 – 29.56%).

The greater number of days on which EMG_{para} could be measured, as well as the younger age in which EMG_{para} could be obtained in comparison to conventional measures of lung function, supports the acceptability of this measure under a wider range of circumstances than current standard measures. Some subjects were unable to perform spirometry to a technically acceptable standard due to age, comprehension or fatigue. Patients' willingness to perform spirometry and IOS was limited in several instances by nausea, a common side effect of many of the frequently-used antibiotics in patients with CF [321]. Although IOS is a tidal breathing technique and therefore thought to be suitable for children unable to perform spirometry [259, 322], four subjects in the current study were nonetheless unable to perform this measure to a satisfactory standard. This is in comparison to only two subjects who were unable to perform spirometry. This was due either to unwillingness to breathe through a mouthpiece or an inability to adopt a sufficiently stable tidal breathing pattern in the presence of the imposed oscillations. EMG_{para} clearly offers an advantage in this respect due to the completely non-volitional nature of the measure. Although some subjects initially demonstrated an awareness of the EMG_{para} measurement and altered their breathing pattern as a result, the provision of DVDs and/or books was sufficient distraction to allow the adoption of a stable tidal breathing pattern within only a few minutes.

The significantly greater change observed in EMG_{para} than FEV₁ supports the initial hypothesis and suggests that EMG_{para} may be more sensitive to the range of pathophysiological changes that occur during treatment of an acute

exacerbation of CF lung disease, though EMG_{para} may also demonstrate greater variability (as suggested by the large range of percentage changes in EMG_{para} from admission to discharge). The complexity of CF lung disease results in heterogeneous changes throughout the lung fields, including chronic bacterial infection, airway wall thickening and mucous plugging. This subsequently leads to inflammation in both proximal and distal airways, which further impairs local host-defence mechanisms and facilitates further infection and inflammation. This ongoing vicious cycle leads to bronchiectasis and gas trapping, resulting in impairment of gas exchange and ultimately respiratory failure [300]. While FEV_1 mainly reflects obstruction in the more proximal airways, EMG_{para} was likely to reflect changes occurring throughout the bronchial tree and lung parenchyma.

The lack of a relationship between changes in FEV_1 and EMG_{para} may be partly a result of the predominance of smaller airways disease and distal gas trapping in early CF lung disease. Impulse oscillometry was used in the current study to allow measurement of small airways resistance, though statistically significant changes in these measures were not seen. The change in resistance at 5Hz approached significance ($p=0.051$), although the changes in other measures of small airways function ($\Delta R5-R20$, AX, RF) were not significant. No relationships were observed between admission to discharge changes in EMG_{para} and any of the IOS parameters, although in the absence of statistically significant changes in these measures, this is perhaps unsurprising.

Data obtained from CT scanning provide insight into the nature of the changes occurring in CF lung disease. Peripheral bronchiectasis, air trapping, airway wall thickening and mucous plugging can be identified using CT in young children, adolescents and adults with both stable CF and during acute exacerbations [289, 323-325]. While bronchiectasis, airway wall thickening and gas trapping showed little reversibility with clinical resolution of an acute exacerbation, mucous plugging improved significantly

[324]. FEV_1 %predicted and $FEF_{25-75\%}$ demonstrated weak or no correlations with the changes seen in CT scores [323, 324]. In a longitudinal study evaluating changes in CT scores and pulmonary function over several years in a cohort of children with CF, de Jong *et al* [289] demonstrated more rapid deterioration in CT scores than in spirometric values. In their cohort, not only was there no agreement between changes in CT score and spirometry, but also in over half of subjects discordance in the direction of the changes was seen, with FEV_1 , FVC and FEV_1/FVC ratio improving in the presence of worsening CT scores in some subjects.

The lack of a relationship between changes in FEV_1 and EMG_{para} may have been a result of changes in other aspects of lung function. Mucous plugging can lead to reabsorption atelectasis and reductions in lung compliance [149]. The multiple therapies instituted, including intensive airway clearance therapy and mucolytic, bronchodilator and antibiotic medications, may have resolved mucous plugging present at admission and while the studies cited above indicate that this may not result in improvements in spirometric parameters, the associated improvements in lung compliance may have resulted in a reduction in the respiratory drive. Few data are available regarding the specific effect of resolution of atelectasis on oscillometric airways resistance, though one study demonstrated minimal change in resistance at 5Hz following alveolar recruitment manoeuvres in a porcine model of acute lung injury [326].

6.6 Conclusion

The reduction in EMG_{para} observed in this cohort of children hospitalised for acute exacerbations of CF lung disease may be related to changes in a variety of physiological parameters, both directly and indirectly related to respiratory system mechanics. The heterogeneity of CF lung disease and

associated treatment response may explain the lack of relationship between the changes observed. EMG_{para} nonetheless demonstrates promise as a measure of respiratory load in the paediatric CF population, having demonstrated greater applicability than conventional measures of pulmonary function.

7. Chapter Seven: EMG_{para} in healthy controls

7.1 Introduction

For a technique to be of benefit in assessing disease severity in patient populations, normative data from a cohort of healthy individuals must be available for comparative purposes. Previous studies investigating EMG_{para} in adult populations have used small groups of healthy controls to provide comparative data. Given the substantial maturational changes that occur in the lungs and ribcage throughout childhood and into adolescence, it is not known whether the level of NRD in healthy children would be the same as that observed in adults. Studying a large cohort of healthy children reduces the potential bias resulting from heterogeneity of growth and development.

To allow comparison between subjects and different testing occasions, the raw EMG_{para} signal can be normalised by expressing it as a percentage of the value obtained during a maximal inspiratory effort (EMG_{para}%max). Reilly *et al* reported the mean EMG_{para}%max to be 5.8% in a group of 15 young healthy adults [172], while Steier *et al* showed a similar mean EMG_{para}%max of 4.9% in 12 healthy subjects [197]. Normalising EMG_{para} to a percentage of maximum, however, limits the use of such measures of NRD in young children unable to perform maximal inspiratory efforts. The ability to produce maximal volitional efforts is a basic requirement of many existing objective measures of pulmonary function and represents the main limitation in younger children. Reilly *et al* [172] reported a mean (SD) value for the raw (non-normalised) EMG_{para} of 4.8 (2.0)µV. The low inter-individual variability in the EMG_{para} signal may, therefore, render normalising EMG_{para} when comparing between subjects unnecessary.

The aim of this study was, therefore, to investigate the utility of the raw EMG_{para} signal in a large cohort of healthy children and develop a reference

range for NRD in healthy children, expressed both as μV and $\text{EMG}_{\text{para}}\%_{\text{max}}$. The study also investigated the effect of growth and maturation on respiratory drive. The repeatability and reproducibility of the EMG_{para} signal were also assessed. Finally, these data were compared to the EMG_{para} values from Chapters Three and Six.

7.2 Subjects

Healthy children were recruited from siblings of children attending for clinical pulmonary function testing or participation in clinical research and from friends and relatives of research staff and students. Children were eligible for inclusion if no history of current or previous respiratory ill health was reported by their parent/guardian. Any child with a previous hospitalisation for a respiratory cause, a diagnosis of any chronic respiratory condition, or a history of recurrent GP visits for infant wheeze or lower respiratory tract infections, was excluded from participation. Any child in whom cough or coryzal symptoms were noted at the time of study was also excluded.

Ethical approval for the study was granted by the King's College Hospital Research Ethics Committee (ref: 09/H0808/100). Informed, written consent was obtained from parents or guardians of all participants.

The children recruited for this study represented a convenience sample; no *a priori* sample size calculation was performed.

7.3 Methods

Children attended for testing on one occasion. Demographic and anthropometric data were collected and ten minutes of EMG_{para} was

recorded during resting tidal breathing as previously described (Chapter Two). Briefly, EMG_{para} was recorded using surface electrodes placed over the second intercostal space. The signal was amplified (gain 1,000), band-pass filtered between 10 and 2,000Hz, and a 50Hz notch filter and a post-acquisition 20Hz digital high-pass filter applied. The signal was converted to root-mean-square (RMS) and mean peak RMS EMG_{para} per breath calculated.

The EMG_{para} value from final minute of the recording was reported as the EMG_{para} for each subject. The EMG_{para} from minute three onwards was analysed to calculate the inter-minute variability of EMG_{para}. In those children able to comply with the manoeuvre, inspirations to total lung capacity (TLC) were also performed to obtain a measure of maximum EMG_{para} (maxEMG_{para}), and the resting EMG_{para} expressed as EMG_{para}%max.

EMG_{para} measurement was repeated within the same testing session in ten subjects to obtain data regarding measurement repeatability. In those subjects, the electrodes were removed following the first recording and a new measurement performed following a break of between ten minutes and one hour. Repeatability data could not be obtained from larger numbers of subjects for pragmatic reasons – many subjects attended with at least one sibling and parents were often not willing to remain in the laboratory for a sufficient time period to allow repeat measurements to be taken.

In order to assess whether any differences in EMG_{para} between older and younger children were due to differences in electrode size relative to the size of the muscle, EMG_{para} was measured in six paediatric and six adult subjects using electrodes of two different diameters (diameter of electrode active area 9mm and 19mm). The two sizes of electrode were of identical construction and supplied by the same manufacturer (Blue Sensor R, Ambu, Ballerup, Denmark).

7.3.1 Statistical analysis

Where the sample size exceeded 30 subjects, data were tested for normality using the D'Agostino Pearson omnibus test. As no data were normally distributed, all data were expressed as median (range). A p value <0.05 was taken as statistically significant. Spearman's rank correlation was used to assess relationships between variables. Linear regression analysis was performed with the Wald-Wolfowitz runs test applied to assess for deviation from linearity.

Non-parametric statistical testing was performed on the repeated measures due to the small number of subjects. Agreement between repeated measures was assessed using the Spearman's rank correlation, Bland-Altman analysis, and the Wilcoxon matched pairs test. Differences between groups were assessed using the Mann-Whitney U test.

7.4 Results

7.4.1 EMG_{para} in healthy children

Of the 87 children studied, 81 acceptable data sets were obtained. Six data sets were rejected, due to either movement artefact or unstable tidal breathing pattern. Subject demographic and anthropometric characteristics are shown in Table 19.

Age (years)	9.63 (1.17 – 17.33)
Height (m)	1.373 (0.800 – 1.846)
Weight (kg)	32.35 (10.80 – 97.00)
BMI (kg m⁻²)	17.0 (13.8 – 35.5)
BMI-for-age percentile	64.4 (3.5 – 100.0)
Ethnicity (n (%))	White: 49 (60.5%) Black: 22 (27.2%) Asian: 4 (4.9%) Mixed: 6 (7.4%)

Table 19 Anthropometric and demographic characteristics of healthy subjects. Data are expressed as median (range).

Seventeen of the 81 subjects (21.0%) were classified as overweight or obese, defined by BMI-for-age >+1SD in children aged 5-19 years, or BMI-for-age >97th percentile in children aged 0-5 years. Of these, five were classified as obese (BMI-for-age >+2SD). The median (range) BMI in these seventeen children was (24.0 (18.2 – 35.5)kg.m⁻²), equivalent to a median (range) BMI-for-age percentile of 93.5 (84.8 – 100.0).

Maximal values of EMG_{para} were obtained in 54 children. The resting EMG_{para}, maxEMG_{para} and EMG_{para}%max are summarised in Table 20.

Mean peak RMS EMG_{para} per breath (µV) (n=81)	4.76 (1.93 – 14.92)
Maximum EMG_{para} (µV) (n=54)	58.19 (22.63 – 253.25)
EMG_{para} %max (%) (n=54)	7.04 (3.46 – 14.43)

Table 20 Resting and maximal EMG_{para} values in healthy children. All data are expressed as median (range).

The median (range) age of children who could reliably perform the maximum EMG_{para} manoeuvre was significantly higher than those who could not (10.26 (2.00 -16.35) vs. 4.47 (1.17 – 17.33) yrs, p=0.007).

Significant negative correlations were found between age (r=-0.644, p<0.0001, Figure 35), weight (r=-0.648, p<0.0001, Figure 36), and height (r=-0.632, p<0.0001, Figure 37) and EMG_{para}. No significant relationships were observed between EMG_{para}%max and age, height or weight (p>0.2). MaxEMG_{para} demonstrated a weak relationship with age (r=-0.358, p=0.008).

If those subjects in whom maxEMG_{para} was not successfully obtained were excluded, the relationships between EMG_{para} and age, height and weight weakened marginally but remained highly significant: r=-0.587, -0.574 and -0.612 respectively (all p<0.0001).

EMG_{para} and maxEMG_{para} were also related to BMI ($r=-0.583$ and -0.556 respectively, both $p<0.0001$), suggesting attenuation of the signal by adipose tissue. When age-related changes in BMI were accounted for by the use of BMI-for-age percentile, the correlations were weaker but remained significant ($r=-0.242$, $p=0.029$ and $r=-0.490$, $p<0.0001$ for EMG_{para} and maxEMG_{para} respectively).

The relationship between age and respiratory drive, as assessed by measurement of $P_{0.1}$, has been described previously using a power function [174]. When assessed for deviation from linearity, the relationships between EMG_{para} and age, height or weight were best fit using linear regression analysis.

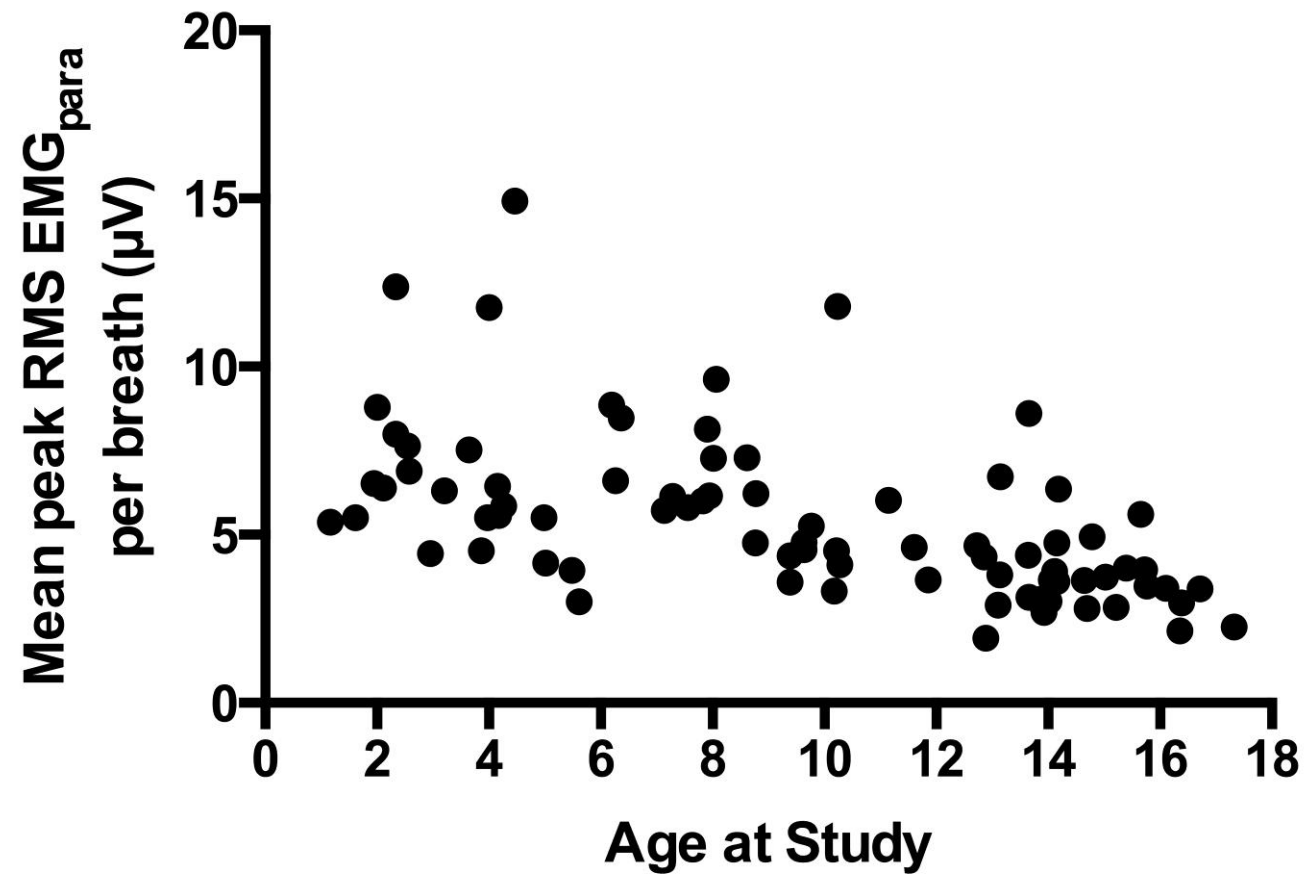


Figure 35 Correlation of EMG_{para} and age.

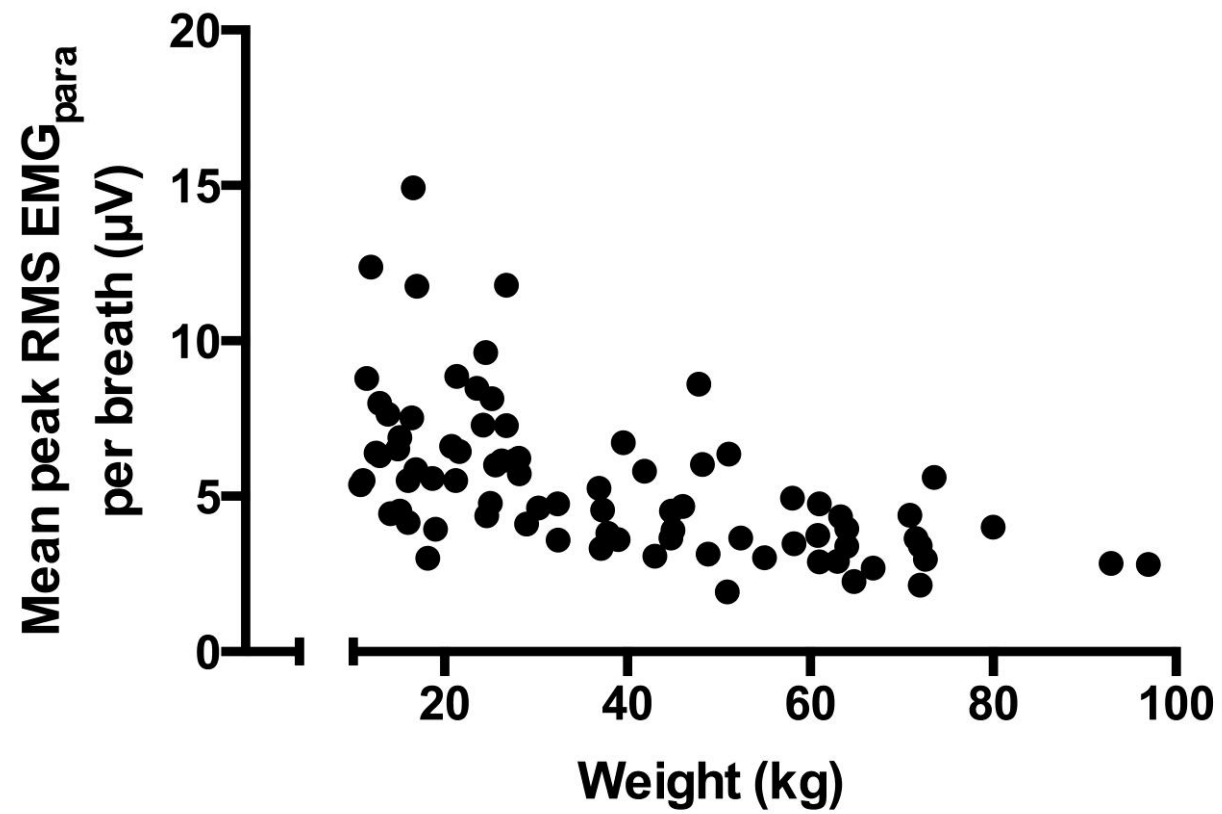


Figure 36 Correlation of EMG_{para} and weight.

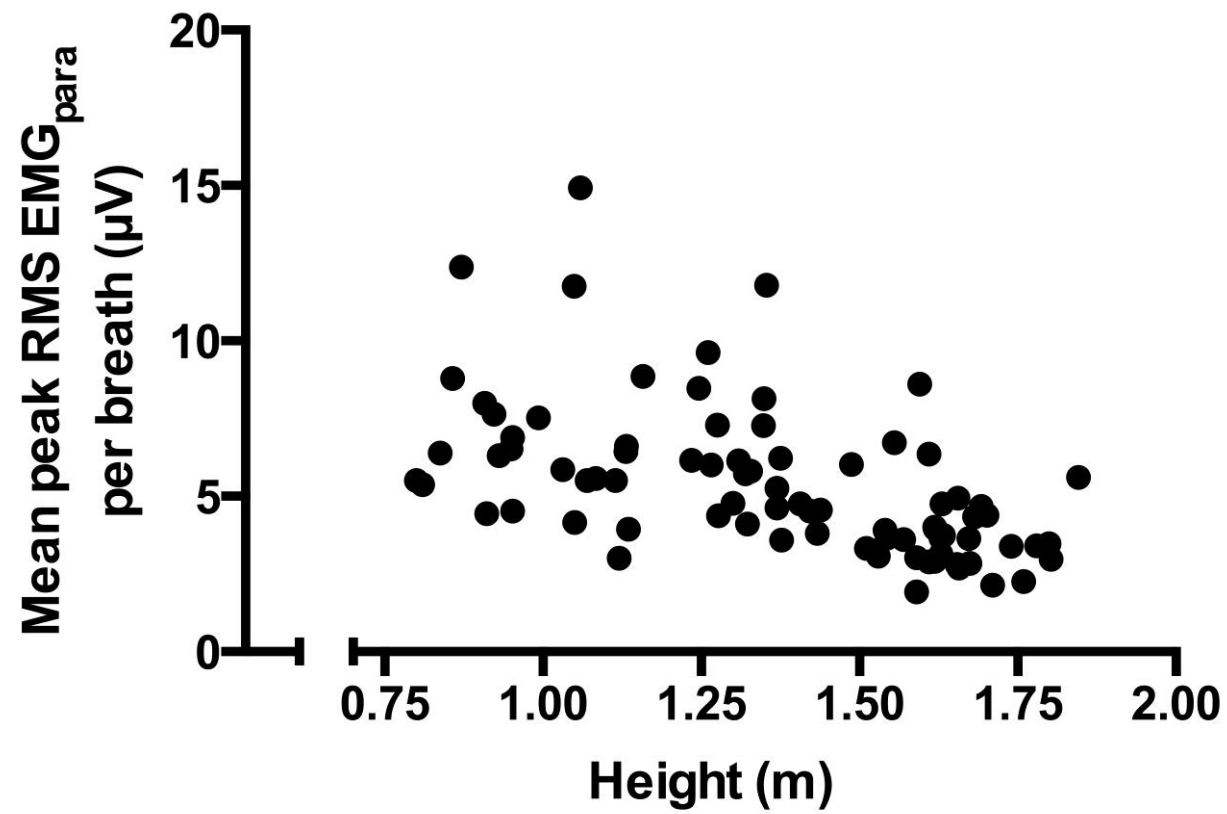


Figure 37 Correlation of EMG_{para} and height.

7.4.2 Variability of EMG_{para}

The inter-subject coefficient of variation for the raw EMG_{para} signal was 45.1%. Due to the influence of age on the EMG_{para} signal, subjects were divided into two-year age subgroups and inter-subject coefficient of variation calculated (Table 21).

Age (years)	Mean peak RMS EMG_{para} per breath (μV)	Inter-subject coefficient of variation (%)
1-2 (n=10)	6.71 (4.44 – 12.37)	31.1
3-4 (n=10)	6.08 (4.52 – 14.92)	44.8
5-6 (n=6)	5.38 (3.00 – 8.85)	42.8
7-8 (n=11)	6.16 (4.76 – 9.63)	20.3
9-10 (n=9)	4.53 (3.32 – 11.79)	49.8
11-12 (n=6)	4.48 (1.93 – 6.03)	32.3
13-14 (n=18)	3.65 (2.69 – 8.61)	38.6
15-17 (n=11)	3.41 (2.14 – 5.61)	27.7
Entire cohort (n=81)	4.76 (1.93 – 14.92)	45.1

Table 21 Raw EMG_{para} values and inter-subject coefficient of variation of resting signal according to subject age. EMG_{para} data are expressed as median (range).

The inter-subject variability in $EMG_{para}\%_{max}$ was also assessed and compared to that for the raw EMG_{para} signal, excluding subjects in whom $maxEMG_{para}$ was not recorded successfully. Despite a trend towards a lower inter-subject coefficient of variation for the raw EMG_{para} signal, there was no statistically significant difference in the median (range) inter-subject coefficient of variation for EMG_{para} (28.07 (7.09 – 51.91)%) and $EMG_{para}\%_{max}$ (36.40 (30.69 – 86.72)%, $p=0.25$) when assessed in two-year age subgroups. When taking the 54 subjects as a single group, the values for inter-subject coefficient of variation for EMG_{para} and $EMG_{para}\%_{max}$ were similar (Table 22).

Age (years)	Raw EMG _{para}	EMG _{para} %max
1-2 (n=2)	17.14	86.72
3-4 (n=4)	7.09	36.65
5-6 (n=5)	48.53	36.15
7-8 (n=9)	21.75	43.16
9-10 (n=8)	51.91	30.69
11-12 (n=4)	33.54	35.02
13-14 (n=15)	26.72	34.61
15-17 (n=7)	29.42	38.91
All subjects (n=54)	41.01	37.76

Table 22 Inter-subject coefficient of variation for raw EMG_{para} and EMG_{para}%max according to subject age.

Stable recordings of a sufficient duration to calculate within-subject inter-minute coefficient of variation were obtained in 55 children. Inter-minute coefficient of variation was examined in the entire cohort and in two-year age subgroups (Table 23). When assessed using correlation analysis, the inter-minute variability of EMG_{para} was not related to age ($r=0.03$, $p=0.8$).

Age (years)	Within-subject inter-minute coefficient of variation (%)
1-2 (n=6)	6.69 (0.50 – 11.90)
3-4 (n=5)	9.08 (6.72 – 11.83)
5-6 (n=5)	7.00 (2.36 – 16.74)
7-8 (n=9)	10.66 (4.55 – 15.53)
9-10 (n=5)	8.44 (3.99 – 11.64)
11-12 (n=6)	8.87 (4.54 – 15.86)
13-14 (n=12)	7.58 (3.09 - 21.89)
15-17 (n=7)	10.36 (2.84 – 12.86)
Entire cohort (n=55)	8.95 (4.14)

Table 23 Within-individual, inter-minute coefficient of variation of the raw EMG_{para} signal according to subject age. Data are expressed as mean (SD) or median (range).

7.4.3 Repeatability of EMG_{para}

The characteristics of the ten subjects in whom repeat measurements of EMG_{para} were performed are shown in Table 24.

Age (years)	8.59 (3.97 – 13.10)
Height (m)	1.314 (1.050 – 1.619)
Weight (kg)	27.65 (16.00 – 63.00)
BMI (kg.m⁻²)	15.6 (14.0 – 24.0)
BMI-for-age percentile	37.6 (15.7 – 99.8)

Table 24 Demographic and anthropometric characteristics of ten subjects undergoing repeat measurements of EMG_{para}.

There was no significant difference between the values for EMG_{para} obtained from the two measurements (4.58 (2.91 – 8.48) μ V *versus* 5.11 (2.88 – 7.82) μ V, $p=0.32$). Bland-Altman analysis showed a small bias of -0.37 μ V (towards a higher second measurement), with 95% limits of agreement of -2.11 – 1.38 μ V (Figure 38). The two measurements were significantly correlated ($r=0.733$, $p=0.02$).

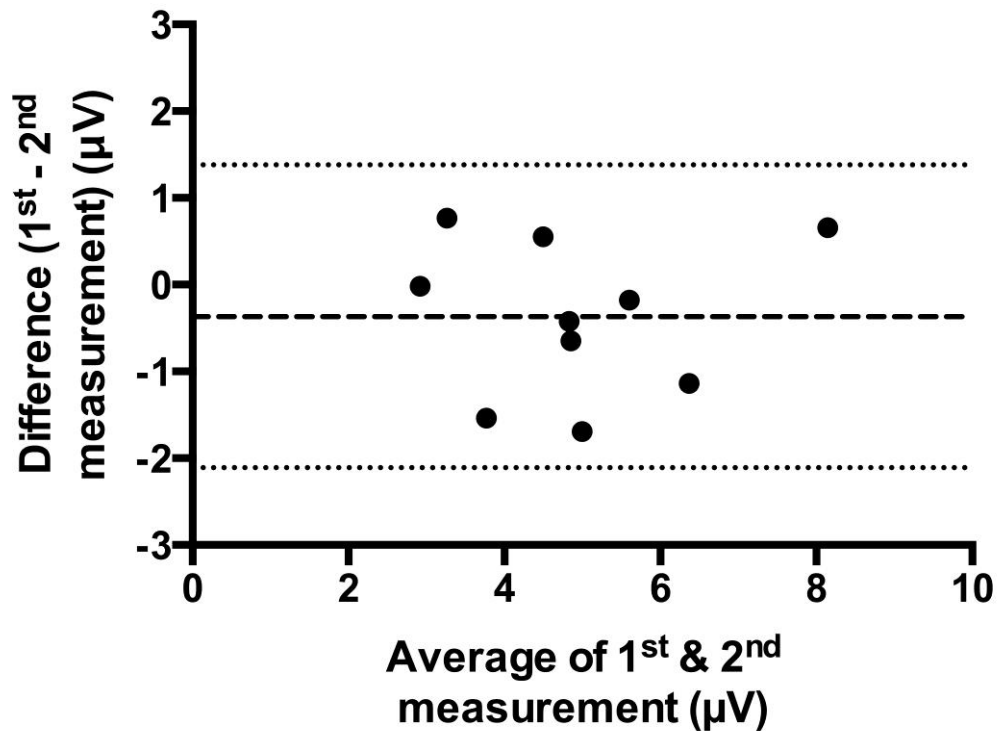


Figure 38 Bland-Altman plot of repeat measures of EMG_{para} in ten children. Dashed line shows bias (-0.37µV); dotted lines 95% limits of agreement (-2.11 – 1.38µV).

Six of the subjects who underwent repeat measurements were able to successfully perform maximal inspiratory efforts. The median (range) EMG_{para}%max from the first and second measurements was 8.85 (5.17 – 13.08)% and 9.59 (4.77 – 12.78)% respectively ($p=0.438$). The median (range) raw EMG_{para} in these six subjects was 4.65 (3.65 – 8.48)µV and 5.51 (2.88 – 7.82)µV from the first and second measurements ($p=0.688$). There was a strong significant correlation between the EMG_{para}%max ($r= 0.886$, $p=0.033$) obtained from the two measurements in these six subjects, although the relationship between the two raw EMG_{para} measurements in these six individuals did not reach significance ($r=0.771$, $p=0.103$). The Bland-Altman analysis indicated a bias of -0.63%max for EMG_{para}%max and -0.25µV for EMG_{para} between measurements (Figure 39 and Figure 40), suggesting good reproducibility.

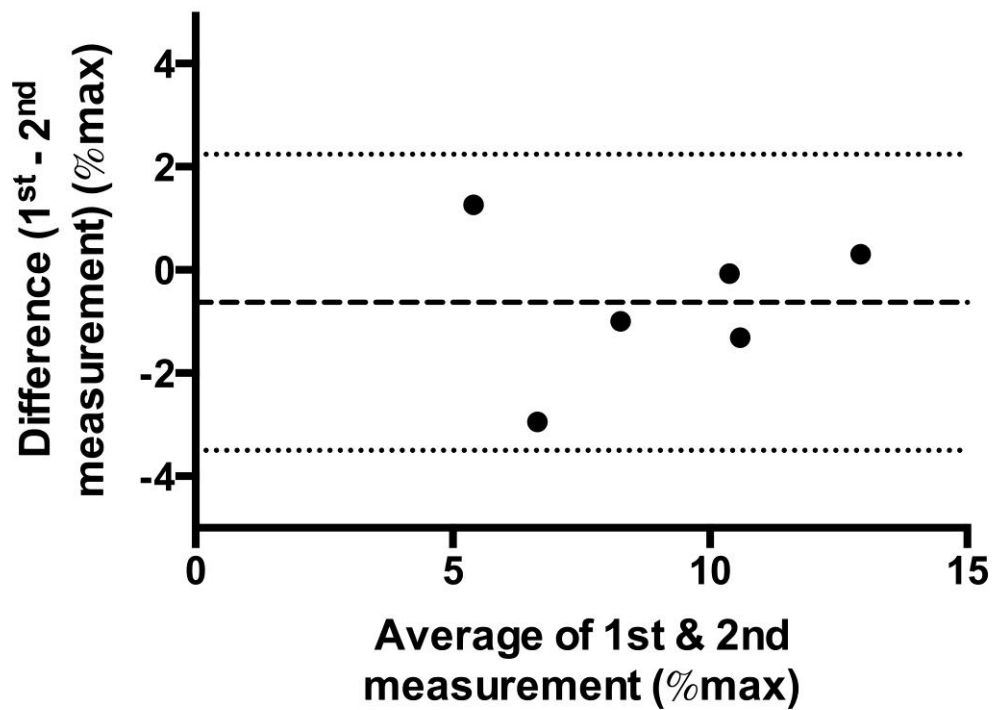


Figure 39 Bland-Altman plot of repeat measures of EMG_{para}%max in six children. Dashed line shows bias (-0.63%max) and dotted lines 95% limits of agreement (-3.5 – 2.24%max).

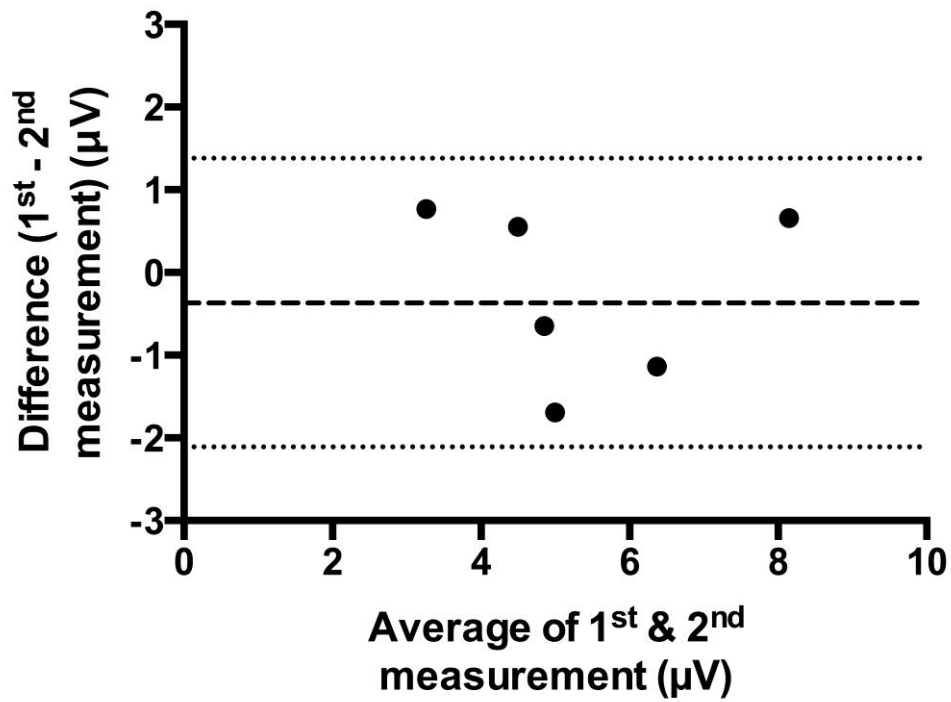


Figure 40 Bland-Altman plot of repeat measures of EMG_{para} in six children in whom maxEMG_{para} values could be obtained. Dashed line shows bias (-0.25µV) and dotted lines 95% limits of agreement (-2.31 – 1.81µV).

7.4.4 Effect of electrode size

The demographic and anthropometric characteristics of the six children and six older children and adults in whom EMG_{para} was measured on a single occasion with two different sized electrodes are shown in Table 25.

	Young children (n=6)	Older children & adults (n=6)
Age (years)	4.79 (3.20 – 8.07)	21.34 (16.53 – 31.24)
Height (m)	1.107 (0.931 – 1.261)	1.65 (1.58 – 1.72)
Weight (kg)	18.65 (12.90 – 24.50)	54.00 (48.00 – 62.00)
BMI (kg.m⁻²)	15.6 (14.6 – 17.1)	20.2 (17.9 – 21.6)

Table 25 Demographic and anthropometric characteristics of subjects in whom EMG_{para} was measured with electrodes of different sizes. Data are expressed as median (range).

No significant difference was found in resting peak RMS EMG_{para} per breath when electrodes of two different sizes were used ($p=0.38$) (Table 26). The median (range) EMG_{para} value was not significantly different between the 19mm and 9mm electrodes in either the young (7.24 (3.10 – 8.62) μ V vs. 5.74 (2.95 – 9.35) μ V, $p=0.313$) or older (5.13 (3.43 – 8.80) vs. 5.23 (3.21 – 9.35) μ V, $p>0.999$) subjects.

	Mean peak RMS EMG_{para} per breath (μV)	
	19mm electrode	9mm electrode
Subject 1	8.62	6.30
Subject 2	8.46	7.48
Subject 3	6.33	5.18
Subject 4	8.14	9.35
Subject 5	3.10	2.95
Subject 6	5.48	4.22
Subject 7	5.27	5.52
Subject 8	3.43	3.21
Subject 9	4.87	4.70
Subject 10	5.00	4.93
Subject 11	6.39	6.35
Subject 12	8.80	10.31
All subjects (median)	5.91	5.35

Table 26 Values of EMG_{para} recorded using electrodes of two different sizes in six young children (subjects 1-6) and six older children and adults (subjects 6-12).

There was a strong correlation between the values of EMG_{para} measured with the two electrode sizes in the twelve subjects ($r=0.895$, $p=0.0002$, Figure 41). In the younger children alone, the correlation did not reach significance ($r=0.771$, $p=0.103$). In the six older children and adults, the correlation coefficient was 1.00 ($p=0.0028$).

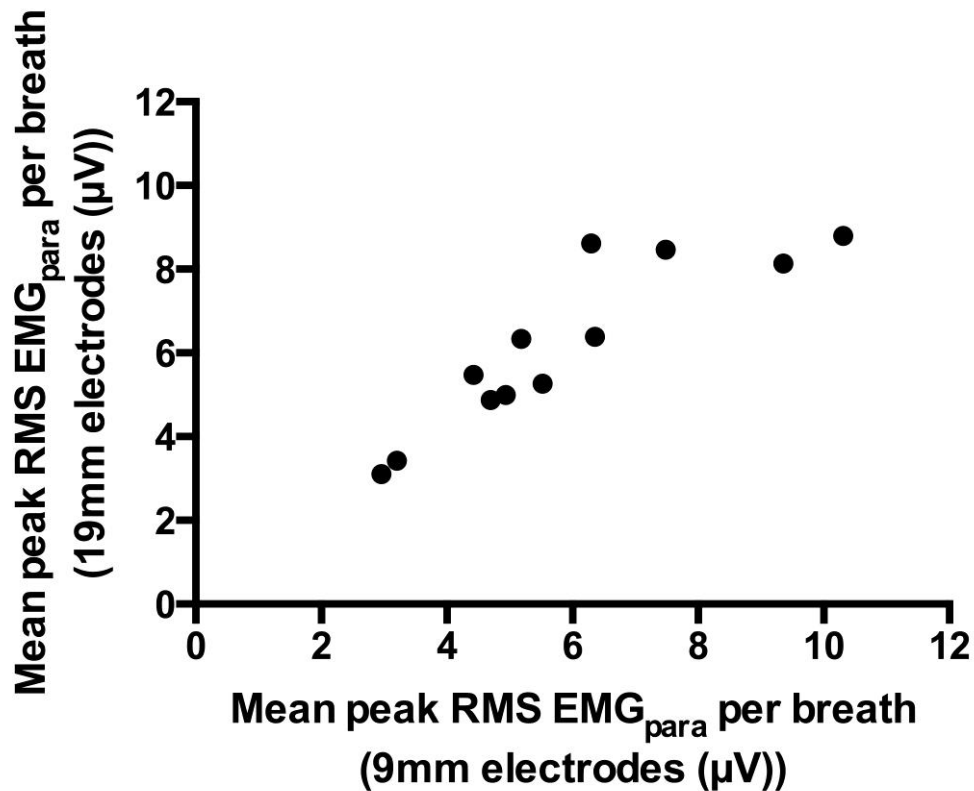


Figure 41 Correlation between EMG_{para} recorded with 9mm and 19mm electrodes.

Bland-Altman analysis was performed to assess the agreement between the measures of EMG_{para} from each electrode size (Figure 42). A bias of 0.26µV was found towards a higher value from the larger, 19mm electrode, though the 95% limits of agreement were relatively wide and did include zero (-1.77 – 2.30µV).

Bland-Altman analyses of the younger children indicated a bias of 0.74µV with 95% limits of agreement of -1.58 – 3.06µV. In the older children and adults, the bias was -0.21µV with 95% limits of agreement -1.50 – 1.08µV.

MaxEMG_{para} was recorded in the six older subjects. Median (range) maxEMG_{para} was not significantly different when measured with 19mm vs.

9mm electrodes (123.6 (59.7 – 146.4) μ V vs. 114.9 (52.7 – 150.6) μ V, $p=0.125$).

Correlation analysis indicated an inverse relationship between age and the difference between the EMG_{para} measured with the two electrode sizes in twelve subjects ($r=-0.643$, $p=0.028$), indicating closer agreement between EMG_{para} measured with the two electrodes with increasing age.

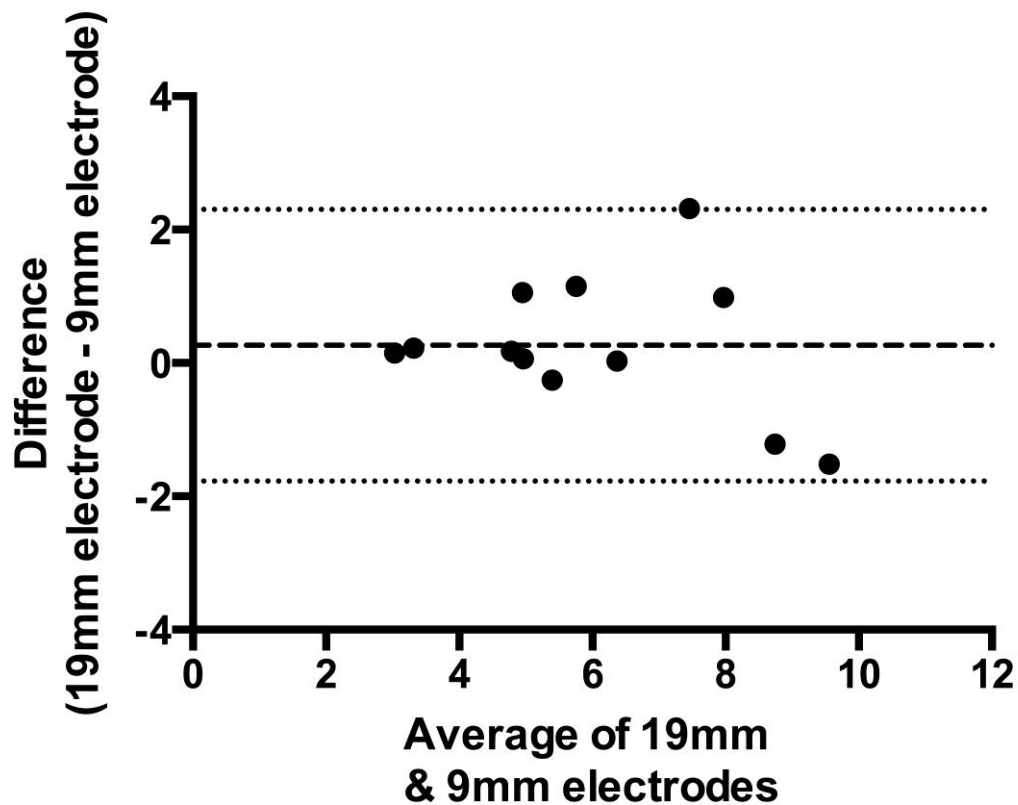


Figure 42 Bland-Altman plot of EMG_{para} recorded with 19mm and 9mm electrodes in twelve subjects.

Dashed line shows bias (0.26 μ V); dotted lines 95% limits of agreement (-1.77 – 2.30) μ V.

7.4.5 Comparison to data from patient populations

The data from the control subjects was compared to those obtained from the children with asthma and CF in Chapters Three and Six respectively. As the control subjects were not recruited to provide a cohort matched to the patients, average values were taken of all control subjects with ages within $\pm$ six months of each patient. The mean age and EMG_{para} value was calculated and then compared to the value obtained for each patient. Comparisons were made for both the pre- and post-bronchodilator values (for the paediatric asthma study) and admission and discharge values (in the CF study) against the control value.

In the 53 children with asthma, median (range) EMG_{para} pre-bronchodilator was significantly greater (8.36 (1.37 – 20.11) μ V) than in controls (5.48 (2.83 – 8.35) μ V, $p < 0.0001$). Despite significant reductions, the post-bronchodilator median (range) EMG_{para} remained significantly higher than in controls (6.30 (1.42 – 20.57) μ V, $p = 0.037$, Figure 43).

The median (range) percentage difference between EMG_{para} in children with asthma and healthy subjects was 67.47 (-82.83 – 573.30)% pre-bronchodilator and 20.62 (-82.21 – 628.10)% following bronchodilator administration.

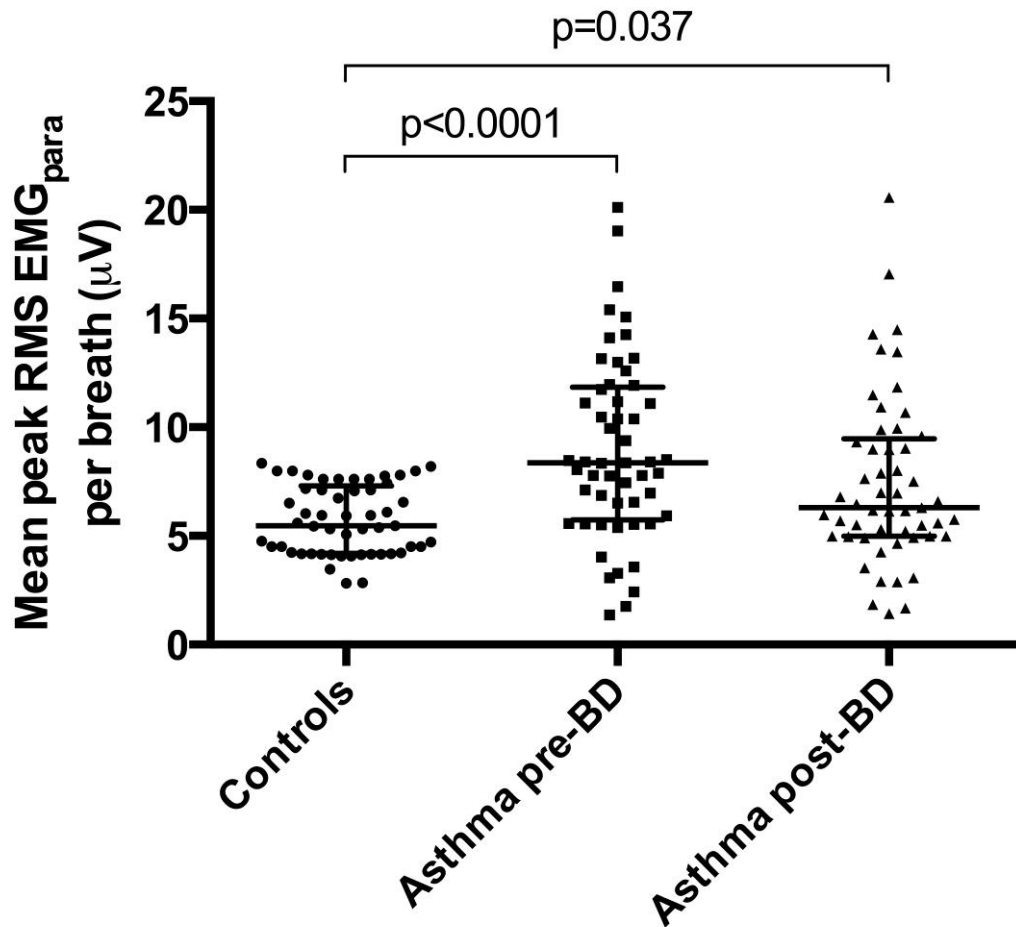


Figure 43 Difference between EMG_{para} in healthy subjects and children with asthma pre- and post-bronchodilator (BD). Lines denote median and inter-quartile range.

Median (range) EMG_{para} was higher in patients with CF at hospital admission (7.13 (3.19 – 13.18)µV) compared to healthy controls (4.40 (3.65 – 8.19)µV, $p=0.018$), but was not significantly different to controls at discharge (5.60 (2.62 – 13.45)µV, $p=0.433$, Figure 44). A retrospective power calculation showed that the data from the 20 children with CF and matched control sample were significantly underpowered to detect such a difference, achieving only 24% power at the 5% level, though 81% power was achieved to detect the difference between the healthy subjects and CF patients at admission.

The median (range) percentage difference between EMG_{para} in children with CF and healthy subjects was 25.97 (-23.59 – 215.70)% at hospital admission and 15.28 (-37.25 – 89.94)% on discharge.

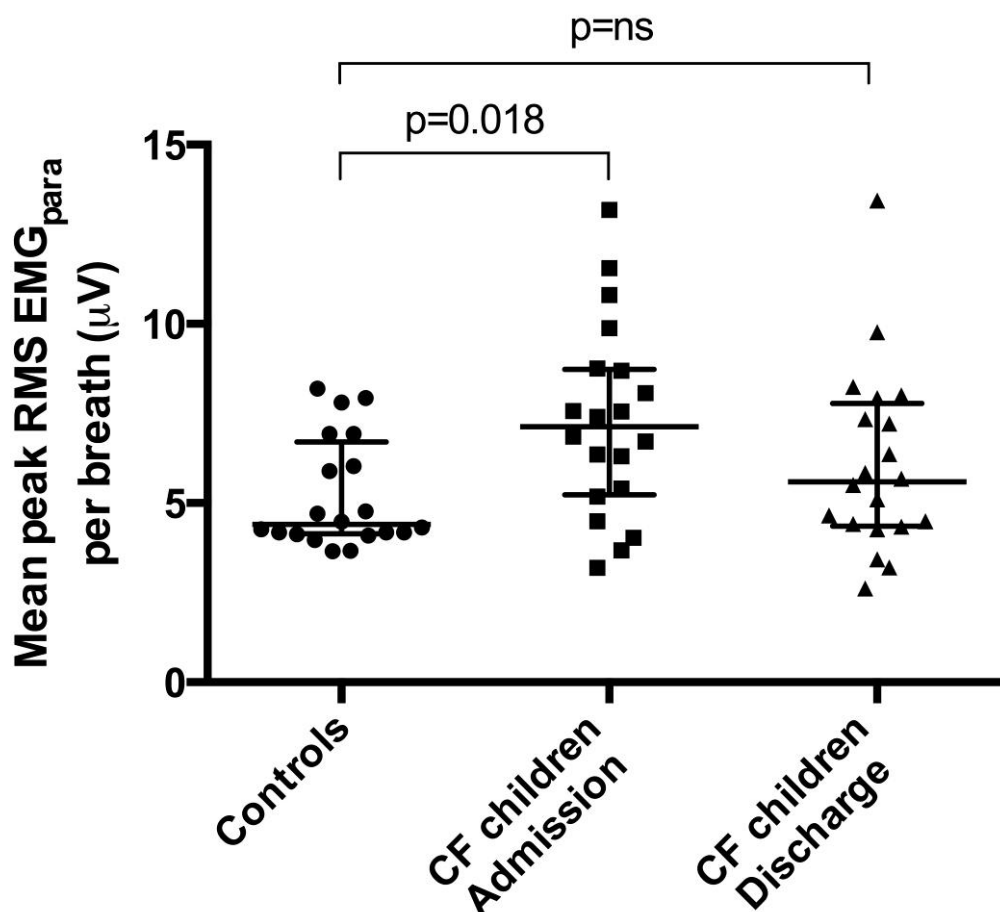


Figure 44 Difference between EMG_{para} in healthy subjects and children with CF at hospital admission and discharge. Lines denote median and inter-quartile range.

Correlation analysis indicated that, at hospital admission, the difference in EMG_{para} between the controls and patients was related to the degree of lung function impairment, as quantified by FEV_1 %predicted: $r=-0.678$, $p=0.002$. Such relationships were not observed at hospital discharge ($r=0.086$,

$p=0.735$) or in the children with asthma, either pre-bronchodilator ($r=-0.165$, $p=0.358$) or post-bronchodilator ($r=-0.21$, $p=0.242$).

7.5 Discussion

This study demonstrates the feasibility of obtaining EMG_{para} in healthy children and provides normative data for comparative purposes. Significant relationships between EMG_{para} and age, height and weight were observed, highlighting that a single reference value for EMG_{para} cannot be applied to children of all ages. The EMG_{para} values in healthy children were significantly lower than those in children with asthma pre- and post-bronchodilator, and lower than those in children with CF at hospital admission.

Although children of a range of ages were studied and despite the requirement to remain still during the recording of EMG_{para} , only six of 87 (6.9%) datasets were rejected due to movement artefact. Maximal EMG_{para} , however, was obtained in only 54 of the 81 successful recordings (66.7%) emphasising the difficulty of obtaining the maximal manoeuvres required to normalise EMG_{para} . Obtaining a maximal volitional manoeuvre when testing skeletal muscle strength in children is difficult [327]. The children who were unable to reliably perform a maximal inspiratory effort were significantly younger than those in whom $maxEMG_{para}$ was successfully obtained, although it should be noted that the upper age in both groups was similar (17.33 vs. 16.35 years). Although these older children were able to perform the manoeuvres required, the maximal EMG_{para} values were felt to have been contaminated by activity of other adjacent muscles and were consequently rejected. This was particularly notable in children with more developed chest wall musculature. It is not possible to confidently eliminate the possibility of EMG_{para} signal contamination during maximal inspiratory efforts from any of the subjects through the use of surface EMG measurements alone; this would necessitate comparison of signals obtained simultaneously from needle and surface electrodes. Future studies could examine the magnitude of EMG_{para} responses obtained during maximal and graded submaximal inspiratory efforts.

The values for raw EMG_{para} activity reported from this cohort of children are broadly in agreement with adult data, although demonstrate a much larger degree of variability. EMG_{para}%max values, however, were somewhat higher (7.04 (3.46 – 14.43)%) than those reported in healthy adult subjects. Reilly *et al* [172] reported a mean (SD) raw value of EMG_{para} of 4.8 (2.0)µV in healthy adults during resting breathing, equivalent to 5.8 (3.0)% of maximum. Similarly, Steier *et al* [197] described mean (SD) EMG_{para}%max values of 4.9 (3.2)%, although raw values of EMG_{para} were not reported. The higher overall EMG_{para}%max values observed in the current study are consistent with previous data showing increased respiratory drive as measured using P_{0.1} as age decreases [174].

Although the standard deviations of the EMG_{para}%max values given by Reilly *et al* and Steier *et al* were not wide in numerical terms, the data from these two studies give coefficients of variation (CV) of 51.7 and 65.3% respectively. The lower inter-subject CV for EMG_{para}%max (37.8%) in the current study may partly be a reflection of the larger sample size (n=54), compared to those in the studies by Reilly *et al* (n=15) [172] and Steier *et al* (n=12) [197].

The ability of adult subjects to perform reliable repeatable maximal manoeuvres allows the EMG_{para} signal to be normalised to maxEMG_{para}. Normalisation provides a means to control for variation in electrode placement within a subject on different measurement occasions as well as to allow comparison between individuals. Such manoeuvres cannot, however, be obtained in all children. In the current study the between-subject CV for the raw EMG_{para} signal across the whole group was 45.1%, indicating that this measure was not substantially more variable between subjects than EMG_{para}%max (37.8%). The between subject CV of the raw EMG_{para} signal in the 54 subjects in whom EMG_{para}%max could be measured was 41.1%, suggesting that subject groups unable to perform maximal manoeuvres do not demonstrate substantially higher variability in their EMG_{para} signal,

supporting the use of the raw EMG_{para} signal in subject groups unable to perform maximal manoeuvres. Slightly lower inter-subject variability is reported in paediatric populations for other measures of pulmonary function, such as R_{int} (27%) [328], impulse oscillometry (33-38%) and spirometry (32-35%) [322], though this may be a reflection of larger sample sizes and narrower age ranges in these other studies.

To evaluate the inter-subject variability of the raw EMG_{para} signal free of the confounding influence of the relationships observed between EMG_{para} and age/height/weight, subjects were divided into two-year subgroups and the between-subject CV for EMG_{para} within each subgroup calculated. No significant difference was found in the between-subject coefficient of variation for the raw EMG_{para} and $EMG_{para}\%max$ signals, indicating that the raw EMG_{para} signal may demonstrate acceptable variability to merit its consideration as a method for quantifying NRD without the need to normalise the signal.

The data regarding signal variability from both raw EMG_{para} and $EMG_{para}\%max$ from the current study and previous work, as indicated above, suggest that $EMG_{para}\%max$ may not confer additional advantages when compared to the raw signal alone in reducing inter-individual variability. The within-occasion repeatability measures of EMG_{para} and $EMG_{para}\%max$ allow the influence of small differences in electrode position on the EMG_{para} signal within individuals to be assessed. The bias of $-0.37\mu V$ obtained from the ten subjects who underwent repeat measurements was felt to be acceptable. The 95% limits of agreement are, however, relatively wide when considered in the context of the magnitude of the values obtained for raw EMG_{para} . If repeated measurements are to be taken from the same subject on different occasions, as in Chapter Six, then the repeatability of the measurement must be considered. The bias and 95% limits of agreement were, however, greater for $EMG_{para}\%max$ in the six subjects in whom repeated measures were performed, suggesting that raw

EMG_{para} may represent a more reproducible measure than EMG_{para}%max in a paediatric cohort.

The current data indicated an inverse relationship between age, height or weight and EMG_{para} but not EMG_{para}%max. The inverse relationship observed between maxEMG_{para} and age was, however, weaker than that between resting EMG_{para} and age, which may have been the result of greater variability in the maximal manoeuvre and hence the maxEMG_{para} signal.

Interference from adjacent muscles can pose a significant problem in the measurement and interpretation of surface EMG. Cross-talk from other muscles was unlikely to be a problem when recording the EMG from the parasternal intercostal muscles during resting tidal breathing in a healthy subject, due to the absence of active overlying musculature. During maximal efforts, however, the accessory muscles of respiration located in close proximity to the parasternal intercostal muscles such as the pectoralis major muscle may have been recruited, and contaminated the EMG_{para} signal. In an attempt to eliminate accessory muscle use subjects were instructed to perform maximal inspiratory efforts without moving or bracing the upper limbs. Such manoeuvres are, however, difficult for a child to perform without the actions that would normally facilitate a maximal effort. A number of maximal efforts were rejected from signal analysis based on the appearance of the EMG signal that suggested the EMG activity was not purely from the parasternal intercostal muscles. As stated above, the variability of the maxEMG_{para} signal was greater than that in the resting EMG_{para} signal, despite efforts to exclude contaminated signals, suggesting potentially greater signal contamination from cross-talk during forced inspiratory efforts.

A standard 19mm diameter electrode was used to record EMG_{para} in all children regardless of age. In younger subjects, thoracic size and hence parasternal intercostal muscle size will be smaller in relation to the size of the electrode. It could be postulated that the magnitude of the EMG signal

would have been directly proportional to electrode size, as in younger children the active area will cover a greater proportion of the muscle and hence sample from a larger number of motor units.

A surface electrode can be viewed as an arrangement of point electrodes and its net potential is equivalent to the mean rather than the sum of the potentials produced by each single electrode [329]. Electrode size in theory, therefore, has little or no influence on the amplitude of the EMG signal [329, 330]. This assumption, however, is based on mathematical modelling and has not previously been confirmed *in vivo*. It is possible that, while the electrode size does not directly influence signal amplitude from the parasternal muscles, the physically smaller thoracic size in young children would place the electrodes in closer proximity to the accessory respiratory muscles, potentially resulting in greater signal contamination when compared to adults. This was tested by obtaining measurements of EMG_{para} in twelve healthy individuals (six young children, three older children and three adults) using electrodes of two different diameters.

Measuring EMG_{para} with electrodes of two sizes showed a bias toward a slightly higher value for EMG_{para} measured using a larger electrode. There was, however, no significant difference between the values obtained with the two electrode sizes and the 95% limits of agreement obtained from Bland-Altman analysis were relatively wide. There was closer agreement between the measurements of EMG_{para} from the two sizes of electrode in the older than in the younger subjects. The bias in the older subjects in fact demonstrated a tendency towards higher values from the smaller electrode. The bias and 95% limits of agreement were similar to those shown in the ten subjects who underwent repeat measurements, suggesting that the difference in EMG_{para} values when measured with the two electrode sizes may represent simply the variability of the measure. Bland-Altman analysis also showed a tendency towards poorer agreement between values at higher levels of EMG_{para} activity. Correlation analysis showed an inverse relationship between age and the difference in the measurements from the

two electrode sizes, indicating greater differences in younger children. A similar rather than a weaker relationship between $\text{maxEMG}_{\text{para}}$ and age (or height/weight) than between resting EMG_{para} and age might have been expected if the change in NRD with age or growth was due to relative electrode-to-muscle size. Under circumstances of maximal muscle activation, it would be expected that any influence of electrode size on signal amplitude would be accentuated. In addition, $\text{maxEMG}_{\text{para}}$ was also not significantly different when measured with two electrode sizes.

Gaultier *et al* [174] demonstrated an inverse relationship between age and respiratory drive, measured as $P_{0.1}$. The reduction in NRD that occurs with increasing age could be due to maturational changes in the respiratory system, including ribcage ossification [48, 49], increases in length of the abdominal hydrostatic column [48], and lung growth and maturation [37-40, 42-47]. It is likely that the substantial increases in airway diameter, and concomitant reductions in airways resistance, occurring with growth has the greatest influence on the changes observed in EMG_{para} with increasing age.

When considering whether the raw EMG_{para} signal or $\text{EMG}_{\text{para}}\%_{\text{max}}$ should be used in future studies, it should be noted that reliance on a normalised signal requires the subject to be able to reliably perform a maximal inspiratory effort, thus limiting the applicability of the measure. The upper limit of the range of $\text{EMG}_{\text{para}}\%_{\text{max}}$ values obtained in the current study (14.43%_{max}) is markedly greater than those reported in adult subjects [172, 197], suggesting that true $\text{maxEMG}_{\text{para}}$ may not have been attained in all subjects in the current study during inspiratory efforts. All recordings of $\text{maxEMG}_{\text{para}}$ manoeuvres were assessed for consistency, with the manoeuvre repeated at least three times, and subjects were given both a practical demonstration and verbal encouragement to promote performance of the optimal technique. Nonetheless, true maxima can be difficult to obtain in many populations, not least children [327, 331], and

reproducibility may not be an indication of maximality when performing maximal respiratory efforts [332].

The effect of differences in BMI is important when considering the use of surface EMG_{para}. Subcutaneous adipose tissue acts as a low-pass filter and decreases the amplitude of the resultant EMG signal [333]. The results from the current study indicated relationships between both EMG_{para} and maxEMG_{para} and BMI. As the change in BMI during childhood is non linear [334], BMI-for-age percentile was calculated for all children to eliminate the effect of age. The strength of the relationships between EMG_{para} and BMI-for-age was weaker but remained significant, with the EMG signal amplitude decreasing with increasing adiposity. Neural respiratory drive, measured using EMG of the diaphragm (EMG_{di}), has previously been shown to be increased in obesity [311]. The use of oesophageal EMG_{di} eliminates the filtering effects of adipose tissue and thus represents a more accurate representation of the effect of increased BMI on NRD. The increases in respiratory load, and hence respiratory drive and respiratory muscle EMG, will be attenuated when using surface EMG techniques due to the filtering effect of the subcutaneous fat. In the current study, 17 of the 81 subjects (21.0%) were classified as overweight, with five of these classified as obese. Steier *et al* [311] examined the influence of obesity on EMG_{di} in a cohort of adult patients with a mean (SD) BMI of 42.8 (8.6)kg.m⁻²; in contrast, the maximum BMI in the current study was 35.5kg.m⁻². The median (range) BMI (24.0 (18.2 – 35.5)kg.m⁻²) in the 17 overweight subjects in the current study may not have been sufficient to cause a substantial increase in respiratory load and hence NRD. The relationship between EMG_{para} and BMI in the current study may be, therefore, a result of signal filtering rather than physiological alterations, though this remains an important consideration when utilising this technique.

7.5.1 Difference between healthy subjects and patients

EMG_{para} values in the patient groups studied in Chapters Three and Five are significantly higher than those obtained from healthy controls in the current study. EMG_{para} remained higher in children with asthma than in healthy subjects even following bronchodilator administration. Although no difference was seen in EMG_{para} in children with CF at hospital discharge when compared to healthy controls, the sample size was too small to achieve sufficient statistical power. The relationship observed between the severity of airways obstruction (as quantified by FEV₁) and the difference between EMG_{para} in healthy children and patients with CF on admission further supports the utility of EMG_{para} as a measure of disease severity.

The use of a large cohort of healthy subjects allowed values from individual patients to be compared to average values calculated from a number of healthy subjects aged within six months of that of the patient. Six months was chosen as a timeframe during which major maturational differences were unlikely to be present. Despite the statistically significant difference between the groups, an overlap is noted, with some patients demonstrating lower EMG_{para} activity than the comparative healthy values; the greatest difference was EMG_{para} activity 82.83% lower in a patient with asthma than in the matched healthy data. Determining the ability of EMG_{para} to discriminate between healthy children and patients with respiratory disease is also confounded by the fact that patients with very mild disease were included in both Chapter Three and Chapter Six. The overlap between groups may reflect an inability of EMG_{para} to detect the small increases in respiratory load associated with mild disease. It would also be preferable to obtain normative data from a larger cohort such that age-specific reference ranges could be developed and EMG_{para} expressed as a percentage of predicted values or z-scores. It is recommended that reference data sets comprise a minimum of 300 subjects in order to avoid significant effects of outliers [335].

7.6 Conclusions

The aims of the study were to produce normative data for EMG_{para} for comparative purposes, and to investigate the utility of the raw EMG_{para} signal. It is clear that use of the raw signal allows the technique to be applied to a wider range of subjects than relying on a value of $EMG_{para}\%max$. The inter- and intra-individual variability of the EMG_{para} signal also appears to be less than that for $EMG_{para}\%max$. Significant relationships between age, height and weight and raw EMG_{para} values indicate that a single reference value for EMG_{para} cannot be applied to all age groups. While the lack of a significant bias between measures of EMG_{para} obtained using different electrode sizes suggests that the relationships are physiological, rather than technical, the small sample size from which to draw conclusions means that this remains to be fully clarified. EMG_{para} is significantly higher in children with asthma and CF than in healthy children, lending further support to the use of this measure to assess respiratory disease in children.

8. Chapter Eight: Discussion

The importance of accurate measurement of pulmonary function in quantifying respiratory disease severity, progression and response to treatment is well documented [11, 303]. Spirometry is currently the technique used most commonly in assessing lung disease, though the requirement for the subject to perform complex, maximal, volitional respiratory efforts limits the applicability of this technique in a range of patient populations, including younger children and those patients with more severe disease [14, 31, 113]. Alteration of the respiratory load-capacity balance that occurs in lung disease and the resultant increase in the neural output from brainstem respiratory centres can be quantified via the electromyogram of selected inspiratory muscles. The parasternal intercostal muscles, as obligate muscles of inspiration, easily accessed with surface EMG techniques, represent a clear choice for assessment of neural respiratory drive.

The data within this thesis have demonstrated EMG_{para} to be a feasible technique in the paediatric setting, in a range of ages and clinical conditions. EMG_{para} changes in response to a variety of interventions that alter the load-capacity balance of the respiratory system. Exploration of the relationship between EMG_{para} and existing measures of pulmonary function has indicated that such relationships are likely to be complex. While previous work has compared measures of neural respiratory drive to spirometry on a single occasion as a marker of disease severity [172, 196, 197, 214], the work contained within this thesis has expanded the understanding of the relationship between dynamic changes in EMG_{para} and conventional measures of pulmonary function.

Previous data examining the use of EMG_{para} have expressed it relative to either a baseline measurement obtained from the same patient (the logarithm of the EMG activity ratio (logEMGAR)) [185-187, 194, 195, 336], or to the signal obtained during a maximal volitional effort ($EMG_{para}\%max$)

[172, 192, 198, 199]. The former method limits the application to assessment of within-subject change, while the latter cannot be used in subjects unable to comply with maximal inspiratory efforts. The studies within this thesis have utilised the raw EMG_{para} signal, expressed in microvolts (μV), in healthy children and in a range of presentations of obstructive lung disease, and the data suggest this to be an acceptable method of expressing the EMG_{para} signal, therefore allowing EMG_{para} to be applied to a wider range of subjects and scenarios than would be possible with the previous methods ($\log EMGAR$ or $EMG_{para}\%max$).

8.1 Response to change

Chapter Three demonstrated the ability of EMG_{para} to detect change in respiratory load in asthmatic children following a relatively simple intervention, administration of a β_2 agonist to reverse bronchoconstriction. This was demonstrated both in children over the age of five, who were able to complete spirometry, and in children of pre-school age. Similarly, significant change in EMG_{para} was demonstrated in children with cystic fibrosis undergoing inpatient treatment for an exacerbation of CF lung disease. In both of these studies, the EMG_{para} technique was shown to be feasible in children unable to complete existing standard measures of lung function, including those as young as eight months of age. Although other measures of pulmonary function are available for use in very young children and infants [236], the techniques may require adaptation, making longitudinal interpretation of the results challenging. For example, while infant lung function testing (including the rapid thoraco-abdominal compression technique and infant plethysmography) is comparable to spirometry, the child must be sedated during the procedure [236]. Similarly, while spirometry in children between the ages of three and five years of age may be feasible in greater than 50% of subjects [111], the small lung volumes and large airway size relative to lung volume results in

expiratory times less than one second. In younger children, $FEV_{0.5}$ and $FEV_{0.75}$ are therefore used [119], making longitudinal interpretation of spirometry values more complex when transitioning to the use of FEV_1 . In contrast, the EMG_{para} technique is consistent regardless of age, disease severity or cognitive ability, meaning results can be continuously interpreted throughout life.

Inter-minute variability of the EMG_{para} signal was also evaluated in a subset of children with asthma, allowing the development of a threshold for statistically significant change in EMG_{para} to be determined. This methodology was based on previous work using the within-subject variability to develop a threshold for significant bronchodilator reversibility based on FEV_1 [217]. Applying the method used to determine a threshold for significant change in FEV_1 , where each measurement is a discrete value obtained from the best of three maximal expiratory efforts, to EMG_{para} , which may be a continuous recording of any duration, may be somewhat problematic. It may be of benefit to examine in future studies the relationship between inter-minute variability and other aspects of signal variability. Future studies may also need to expand on this to develop a minimal clinically important difference in EMG_{para} by anchoring against other outcome measures [337]. Nevertheless, knowledge of the variability of the measure represents an important early step in potentially moving this technique towards clinical use. Inter-minute variability of the EMG_{para} signal was also assessed in the large cohort of healthy children included in Chapter Seven, and was shown to be similar to that observed in the children with asthma (8.95% compared to 9.24%), suggesting little difference in within-occasion variability of NRD between asthmatic and healthy children.

8.2 Relationship with conventional measures of pulmonary function

While previous studies have shown clear relationships between measures of NRD (both EMG_{di} and EMG_{para}) and conventional measures of pulmonary function [172, 189, 194], the studies contained within this thesis did not observe consistent relationships between changes in spirometry, oscillometry or end expiratory lung volume and those in EMG_{para} . In Chapter Three, no relationship was observed between changes in FEV_1 and EMG_{para} in 33 children over the age of five years undergoing bronchodilator reversibility testing. EMG_{para} was also shown to be poorly predictive of a significant ($\geq 12\%$) increase in FEV_1 following β_2 agonist administration. Similarly, in the subset of twelve subjects in whom impulse oscillometry was measured, no relationship was observed between changes in IOS parameters and changes in EMG_{para} . These results were reflected in those from Chapter Six, with no relationship between the admission to discharge changes in EMG_{para} and spirometric or oscillometric parameters. There was a relationship observed, however, between the change in EMG_{para} and the FEV_1 on admission, indicating that those children with the greatest lung function impairment experienced the greatest reduction in respiratory load. There was also a trend observed towards a relationship between EMG_{para} and FEV_1 on admission, though this did not reach significance.

To investigate further the relationship between conventional measures of pulmonary function and EMG_{para} in an asthmatic phenotype, incremental changes in spirometry and EMG_{para} were induced using methacholine challenge testing. Chemically induced bronchoconstriction may differ somewhat from the spontaneous airways obstruction occurring in asthma. Pragmatic reasons also necessitated the use of adult subjects in this study, thus caution must be exercised in using the results to inform and explain those obtained in asthmatic children with spontaneous bronchoconstriction.

A moderate correlation was observed between incremental changes in FEV_1 and those in EMG_{para} from the first methacholine dose to end of test, as

assessed by area under the curve analysis. The magnitude of the change in EMG_{para} was significantly greater than that in FEV_1 , when examined from first methacholine dose to end of test both using overall percentage change and area under the curve analysis. The percentage change in EMG_{para} was also greater than that in FEV_1 in both children with cystic fibrosis and those with asthma, though the difference did not reach significance in the asthmatic group. These data indicated that factors additional to the larger airways obstruction measured by FEV_1 may be responsible for the changes in EMG_{para} observed in airways obstruction.

Hyperinflation is known to be present in a range of obstructive lung diseases and may occur in the absence of overt airways obstruction as quantified by FEV_1 [288]. Hyperinflation has previously been shown to relate to increases in NRD [172, 189], though the relationship between dynamic changes in end expiratory lung volumes and EMG_{para} has not previously been reported. Significant small airways obstruction has also been demonstrated in, among other diseases, asthma [30] and cystic fibrosis [31, 338], and may contribute to respiratory load and hence EMG_{para} . Chapter Five was therefore designed to investigate in depth the pathophysiological changes occurring during induced bronchoconstriction, and to relate these to the changes in EMG_{para} .

The results of Chapter Five indicated, however, that evaluating the relative influences of small and large airways obstruction and end expiratory lung volume on EMG_{para} is made difficult by the simultaneous and heterogeneous nature of these changes. Subjects demonstrated differing degrees of change in each parameter; isolating the effect of changes in each parameter was therefore not possible with the sample size used. The study was able to demonstrate clear changes occurring in small airways resistance and end expiratory lung volume, in addition to the changes previously observed in FEV_1 in Chapter Four. Although clear relationships have not been demonstrated between these measures and EMG_{para} , the presence of such changes nonetheless suggests that these may well be factors in determining the magnitude of changes in EMG_{para} . Formulating a study design that

allows the influence of such measures to be evaluated in isolation is likely to be difficult; larger studies that allow multiple regression analysis to be undertaken are likely to represent the best method, although the current reliance on manual analysis of the EMG_{para} data makes such studies formidable.

The lack of strong and/or consistent relationships between EMG_{para} and conventional measures of pulmonary function in the studies included within this thesis must also be considered in the context of the populations studied, most of which had markedly less pulmonary function impairment compared to previous studies. Reilly *et al* [172] included patients with CF with a mean (SD) FEV₁ of 53.5 (24) %predicted. Similarly, Jolley *et al* [189] showed relationships between EMG_{di} and FEV₁, inspiratory capacity and vital capacity in 30 subjects with COPD with a mean (SD) FEV₁ of 34.8 (13.9) %predicted. Jolley *et al* also described the relationship between EMG_{di} and FEV₁, IC or VC to be curvilinear. It may be that the majority of the subjects included in this thesis, due to their milder disease, are situated on the flatter portion of the FEV₁-EMG curve.

The most likely factor underlying the lack of relationship between conventional pulmonary function measures and EMG_{para}, however, is the consideration that such measures are unidimensional, assessing only a single aspect of respiratory physiology. EMG_{para} can be considered likely to provide a composite measure of the overall load-capacity balance of the respiratory system, thus reflecting in a single measure all of the pathophysiological changes in obstructive lung disease. FEV₁ is a measure of obstruction in predominantly proximal airways, and is therefore relatively insensitive to the substantial airflow limitation in smaller, more distal airways, such as occurs in asthma [30] and early CF lung disease [31]. Changes in small airways function have been demonstrated in the studies contained within this thesis, in addition to significant changes in FEV₁ and, in Chapter Five, end expiratory lung volume. These changes may occur to differing extents within individual subjects, resulting in a lack of direct

relationships between individual measures and EMG_{para} . The multidimensional nature of EMG_{para} , however, represents the major advantage of this measure over existing techniques, and the lack of relationship between the different variables should not, perhaps, be viewed as a major weakness of the EMG_{para} technique.

The studies within Chapters Four and Five, in which an expected fall in EMG_{para} was observed between the saline and first methacholine inhalations in adults undergoing bronchial challenge testing, indicate another potential weakness of the EMG_{para} technique. Although EMG_{para} appears to have clear potential as a marker of respiratory load, it is strictly a measure of respiratory muscle activity and can therefore be influenced by higher centres as well as by the metabolic drive to breathe. We hypothesised that anxiety may have been the reason for the fall in EMG_{para} observed between the saline and first methacholine step in Chapters Four and Five and this is an important consideration for future studies. Subjects should be given sufficient time to acclimatise to the environment and familiarise themselves with the equipment before data acquisition begins, and may benefit from distraction, as was provided for the paediatric subjects. Simultaneous measurements of end tidal CO_2 may assist in screening for the presence of hyperventilation, though the behaviour of EMG_{para} during hypocapnic ventilation has not been investigated to date.

8.3 Technical and methodological considerations

Chapter Seven provided normative data regarding levels of EMG_{para} activity in children over a range of ages. While the cohort is insufficient in size to develop true reference values, it nonetheless represents a much larger cohort of healthy subjects than have been measured previously [172, 192, 197, 198]. This large data set has provided insight into the changes in EMG_{para} that occur with growth and development, emphasising that a single

reference value cannot be applied to all children. The reduction in EMG_{para} activity with increasing age is likely to be multifactorial, related to changes in chest wall configuration, muscle development and maturation of lung tissue and vasculature [41-43, 48, 55], and mirrors the reduction in respiratory drive as quantified using $P_{0.1}$ shown in previous work [174]. A small sub-study was performed to evaluate whether the differences in EMG_{para} signal magnitude may have been due to the relative muscle to electrode size; the results of this small laboratory study provide *in vivo* support for previous modelling data suggesting that electrode size does not affect signal magnitude [329].

The data from Chapter Seven also indicate that the raw EMG_{para} signal demonstrates acceptable reproducibility when compared to $EMG_{para}\%max$. Although in the healthy subjects, the difference in inter-subject variability of the raw EMG_{para} and $EMG_{para}\%max$ signals did not reach statistical significance, the data in Chapter Six showed significantly greater variability in the $EMG_{para}\%max$ signal. This variability was influenced predominantly by variation in $maxEMG_{para}$. Although previous studies have suggested that, in order to successfully interpret EMG_{para} data obtained on different occasions, the EMG_{para} signal must be normalised to that obtained during a maximal manoeuvre ($EMG_{para}\%max$) to account for differences in electrode placement and relative skin to muscle geometry [172, 192, 198, 199], the data contained within this thesis would appear to contradict this.

Given these data regarding the increased variability of $EMG_{para}\%max$, as well as the apparent utility of the raw EMG_{para} signal, it would appear that the raw EMG_{para} signal warrants further investigation. EMG_{para} can be used for within-occasion assessment of change without requiring normalisation (for example during assessment of bronchodilator response, or in airway challenge testing), but in order to allow assessment of disease severity or longitudinal monitoring of individual subjects, further data will be required regarding the reproducibility of the resting EMG_{para} signal. That a significant difference was seen between raw EMG_{para} from children with asthma or CF

and age-matched data from healthy subjects supports further investigation of the raw signal. Furthermore, the use of raw EMG_{para} allows the technique to be applied to a much wider range of individuals. The substantial number of healthy children in whom maxEMG_{para} could not be obtained (27 of 81 children), including children able to perform the manoeuvre but only with levels of signal contamination deemed unacceptable, as well as younger children, highlights the weakness of relying on maximal volitional efforts for the measurement of EMG_{para}. Similarly, 20% of the subjects included in Chapter Six were unable to consistently perform maximal manoeuvres to a satisfactory standard. The use of the raw EMG_{para} signal therefore would allow the clinical application of this technique not only to very young children and infants, but also to individuals with significant cognitive impairment, including children with severe developmental delay who may develop respiratory diseases such as bronchiectasis [339], and sedated patients in critical care environments. Indeed, the Neurally Adjusted Ventilatory Assist (NAVA) technology already in use clinically utilises the raw EMG_{di} signal [340]. One major consideration when using the raw EMG_{para} signal is the effect of chest wall adiposity on the magnitude of the signal (as observed in Chapter Seven); with increasing obesity in the paediatric population [341, 342], this is likely to be an important matter and warrants further attention.

8.4 General critique of the method

Although the sample sizes within the clinical studies included within this thesis were larger than those in many previous studies, some studies, or subanalyses therein, remained underpowered, therefore limiting the conclusions that can be drawn from the data. Larger sample sizes were not feasible for a number of reasons, primarily the time required for analysis of the EMG_{para} signal. Development of automated analysis algorithms may facilitate larger studies.

The patient populations studied in Chapters Three and Six had relatively mild and/or well controlled respiratory disease, which may have impacted on the ability to detect relationships between variables. Similarly, the magnitude of respiratory load induced by the methacholine challenge test was relatively small due to most of the subjects having normal or even supra-normal lung function at baseline.

Inter-rater reproducibility of the EMG_{para} signal analysis was not assessed during the undertaking of these studies. Although good inter-rater reliability has previously been demonstrated for respiratory EMG [172], this has not been assessed as part of the work undertaken within this thesis.

8.5 Future work

The successful use of EMG_{para} in the studies contained within this thesis in younger children who were unable to perform conventional measures of pulmonary function suggests that this population represents the first in which EMG_{para} should be explored clinically. The data from Chapter Three demonstrated a fall in EMG_{para} occurring following administration of bronchodilator in pre-school children with asthma or wheeze. Bronchodilator reversibility testing is used in older children and adults as a marker of asthma control and can help to guide decisions regarding the need for medication changes. To build on the data from Chapter Three, the utility of EMG_{para}-based bronchodilator reversibility testing in predicting treatment outcome in children with pre-school wheeze commenced on preventer medication could be evaluated. We would hypothesise that children with a significant reduction in EMG_{para} following bronchodilator administration would be more likely to benefit from inhaled corticosteroids, and we propose a study design whereby the ability of the EMG_{para} reversibility test to predict clinical improvement would be evaluated using receiver-operator characteristic analysis.

The data from Chapter Six indicate that EMG_{para} may have promise in monitoring patients hospitalised with an acute respiratory illness. This thesis has demonstrated that EMG_{para} can be undertaken in subjects who are either unable to perform conventional measures of pulmonary function. Infants with bronchiolitis represent such a group. One-third of all infants are thought to develop bronchiolitis within the first year of life, and approximately nine percent of these will require hospitalisation, although this rate is higher in infants with pre-existing medical conditions [2, 343]. Rapid clinical deterioration can occur in some infants and EMG_{para} may represent a method for identifying this deterioration, thereby allowing earlier escalation of care and possibly improving outcomes.

The non-volitional nature of the technique also holds promise in areas such as intensive care. Neurally adjusted ventilatory assist (NAVA), whereby the delivered ventilatory support is titrated according to the magnitude of the EMG_{di} signal [340], has been in clinical use for a number of years. However, NAVA utilise the EMG signal from the diaphragm and therefore necessitates the use of an oesophageal catheter, and can only be used in conjunction with the NAVA ventilator, limiting flexibility and applicability of the technique. Accurately predicting extubation outcome and optimising weaning of ventilatory support present significant and important clinical problems in paediatrics [344]. As a marker of respiratory load relative to capacity, EMG_{para} may have potential in informing decisions regarding ventilator weaning and readiness for extubation, as well as guiding the need for post-extubation respiratory support. The non-invasive nature of the technique and independence from any other equipment makes it well suited to brief measurements rather than the ongoing monitoring offered by the NAVA system. Future studies should evaluate the feasibility of performing EMG_{para} measurements within the paediatric intensive care environment, as well as the response of the EMG_{para} signal to changes in ventilatory support, with a view to considering the technique as a potential predictor of extubation outcome or to facilitate decisions about optimal ventilator settings.

8.6 Conclusion

The data within this thesis have demonstrated that EMG_{para} is sensitive to change in respiratory load in a range of clinical scenarios. It is a feasible tool for use in populations in whom assessment of pulmonary function has, to date, been difficult to objectively assess. Although conducted in adult, rather than paediatric populations, the data obtained during methacholine challenge testing highlights the complexity of the pathophysiological changes occurring in obstructive lung disease and may help to explain the lack of clear relationships observed between EMG_{para} and existing conventional measures of pulmonary function in the paediatric studies. Technical aspects of EMG_{para} signal acquisition have been explored and, together with differences observed between the EMG_{para} signals obtained from healthy children and those with asthma and CF, the use of the raw EMG_{para} signal has been supported, opening up the use of this technique to virtually any patient population.

9. Chapter Nine: References

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List of abstracts published during this thesis

MacBean, V., A.C. Lunt, V. Palit, C.J. Jolley, A. Greenough, J. Moxham, G.F. Rafferty *Electromyography Of The Parasternal Intercostal Muscles Detects Change In Neural Respiratory Drive In Asthmatic Children* Am J Resp Crit Care Med, 2011. **183**: A5428

MacBean, V., M.A.F. Siddiqui, A. Greenough, J. Moxham, G.F. Rafferty *Neural Respiratory Drive In Healthy Children* Am J Resp Crit Care Med, 2012. **185**: A6145

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