Non-invasive measures of neural respiratory drive in children: the utility of respiratory muscle electromyography

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Background and aims

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The reliability of a developed method to quantitatively assess sEMG of the diaphragm (sEMGdi) recorded using a commercial sleep study set up was examined. Surface EMG of the diaphragm recorded from snoring children with and without obstructive sleep apnoea, and also from children with sleep-disordered breathing before and after treatment with pressure support were compared. Inspiratory muscle (scalene, parasternal intercostal, and diaphragm) sEMG recorded from healthy children during tidal breathing and maximal inspiratory ramps were evaluated. Variability in peak inspiratory muscle sEMG recorded during different maximal inspiratory manoeuvres were assessed. Linear mixed models were used to assess factors affecting sEMG magnitude.

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A reliable method using sEMGdi to assess NRD was developed and demonstrated that NRD was significantly higher in children with obstructive sleep apnoea and increased work of breathing compared to healthy snorers. Provision of positive airway pressure support decreased NRD as reflected by decreased sEMGdi from baseline in children with sleep-disordered breathing. Inspiratory muscle sEMG had a curvilinear/linear relationship with increasing lung volume and pressure. Inspiratory muscle tidal sEMG had a negative linear relationship with age and body mass index (BMI), but not biological sex. Postural changes can affect inspiratory muscle sEMG. Peak sEMG of the inspiratory muscles were recorded from the majority of the children when performing the maximal sniff inhalation manoeuvre. Normalising sEMG to a maximal value abolished the influence of BMI, but not age, on sEMG.

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Diaphragm sEMG recorded from children with and without sleep-disordered breathing using commercial equipment can be quantitively measured to evaluate NRD. Inspiratory muscle sEMG in children is affected by age and BMI. Future work needs to address standardisation of the methodology in order to translate this application into routine clinical practice.

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*Authors :*Chuang SY, Teng A, Butler JE, Gandevia SC, Selvadurai H, Jaffe A
Journal or book name: Respiratory Physiology & Neurobiology
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This paper will be in lieu of Chapter 2 Methods. The paper describes the development of the methodology used to quantitatively assess diaphragm electromyography recorded using clinical polysomnography set up and equipment. The method detailed was subsequently used to assess diaphragm EMG recorded in children during sleep study for the result chapters, Chapter 3 Quantitative assessment of nocturnal neural respiratory drive in children with and without obstructive sleep apnoea using surface EMG; and Chapter 4 Non-invasive respiratory support improves neural respiratory drive in children with sleep- disordered breathing.

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This paper will be included in lieu of Chapter 3, the first of the result chapters. The paper discussed the use of surface electromyography of the diaphragm (sEMGdi) as a quantitative index for assessing neural respiratory drive in snoring children during overnight sleep studies. The paper demonstrated significant higher level of sEMGdi in children with obstructive sleep apnoea and increased work of breathing, compared to healthy snorers. The paper highlights the potential clinical application of sEMGdi as a complementary respiratory variable in clinical sleep studies to assess breathing in children during sleep.

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SC was involved in the conception of the paper, performed all data collection, performed analysis including statistical analysis on all samples, interpreted data, wrote manuscript, and acted as corresponding author. Overall percentage of SC's contribution to the chapter 80%.

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This paper will be included in lieu of Chapter 4, the second of the result chapters. The paper discussed the use of surface electromyography of the diaphragm (sEMGdi) as a quantitative index for assessing changes to respiratory load (and hence neural respiratory drive) when children with sleep-disordered breathing are treated with positive airway pressure support. The paper demonstrated significant improvement in sEMGdi after positive airway pressure was applied during sleep in children with obstructive and non-obstructive sleep-disordered breathing. The paper highlights another potential clinical application of sEMGdi as a complementary respiratory variable in clinical sleep studies to apnoea hypopnoea index and gas exchange parameters when determining optimal pressure for treatment of children with sleep-disordered breathing.

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Supervisor's name Prof Adam Jaffe	*Supervisor's signature*	*Date (dd/mm/yy)* 17/07/2019

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Abstract

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Chuang S, Luu B, Butler J, Gandevia S, Jaffe A. (2017). Inspiratory muscle recruitment during dynamic inspiration in healthy children'. *European Respiratory Journal*, 50(supp61), PA1302

Chuang S, Teng A, Butler J, Gandevia S, Selvadurai H, Jaffe A. (2016). CPAP can improve respiratory effort as measured by surface electromyogram of the diaphragm (sEMGdi) in children with sleep-disordered breathing. *European Respiratory Journal*, 48 (supp60), PA 4351

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List of Abbreviations

%max	Percentage of maximum
ΔPao	Change in airway opening pressure
ΔPdi / EMGdi	Change in transdiaphragmatic pressure for a given level of diaphragm electromyography
AASM	American Academy of Sleep Medicine
AB	Abdominal
AHI	Apnoea hypopnoea index
ANOVA	Analysis of variance
BMI	Body mass index
BPAP	Bilevel positive airway pressure
CAI	Central apnoea index
cathEMG	Electromyography recorded using catheter mounted electrodes
cathEMGdi	Crural diaphragm electromyography recorded using catheter mounter electrodes
CF	Cystic fibrosis
CMAP	Compound muscle action potential
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure support
CV	Coefficient of variation
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electro-oculogram
FEV1	Forced expiratory volume in 1 second
FRC	Functional residual capacity

FVC	Forced vital capacity
IC	Inspiratory capacity
IQR	Interquartile range
imEMG	Electromyography recorded using intramuscular electrodes
incWOB	Increased work of breathing
LnEMG%max	Logarithmic transformation (natural log) of the variable sEMG%max
MIP	Maximal inspiratory pressure
MEP	Maximal expiratory pressure
MUAP	Motor unit action potential
mV	Millivolts
NAVA	Neurally adjusted ventilatory assistance
NRD	Neural respiratory drive
NREM	Non rapid eye movement sleep
O_2 sat	Oxygen saturation
OAHI	Obstructive apnoea hypopnoea index
OAI	Obstructive apnoea index
ODI3	3% Oxygen desaturation index
OEP	Optoelectronic plethysmography
OSA	Obstructive sleep apnoea
Pab	Abdominal pressure
PAP	Positive airway pressure therapy
Pdi	Transdiaphragmatic pressure
Pgas	Gastric pressure
Pmo	Mouth pressure
Poes	Oesophageal pressure
Ppl	Pleural pressure

PSG	Polysomnography, overnight sleep studies
PTPdi	Pressure time product of the diaphragm
PTPoes	Pressure time product of the oesophageal pressure (i.e. index of global inspiratory muscle load)
RC	Rib cage
REM	Rapid eye movement sleep
RIP	Respiratory inductance plethysmography
RMS	Root mean square
RR	Respiratory rate
RV	Residual volume
SD	Standard deviation
sEMG	Surface electromyogram; electromyogram recorded using transcutaneous electrodes
sEMG%max	Magnitude of the surface electromyogram from a muscle presented as a percentage of the maximal EMG value recorded from the same muscle during a maximal inspiratory manoeuvre.
sEMGcw	Surface electromyogram of the lower chest wall (electrode placed on the skin overlying costal diaphragm; an alternative term for sEMGdi)
sEMGdi	Surface electromyogram of the diaphragm
sEMGdi%max	Surface electromyogram of the diaphragm presented as a percentage of the maximal diaphragm EMG value recorded during a maximal inspiratory manoeuvre.
sEMGdi,peak	Peak or maximal surface electromyogram of the diaphragm recorded during a maximal inspiratory manoeuvre
sEMGpara	Surface electromyogram of the parasternal intercostal muscle

sEMGpara%max	Surface electromyogram of the parasternal presented as a percentage of the maximal parasternal EMG value recorded during a maximal inspiratory manoeuvre.
sEMGpara,peak	Peak or maximal surface electromyogram of the parasternal recorded during a maximal inspiratory manoeuvre
sEMG,peak	Peak or maximal surface electromyogram recorded when performing maximal inspiratory manoeuvres
sEMGsc	Surface electromyogram of the scalene
sENGsc,peak	Peak or maximal surface electromyogram of the scalene recorded during a maximal inspiratory manoeuvre
sEMGsc%max	Surface electromyogram of the scalene presented as a percentage of the maximal scalene EMG value recorded during a maximal inspiratory manoeuvre.
shMIP	Short (standard) maximal inspiratory pressure manoeuvre
sitToEMGdi	Onset time of the diaphragm surface EMG in the sitting position
sitToEMGpara	Onset time of the parasternal surface EMG in the sitting position
sitToEMGsc	Onset time of the scalene surface EMG in the sitting position
sniffPdi	Transdiaphragmatic pressure produced during sniff inhalation
sniffPoes	Oesophageal pressure produced during sniff inhalation
SNIP	Sniff nasal inspiratory pressure
S_{pO2} nadir	Oxygen saturation nadir during overnight sleep study
supToEMGdi	Onset time of the diaphragm surface EMG in the supine position
supToEMGpara	Onset time of the parasternal surface EMG in the supine position
supToEMGsc	Onset time of the scalene surface EMG in the supine position
$TcCO_2$	Transcutaneous carbon dioxide
TEF / TOF	Tracheoesophageal fistula / Tracheo-oesophageal fistula
TLC	Total lung capacity

TST	Total sleep time
TwPdi	Twitch transdiaphragmatic pressure
UAR	Upper airway resistance
VC	Vital capacity
V_{O2} peak	Peak aerobic capacity

Chapter 1. Introduction and Literature Review

1.1 Introduction

Human ventilation is a sequential process involving 1) rhythmic activation of motoneurones innervating respiratory pump muscles, 2) contraction of respiratory muscles, 3) inducing expansion of the thorax and abdomen, 4) generation of negative intrathoracic pressure and 5) influx of air into the lung for gas exchange. Assessment of the respiratory system can occur at any of the steps outlined, with the measurement and quantification of changes in lung volume and intrathoracic pressure as the most commonly adopted method in clinical settings. The majority of respiratory function tests require a high level of motivation and cooperation, limiting its use to children at least 5 years of age and adults who are conscious and understand instructions to produce repeatable and reliable measurements (Miller et al., 2005).

Monitoring electrical activity of the respiratory muscle using electromyography (EMG) is the most direct way of assessing respiratory muscle function. Measurement of the electrical activity delivered by respiratory muscles such as the diaphragm reflects the neural output from the respiratory centre in the brainstem, also known as the neural respiratory drive (NRD) (American Thoracic Society/European Respiratory Society, 2002). In the clinical settings, recording of diaphragmatic EMG is already included in overnight sleep studies in both adults and children as a method of assessing respiratory effort to differentiate between obstructive and central sleep-disordered breathing events (Berry et al., 2013). Diaphragm EMG is also widely

used in the field of intensive care medicine in both children and adults as a method of improving patient-ventilator interaction and comfort by matching ventilatory support with individual patient's estimated respiratory effort (Baudin et al., 2015; Beck et al., 2001; Kallio, Peltoniemi, Anttila, Pokka, & Kontiokari, 2015; Sinderby et al., 1999). In the research setting, diaphragm and other respiratory muscle EMGs have been used as an alternative indicator of lung function, including in children with wheeze and asthma and adults with chronic obstructive pulmonary disease (COPD) (Jolley et al., 2015; Jolley et al., 2009; Maarsingh et al., 2002; Maarsingh, van Eykern, Sprikkelman, & van Aalderen, 2004; MacBean, Jolley, et al., 2016).

Despite the increasing interest in the use of inspiratory muscles EMG as a method for assessing respiratory muscle function and estimation of respiratory motor output, there are gaps in existing knowledge in the recording and measurement of inspiratory muscle EMG which need to be addressed to support translation of inspiratory muscle EMG into clinical care. This review will examine the current knowledge on the assessment of respiratory muscle function in children and in particular the use of EMG recorded using transcutaneous electrodes, also known as surface EMG (sEMG), in children. It will review the components of the respiratory muscle pump, drive to the human respiratory muscles, and the factors affecting the function of our respiratory muscles including developmental and maturational changes associated with age. The review will then discuss different techniques of assessing respiratory muscle function in children including respiratory EMG. The methods of recording and reporting respiratory muscle EMG will be examined, including a discussion of factors which can affect recording and interpretations of EMG. Respiratory diseases which have been evaluated using respiratory muscle sEMG

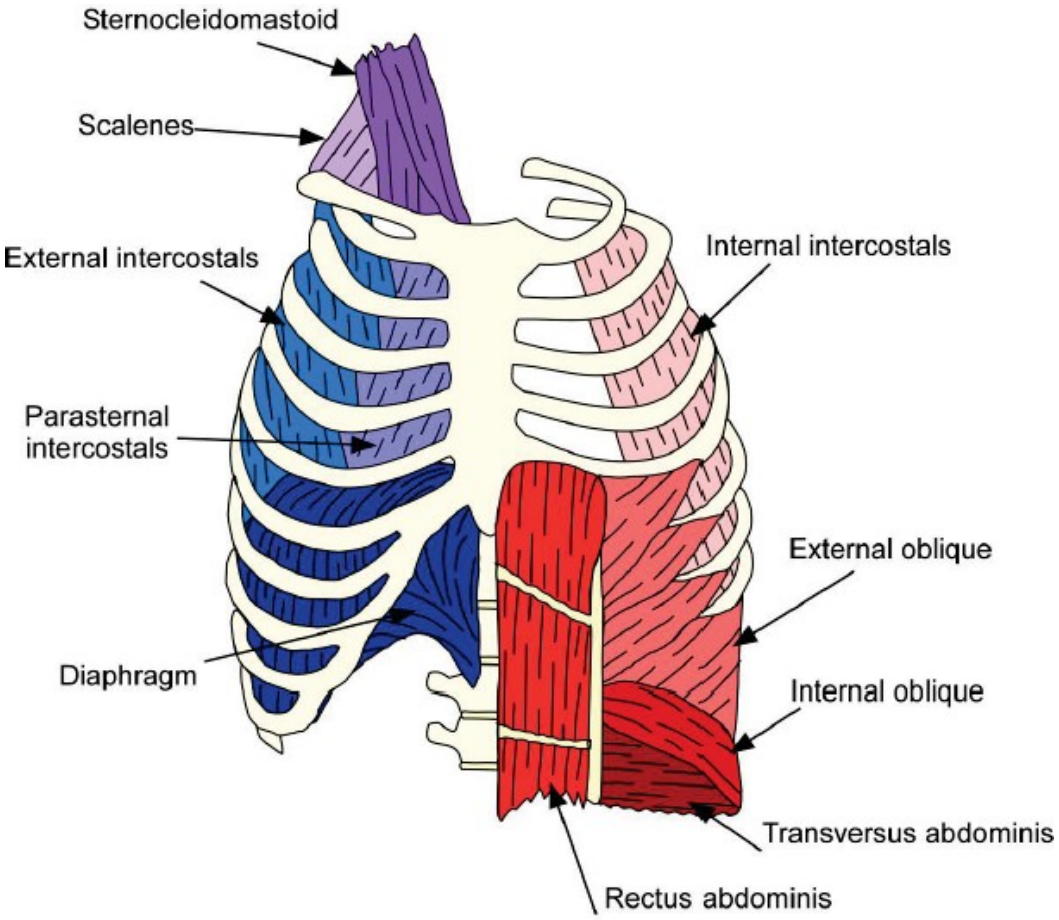
will be considered to determine potential translational applications of respiratory muscle EMG in the clinical setting. Throughout this review, deficiencies in existing knowledge and potential areas for future research will be identified.

1.2 Respiratory muscle pump – anatomy and mechanics of the respiratory muscles

The respiratory muscles are skeletal muscles that drives chest wall expansion or contraction during breathing, pumping air in and out of the lung. Inspiration is an active process whereas expiration occurs mainly through passive elastic recoil of the chest wall. Therefore, the focus of this thesis will be on the inspiratory muscles. The diaphragm is the main muscle involved in inspiration, however it also receives support from other inspiratory muscles including those from the neck, the rib cage and the chest wall, depending on the demand imposed on the respiratory system (De Troyer & Boriek, 2011) (Figure 1.1). Respiratory muscles which contract in every breath during tidal breathing are termed 'obligatory' muscles of respiration (De Troyer, Kirkwood, & Wilson, 2005; Saboisky, Gorman, De Troyer, Gandevia, & Butler, 2007). Diaphragm, scalene from the neck, and parasternal intercostal muscles from the rib cage are known obligatory inspiratory muscles (Figure 1.1). Additional muscles which contract when the demand on the respiratory system changes, such as when taking larger breaths or when there is increased ventilatory load, are termed 'accessory' respiratory muscles. Sternomastoid muscle from the neck is an example of an accessory respiratory muscle. Age-related changes to respiratory muscle function and respiratory mechanics will be discussed in detail in Section "1.4 Developmental changes and maturation of the respiratory system".

Figure 1.1 The rib cage and the respiratory muscles.

The muscles shown on the left (in shades of blue) are the inspiratory muscles. The muscles shown on the right (in shades of red) are the muscles of expiration (Adapted from Butler (1999)).

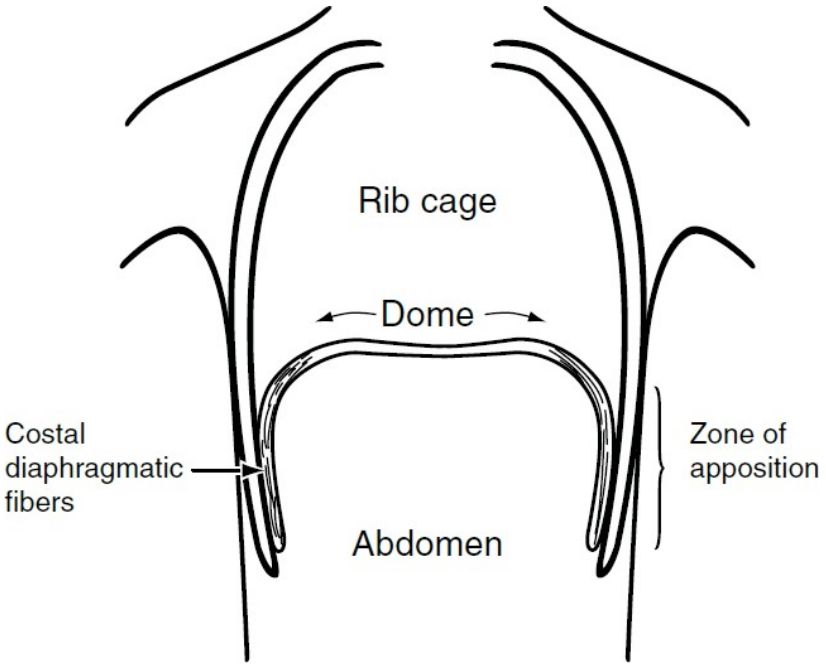


Our chest wall can be regarded as two compartments working in parallel – the rib cage and the abdomen, which are separated by the thin musculotendinous diaphragm muscle (Figure 1.2) (De Troyer, 2005; Konno & Mead, 1967). Expansion of the lungs are therefore achieved by expansion of either the rib cage, the abdomen, or both compartments simultaneously. The actions of inspiratory muscles will be discussed in detail in section "1.2.1 Inspiratory muscles". In brief, rib cage displacement during breathing is related to the motion of the ribs, with cranial displacement of the ribs causing

an increase in both the lateral and the antero-posterior diameters of the rib cage during inspiration. Displacement of the abdominal compartment occurs when the diaphragm contracts during inspiration, descend into the abdominal compartment, resulting in an outward displacement of the anterior abdominal wall. However, recent evidence that the rib cage's action does not move as one single unit, but can be separated into the upper (acted on by the rib cage inspiratory muscles) and lower rib cage (with action by the diaphragm muscle) suggests a three-compartment model of the chest wall may be a more accurate representation(De Troyer & Wilson, 2016; Ratnovsky, Elad, & Halpern, 2008).

Figure 1.2. Frontal section of the chest wall at end-expiration.

The diaphragm, our main inspiratory muscles, is positioned between the chest and abdominal cavity in the two-compartment model. Note the cranial orientation of the costal diaphragmatic fibres and their apposition to the inner aspect of the lower rib cage (zone of apposition) (adapted from De Troyer, (2005))



1.2.1 Inspiratory muscles

1.2.1.1 Diaphragm

The diaphragm is our primary muscle of respiration, a dome-shaped structure consisting of a peripheral muscular part, and a central part where the fibres of the muscular part converge to form the central tendon (Sandring, 2016). The muscular part is further divided into two main components, the costal (sternal) part, and the crural (vertebral) part (De Troyer & Boriek, 2011). The costal part consists of wide muscular slips arising from the internal surface of the inferior six ribs and associated costal cartilages inserting on the posterior aspect of the xiphoid process of the sternum (Figure 1.2). These muscular slips interdigitate with slips of the transversus abdominis muscle. It is the costal part of the diaphragm which forms part of the left and right domes with the central tendon which move during respiration. The part of the costal diaphragm which directly opposes the inner aspects of the lower rib cage (or the cylindrical part of the diaphragm) is also referred to as the "zone of apposition" (Figure 1.2). The crural (or lumbar vertebral) part of the diaphragm arises from the ventrolateral aspect of the first three lumbar vertebrae and the aponeurotic arcuate ligaments as left and right musculotendinous crura on each side of the descending aorta (Sandring, 2016). The motor supply of the diaphragm is through the phrenic nerves, originating from the third, fourth and fifth cervical segments (C3 to C5).

The diaphragm can be visualized as an elliptical cylindroid capped by a dome, with the cylindrical part corresponding to the zone of apposition, and the dome being primarily made of the central tendon (De Troyer, 2005; De Troyer & Boriek, 2011). When the muscle fibres of the diaphragm contracts and shortens, the dome of the diaphragm

descends relative to its costal insertion. The descent of the dome produces both an expansion of the pleural cavity along its craniocaudal axis, and a caudal displacement of the abdominal viscera leading to an outward motion of the anterior abdominal wall. Consequently, intrapleural pressure (Ppl) falls, abdominal pressure (Pab) rises, and lung volume increases if the airway is patent. Tension in the diaphragm sheet generates a transdiaphragmatic pressure (Pdi) that balances the difference between Pab and Ppl (Pdi = Pab – Ppl).

The diaphragm also exerts an inspiratory action on the lower ribs when it contracts, increasing the anteroposterior and the transverse diameter and hence the cross-sectional area of the lower ribs. The insertion of the diaphragm on the upper margins of the lower six ribs exerts a lifting and outward rotating force on the lower rib cage, known as an "insertional force" (De Troyer & Boriek, 2011; De Troyer & Wilson, 2016). The rise in Pab when the diaphragm contracts is also transmitted through the apposed diaphragm to expand the lower rib cage at the zone of apposition, termed the "appositional" force. The inspiratory action of the diaphragm on the lower rib cage is the combined result of the insertional and appositional forces (De Troyer & Wilson, 2016). The magnitude of the appositional force is directly related to the size of the zone of apposition. The zone of apposition (and hence appositional force) increases as lung volume decreases from functional residual capacity (FRC, the lung volume at the end of a normal expiration) to residual volume (RV, the lung volume at the end of a forced expiration). Hence the inspiratory action of the diaphragm on the rib cage is enhanced when breathing in from RV instead of FRC.

With changes in posture such as changing from seating to supine position, diaphragm mechanics also changes due to the changed effect of gravity on the abdominal

contents and abdominal wall resulting in marked increase in abdominal compliance. There is less resistance from the abdominal contents and hence greater descent of the diaphragm when supine, with greater lung expansion and change in Ppl, but also decrease in zone of apposition and appositional forces. At the lower rib cage, the anteroposterior diameter decreases and less increase occurs in the transverse diameter (De Troyer, 2005). On the other hand, in supine posture, diaphragm fibres are lengthened due to the reduction in FRC. Based on the length-tension characteristics of muscle, the force generated by the contracting diaphragm when supine would potentially be greater than when seated (De Troyer, 2005; De Troyer & Boriek, 2011). In patients with transection of the upper cervical cord and bilateral pacing of the diaphragm to allow constant activation of the diaphragm, tidal volume is progressively reduced when changing from a supine to a tilted head-up seated posture, demonstrating the influence of posture on the inspiratory action of the diaphragm (Danon, Druz, Goldberg, & Sharp, 1979; Mead, Banzett, Lehr, Loring, & O'Cain, 1984). These changes in lung volume and pressures that occurs with postural changes suggest that there may also be associated changes in NRD which has not been previously explored in children.

1.2.1.2 Rib cage – external intercostal and parasternal intercostal muscles

The intercostal muscles form two thin layers that spans across each of the intercostal spaces between the ribs (Sandring, 2016). The external intercostal muscles extend from the tubercles of the rib posteriorly to the costochondral junction anteriorly (Figure 1.1). Its muscle fibres run obliquely downward and anteriorly from the rib above to the rib below. Contraction of this muscle exerts a torque force acting on the lower rib, raising

the lower rib with respect to the upper one. The net effect is cranial displacement of the ribs and inflating the lung (De Troyer et al., 2005). The inner layer of the intercostal muscles, termed internal intercostals, extends from the chondrosternal junction to near the tubercle of the ribs with its fibres running at right angle to the external intercostals (Figure 1.1). Its action is an expiratory one and is discussed below in section "1.2.2 Expiratory muscles". Anteriorly between the sternum and chondrocostal junction, the external intercostal muscles are replaced by a fibrous aponeurosis overlying the muscle fibres of the internal intercostals. The internal intercostals in this region are conventionally called the "parasternal intercostals" with differing function to the interosseous intercostals (Figure 1.1). The fibres of the parasternal intercostal muscle run obliquely in the caudal-lateral direction from the sternum to the costal cartilages. Contraction of the parasternal intercostal muscle causes a cranial and outward displacement of the ribs together with a caudal displacement of the sternum, and inflation of the lungs (De Troyer & Boriek, 2011; De Troyer, Legrand, Gevenois, & Wilson, 1998). All intercostal muscles are innervated by the intercostal nerves from the corresponding thoracic segment.

Electromyographic studies in animals and humans have established that the parasternal intercostal muscles invariably contract during the inspiratory phase of the breathing cycle, hence they are "obligatory" inspiratory muscles (De Troyer & Estenne, 1984; De Troyer & Kelly, 1982; Taylor, 1960). It should also be noted in both canine and also human adult studies that there is a rostro-caudal gradient in the inspiratory effect of the parasternal intercostal muscle, where the inspiratory effect of the parasternal intercostals is greatest in the second through fourth interspaces and declines from the fourth to the eighth interspace (De Troyer, Legrand, & Wilson, 1996; Hudson, Gandevia, & Butler, 2011a; Legrand, Brancatisano, Decramer, & De Troyer, 1996). The external

intercostal muscles are also active only during inspiration, with a rostro-caudal gradient of neural drive where the greatest activity occurs in the dorsal portion of the upper rib interspaces (De Troyer, Gorman, & Gandevia, 2003; Taylor, 1960). There is also a posterior-anterior gradient within individual rib space which parallels gradients of inspiratory mechanical advantage (De Troyer et al., 2003; Taylor, 1960). During tidal breathing the contribution of the parasternal intercostal muscles are much greater than that of the external intercostal muscles (De Troyer, 2005). Parasternal intercostal muscles are activated in conjunction with the diaphragm, and a strong agreement between both the amplitude and timing of peak diaphragm and parasternal intercostal muscle's activity has previously been reported from animal models (Darian, DiMarco, Kelsen, Supinski, & Gottfried, 1989; van Lunteren, Daniels, Deal, & Haxhiu, 1989). There are no studies on the interactions between diaphragm and parasternal intercostal EMG activity in children. Surface EMG would be an ideal method to further understanding of respiratory mechanics and functionality for children as it is non-invasive and can assess muscle activation profiles from various muscles simultaneously.

The respiratory mechanics of the intercostal muscles change with alterations in lung volume. Based on canine models, the parasternal and upper external intercostal muscles shorten by ~ 10% during inflation from FRC to total lung capacity (TLC), smaller than the 30-40% of shortening seen in diaphragm muscle (De Troyer, 2005). While the diaphragm's resting length at FRC in supine dogs is close to its optimal length for force generation, the parasternal intercostal muscles at FRC are 10 to 15% longer than its optimal length (De Troyer, 2005). Therefore, since the parasternal intercostal muscle remains relatively close to their optimal length at all lung volumes, it is able to preserve or even increase its force-generating capacity at

higher lung volumes near TLC than at FRC (Jiang, Deschepper, Demedts, & Decramer, 1989). On the other hand, increasing lung volume above FRC causes the upper external intercostal muscles to move away from the muscles' optimal length (De Troyer, 2005; De Troyer & Boriek, 2011). Hence the force-generating ability of the upper external intercostal muscles diminishes as lung volume increases from FRC to TLC. The fall in pleural pressure produced by the contracting parasternal and external intercostal muscles is also determined by the interaction between the force developed by the muscles and the ability of the ribs to move cranially and outward. With lung inflation, the ribs become oriented more transversely which is associated with a smaller outward displacement and hence less lung-expanding action when the intercostal muscles contracts (De Troyer, 2005; De Troyer & Boriek, 2011). Overall, the degree of fall in pleural pressure obtained by contraction of the chest wall muscles decreases as the lung volume increases above FRC due to the combined effect of preserved force generation in parasternal, decreased force from the external intercostal muscles, and smaller degree of outward and cranial displacement of the ribs. It is expected that similar to adults, the activation profiles of the various inspiratory muscles would alter with changes in lung volume in children however this topic has not been explored in children previously (Beck, Sinderby, Lindstrom, & Grassino, 1998; Butler, McKenzie, & Gandevia, 1999).

1.2.1.3 Neck - scalene

There are many muscles that connect the head and the rib cage, or the cervical spine and the rib cage. Out of all these muscles, only the scalene and the sternomastoid

have a significant respiratory function (Figure 1.1) (De Troyer, 2005). The scalene muscle comprises of three bundles of muscles that run from the transverse process of the lower five cervical vertebrae (C3 to C7) to the upper surface of the first two ribs (Sandring, 2016). It is innervated by the lower five cervical nerves between C3 and C8. Contraction of the scalene raises the sternum and the first two ribs, thus assist in expanding the rib cage in the cranio-caudal axis and the anterior-posterior diameter, increasing lung volume (De Troyer & Boriek, 2011; Legrand, Schneider, Gevenois, & De Troyer, 2003). The scalene in humans is another "obligatory" inspiratory muscle, always active during inspiration even when the increase in lung volume is very small (De Troyer & Estenne, 1984; Gandevia, Leeper, McKenzie, & De Troyer, 1996; Raper, Thompson, Shapiro, & Patterson, 1966). Scalene not only helps expansion of the rib cage, it also reduces distortion of the upper chest wall, preventing inward displacement of the upper rib cage in quadriplegic subjects whose innervation to the scalene is preserved (ie. transection of the cervical cord at C7 or below) compared to those with transection of the lower cervical cord (Estenne & De Troyer, 1985).

The sternomastoid muscle descends from the mastoid process to the anterior surface of the manubrium sterni and the medial third of the clavicle. It is innervated by the 11th cranial nerve. Contraction of the sternomastoid muscle is associated with a marked cranial displacement of the sternum and also expansion of the upper portion of the rib cage (De Troyer & Boriek, 2011). The sternomastoid contracts in human only during strong inspiratory efforts, hence it is an "accessory" muscle of inspiration (Hudson, Gandevia, & Butler, 2007; Raper et al., 1966).

1.2.2 Expiratory muscles – abdominal muscles, internal intercostal muscle

The expiratory muscles comprise of mainly the abdominal muscles (including rectus abdominus, internal and external obliques, and transversus abdominis) (Figure 1.1). When the abdominal muscles contract, the anterior abdominal wall moves inwards, Pab rises, and the diaphragm is displaced cranially into the thoracic cavity (De Troyer & Boriek, 2011). This leads to an increase in Ppl, and a decrease in lung volume. Also, these muscles displace the rib cage through their insertions on the ribs, pulling the lower rib cage caudally to deflate the rib cage (De Troyer & Boriek, 2011).

The internal intercostal muscles, which runs deep to and at right angles to the external intercostal muscles, also has a role in expiration (Figure 1.1). Contraction of the internal intercostal muscle causes caudal displacement of the ribs and a decrease in rib cage diameter (De Troyer et al., 2005).

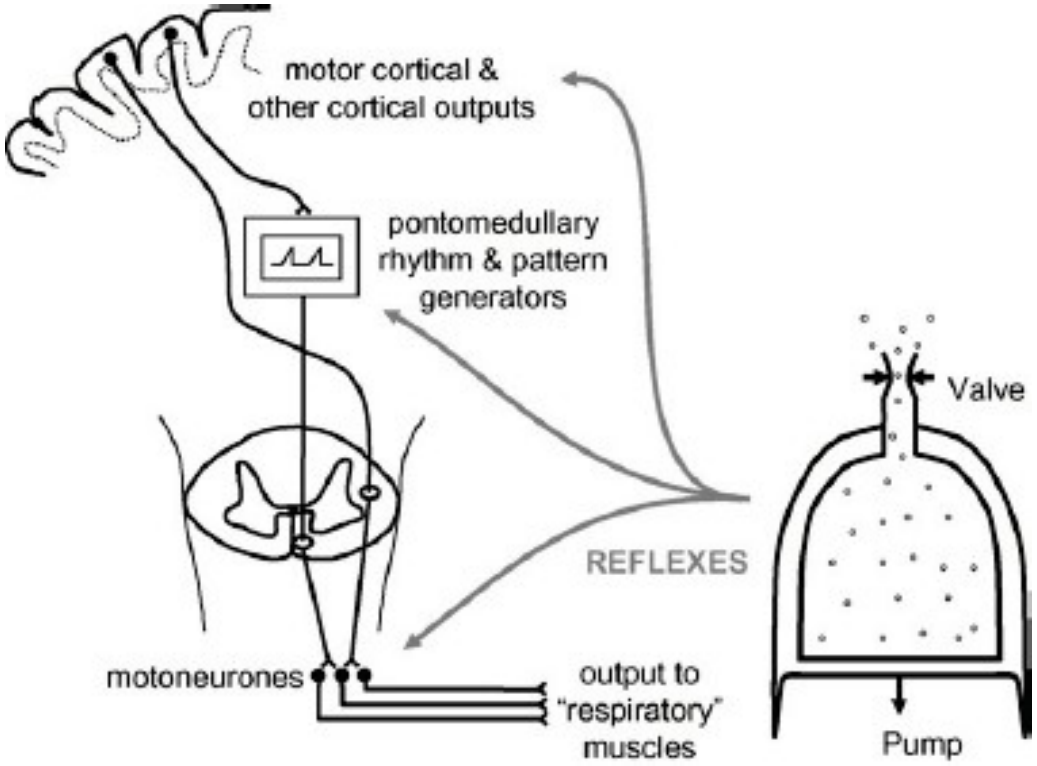
1.3 Neural drive to the human respiratory muscles

Breathing is usually a rhythmic activity that requires activation of motoneurones that innervate the respiratory pump muscles to expand the chest wall and subsequently initiates lung inflation. Respiratory muscles are controlled via two major descending pathways: from the medullary respiratory centres acting via bulbospinal pathways during spontaneous quiet tidal breathing, and from the motor cortex via corticospinal pathways during behavioural or voluntary breathing (i.e. contractions in which air is voluntary drawn into the lungs, such as during a sniff or other maximal inspiratory manoeuvres) (Butler, 2007; Hudson, Gandevia, & Butler, 2011b; Richter & Smith, 2014) (Figure 1.3). The motoneurones are the final

determinant of the neural output from the respiratory centres. A motoneurone (i.e. anterior horn cell), its axon, the neuromuscular junction, and the muscle fibres it innervates are defined as a motor unit (American Thoracic Society/European Respiratory Society, 2002). Motoneurone output can be assessed by measuring the electrical activity in the muscle fibre, i.e. electromyography (EMG).

Figure 1.3 Schematic representation of the neural control of respiratory muscles.

Direct corticospinal and bulbospinal pathways to the respiratory motoneurones are shown. The output from the motoneurones to respiratory muscles including the respiratory pump muscles and also the 'valve' muscles of the upper airway. Feedback are received via reflex pathways from the lung, airway, and muscle afferents to the cortex, medulla, and motoneurones. Adapted from Butler et al (2007).



1.3.1 Control of breathing during quiet breathing

During quiet breathing, breathing occurs automatically under resting condition based on a rhythm generated from the rostral ventro-lateral medulla (Butler, 2007; Smith, Ellenberger, Ballanyi, Richter, & Feldman, 1991; West & Luks, 2016). One region of the medulla, the pre-Botzinger complex, act as the main pacemaking rhythm generator to ensure continuous rhythmic action of the respiratory muscle pump muscles (Smith et al., 1991). It may act in conjunction with the nearby parafacial respiratory group in ventral medulla for expiratory rhythm generation (Onimaru & Homma, 2003). Inspiration is an active process while expiration is largely passive. The timing of inspiratory bursts of activity in the medulla can be altered by reflex input coming from the lungs and from central and peripheral chemoreceptors in response to arterial carbon dioxide levels (Butler, 2007; Gandevia, Gorman, McKenzie, & De Troyer, 1999; West & Luks, 2016).

During tidal breathing in adults, the output from the motoneurone pools to each of the obligatory inspiratory muscles are not uniform with differences in the timing, discharge frequency, and pattern of activity (Saboisky et al., 2007). Using standard single motor unit recording of the obligatory inspiratory muscles, Saboisky et al demonstrated that the diaphragm and 3rd dorsal external intercostal muscle are activated earliest, before the onset of inspiratory airflow (Saboisky et al., 2007). Also, the average discharge rates of motor units from the diaphragm and 3rd dorsal external intercostal are higher compared to other main obligatory inspiratory muscles (including scalene and parasternal intercostal muscles) at the onset and at peak of EMG activity (Saboisky et al., 2007). Variation in inspiratory muscle activation pattern not only differs between different inspiratory muscles but also within the same group of muscle. As discussed before in section "1.2.1.2 Rib cage", during tidal breathing a rostrocaudal gradient of inspiratory neural drive exist

within the parasternal intercostal muscles which are functionally linked to the gradients of mechanical advantage across and along intercostal spaces (De Troyer & Boriek, 2011; De Troyer et al., 2003; De Troyer et al., 1998). The variance in motoneurone output from different inspiratory muscles in Saboisky et al's study may reflect differential neural drive matched to individual muscles' mechanical advantage during resting breathing, similar to the neuromechanical matching seen in the intercostal muscles (Butler, 2007; Saboisky et al., 2007). The exact location where this matching process is organised may potentially be in the spinal cord although the exact mechanism is unknown (De Troyer et al., 2005). It is not known in children whether variations in neural respiratory drive exist between different inspiratory muscles and whether there are differences muscle activation time similar to those reported in adults.

1.3.2 Control of breathing during 'voluntary' volitional respiratory contractions

The discharge properties of human diaphragm motoneurones were previously studied in voluntary breaths of different air flows and target lung volumes by Butler et al (1999). Based on single motor unit recordings from the diaphragm of adults when performing voluntary inspiratory tasks, diaphragm motor units were recruited in order of increasing motoneurone size, also known as the Henneman's size principle (Henneman, 1957). Most diaphragm motor units were recruited by 50% of inspiratory time with increase in discharge rate compared to baseline throughout the breaths (Butler et al., 1999). There was significant variability in recruitment order of the diaphragm motor units between different voluntary tasks, especially in breaths with high flow rates (Butler et al., 1999). The discharge behaviour between tidal breathing was also compared to voluntary

breaths with matched volume. Although the initial and peak discharge rates of motor units were similar, the recruitment order in tidal breaths was different compared to that in voluntary breaths suggesting potential subtle differences in the distribution of voluntary and involuntary (i.e. tidal breathing) neural drive to the phrenic motoneurones (Butler et al., 1999).

Discharge properties of parasternal intercostal motor units in different interspace during tidal breathing and also during targeted voluntary breaths have also been compared in adults (Hudson et al., 2011a). Most of the parasternal intercostal motor units were activated in both tidal breaths and voluntary breaths. Like tidal breathing, a rostrocaudal gradient of motoneurone discharge was observed when performing voluntary breaths matched in volume. Motoneurones in the first interspace were discharged earlier relative to inspiratory airflow and reached greater peak inspiratory firing frequencies than parasternal intercostal motoneurones from caudal interspaces (Hudson et al., 2011a). It is possible the rostrocaudal gradient of neural drive in parasternal intercostal muscle during voluntary breaths is also related to inspiratory mechanical advantage, similar to that observed during tidal breathing.

Activation profiles of scalene have been compared to those of sternomastoid muscle during large voluntary breaths and maximal static manoeuvres using intramuscular multiunit EMG recordings in adults (Hudson et al., 2007; Raper et al., 1966; Washino, Kanehisa, & Yoshitake, 2017). Scalene consistently activated earlier than sternomastoid muscle, whether when breathing from FRC to TLC, or in maximal inspiratory pressures manoeuvres (Hudson et al., 2007; Raper et al., 1966; Washino et al., 2017). In Hudson et al's study, the profile of activation of these two neck muscles were unaltered when performing static maximal inspiratory pressure ramps at different lung

volumes despite the changes in afferent muscle feedback expected with change in lung volumes (Hudson et al., 2007). At all lung volumes, scalene and sternomastoid were recruited according to their relative mechanical advantages for inspiration, another example of neuromechanical matching in distribution of neural drive to the respiratory muscles (Hudson et al., 2007).

There are currently no data available in children on the inspiratory muscle activation profile during voluntary and involuntary breathing tasks. It is not known whether there is a difference in neural drive between tidal breathing or voluntary breathing tasks as demonstrated in adults for diaphragm muscle. It is also not known whether the muscle activation profile would differ between different inspiratory muscles, depending on the individual muscle's relative mechanical advantage for the different inspiratory tasks as shown in adults. Potentially simultaneous surface EMG recordings from multiple inspiratory muscles combined with lung volume and pressure measurements when children perform various breathing tasks can promote understanding of inspiratory muscle activation patterns and details of the muscle interactions associated with changes in lung volume and pressure.

1.3.3 Control of breathing during sleep

Sleep can be broadly divided into 2 main stages based on physiological characteristics (including electroencephalogram (EEG) changes): rapid eye movement (REM) sleep and non-REM (NREM) sleep (Berry et al., 2013). REM sleep is characterized by an activated pattern of EEG, associated with marked decrease in muscle tone and episodic bursts of rapid eye movement on electro-oculogram (EOG) (Berry et

al., 2013). Throughout the night, each individual will go through multiple discrete cycles of NREM and REM sleep, with a single cycle lasting approximately 90-120 minutes (Benca, 2011). As the cycle continues during the night, the percentage of time spent during REM sleep in each cycle generally increases. Sleep architecture varies across the lifespan (Andres, Sadeh, & Appareddy, 1995). Newborn infants spend 16-18 hours per day sleeping, entering sleep through REM rather than NREM sleep and also spending a greater proportion of sleep time in REM sleep (Andres et al., 1995; Iglowstein, Jenni, Molinari, & Largo, 2003). Around 2-3 months of age, infants start to develop a day/night cycle and enter sleep through NREM sleep (Andres et al., 1995). Total sleep time slowly decreases with age, eventually reaching adult norms of approximately 8 hours per night post-adolescence (Iglowstein et al., 2003).

During sleep in normal children and adults, there is a modest increase in upper airway resistance and a small decrease in nocturnal ventilation (with a small decrease in oxygen saturation between 1-3%, and a rise in end-tidal PCO_2 of up to 13 mmHg in children) (Hudgel, Martin, Johnson, & Hill, 1984; Phillipson & Bowes, 2011; Stradling, Chadwick, & Frew, 1985; Tabachnik, Muller, Bryan, & Levison, 1981). Our breathing varies between sleep stages, relying mainly on the action of the diaphragm to maintain ventilation (Phillipson & Bowes, 2011). During NREM sleep, there is slightly diminished tonic activity (i.e. muscle activity which is present throughout the respiratory cycle) in the intercostal, laryngeal, and pharyngeal muscle compared to wakefulness, whereas diaphragm tonic activity remains basically unaltered (Phillipson & Bowes, 2011; Tobin, Cohn, & Sackner, 1983). Phasic activity (i.e. synchronized muscle contraction during inspiration) of intercostal muscle increases during NREM sleep compared to wakefulness in healthy adults and adolescents, likely in response to the increase in airway resistance

from decreased pharyngeal dilator muscle tone and hence increased respiratory load during NREM sleep (Lopes, Tabachnik, Muller, Levison, & Bryan, 1983; Phillipson & Bowes, 2011; Tabachnik, Muller, Bryan, et al., 1981).

During REM sleep, tonic and phasic activity is virtually abolished in all respiratory muscles (including pharyngeal dilator muscles and intercostal muscles) except for the diaphragm (Phillipson & Bowes, 2011; Tabachnik, Muller, Bryan, et al., 1981). As a result of the inhibition of intercostal muscle activity during REM sleep, paradoxical rib cage motion occurs during REM sleep in newborns, whereas in adolescents and adults the rib cage contribution to tidal volume is diminished or absent (American Thoracic Society/European Respiratory Society, 2002; Phillipson & Bowes, 2011; Prechtl, van Eykern, & O'Brien, 1977; Tabachnik, Muller, Bryan, et al., 1981). Although there is a loss of diaphragm tonic activity during REM sleep in both newborn and adults, there is an increase in phasic diaphragmatic activity during REM sleep which help maintains tidal volume (Muller, Volgyesi, Becker, Bryan, & Bryan, 1979; Phillipson & Bowes, 2011; Prechtl et al., 1977; Tabachnik, Muller, Bryan, et al., 1981).

Although more data is available regarding respiratory muscle activity during sleep in children compared to when awake, most of the research were conducted in neonates or children with obstructive sleep apnoea (OSA) with little data available on healthy children (Gauda, Miller, Carlo, DiFiore, & Martin, 1989; J. Lopes, Muller, Bryan, & Bryan, 1981; Praud, D'Allest, Delaperche, Bobin, & Gaultier, 1988; Tabachnik, Muller, Bryan, et al., 1981). The main respiratory muscles investigated are the genioglossus and the diaphragm, with little or no data available on parasternal or scalene muscle activity during sleep in healthy children (Gauda et al., 1989; Praud et al., 1988). Also, respiratory muscle EMG activity were usually reported using arbitrary units (such as “peak to peak pen deflection

in mm") rather than a quantitative value in microvolt due to limitation of the recording or analysis method (Jeffries, Brouillette, & Hunt, 1984; Tabachnik, Muller, Bryan, et al., 1981). Since diaphragm sEMG is a respiratory variable that is already recorded using clinical polysomnography software and equipment, quantitative evaluation of diaphragm sEMG is a logical first step into exploring potential clinical application of sEMG in children during sleep.

1.4 Developmental changes and maturation of the respiratory system

The structure and mechanical properties of the respiratory system change with growth and development throughout childhood (American Thoracic Society/European Respiratory Society, 2002; Bryan & Wohl, 2011). Most alterations in the configuration of the rib cage, maturation and stiffening of the chest wall, developmental changes of the respiratory muscles, and changes in lung structure occur within the first few years of life (American Thoracic Society/European Respiratory Society, 2002). At birth, an infant has approximately 10% of the adult complement of alveoli. Rapid alveolarization occur in the following 18 -24 months of life, but may continue up to adolescence (Narayanan et al., 2012; Thurlbeck, 1982). After the age of 2, further lung growth consists mainly of dimensional growth of alveoli with alveolar volumes doubling by the teenage years (American Thoracic Society/European Respiratory Society, 2002; Calogero & Sly, 2010). Based on lung function measurements (such as the measure of forced expiratory volume in 1 second (FEV1) from spirometry which mainly reflects airway size), it is known that the "normal range" of lung function is highly age dependent (Stanojevic et al., 2008). Lung function trajectory has three main phases: a growth phase (during childhood until

early adulthood), a plateau phase, followed by a decline phase from physiological aging (Agusti & Faner, 2019; Stanojevic et al., 2008). During adolescence, lung function growth stops earlier in girls (around 16 years of age) but continues into early adult life in boys (Agusti & Faner, 2019; Hopper, Hibbert, Macaskill, Phelan, & Landau, 1991). Lung function can continue to increase for up to 3 years after body height plateaus in adolescents (Xuan et al., 2000).

Respiratory mechanics also vary from birth to adulthood. At birth, the ribs extend almost at right angles from the vertebral column. As a result, the rib cage is more circular and elevated than in adults (Bryan & Wohl, 2011). In the adult the volume of the rib cage can be increased by raising the ribs in both a "bucket handle" and a "pump handle" movement, which is not possible in infants due to the more horizontal position of the ribs. Also, the diaphragm is more horizontal and flattened in infants than in adults, with a very wide angle of insertion on the rib cage, resulting in the absence of the zone of apposition (Devlieger et al., 1991). The respiratory pump consequently lacks mechanical efficiency during infancy. With growth the thoracic cavity enlarges, changing in shape and texture. There is progressive mineralization of the ribs with an increase in the bone to cartilage ratio with age which can continue up to the age of 25 (Sandring, 2016). The orientation of the ribs does not change substantially until the infant assumes an upright posture around six months of age when the forces acting on the rib cage changes. The addition of gravitational forces and a pulling force on the ribs from the sacrospinal and abdominal muscles causes the ribs to start sloping downwards and a relative decrease in the anteroposterior diameter of the rib cage. With the change in the orientation of the ribs from adopting an upright posture, the 'bucket handle' movement gradually develops with increasing contribution of the rib cage to tidal volume generation by 1-2 years of age. As

thoracic length increases with age, and greater gravitational pull on the abdominal contents in the upright posture, the inspiratory action of the diaphragm is augmented resulting in reductions in the effort required to affect lung volume changes (Bryan & Wohl, 2011).

Chest wall compliance also has a major influence on maintenance of lung volume. The rib cage of a newborn and infant is softer and thus more compliant compared to an adult (Bryan & Wohl, 2011). Usually, FRC is reached at the point where the outward elastic recoil of the thoracic rib cage counterbalances the inward lung recoil in adults (West & Luks, 2016). In infants the outward elastic recoil of the chest wall is very low with high chest wall compliance. Consequently, infants' FRC is expected to be at a low lung volume because of the high chest wall compliance compared to older children. However, in newborns and infants, the dynamic end-expiratory lung volume is substantially above the passively determined FRC (Kosch & Stark, 1984). It has been shown in newborns that expiration is terminated at substantially higher flows compared to adults. This suggests active interruption of a relaxed expiration in newborns through potentially two mechanisms, post inspiratory activity of the diaphragm, and laryngeal narrowing during expiration to actively slow expiration (Harding, Johnson, & McClelland, 1980; Kosch, Hutchinson, Wozniak, Carlo, & Stark, 1988). Dynamic elevation of end-expiratory lung volume above passive FRC persists until around the end of the first year of life (Colin et al., 1989). During childhood, the compliance of the chest wall gradually decreases as the rib cage stiffen due to rib cage ossification and increases in muscle mass. Concurrently lung compliance increases with growth in airway size and decrease in airway resistance (Stocks, 1999).

With growth, there is also progressive changes in the respiratory muscles including changes in the fibre composition, fibre size, oxidative capacity and hence contraction characteristics. Respiratory muscles usually contain a mixture of type I and type II fibres (West & Luks, 2016). Type I fibres are slow-twitch and high-oxidative in nature, with low contractility but greater resistance to fatigue (West & Luks, 2016). Type II fibres are fast-twitch and low-oxidative, with high contractility but are more prone to fatigue. In preterm infants, the diaphragm is composed of less than 10% of type I fibre, increasing to around 25% in full term newborns, ~50% in children older than 2 years of age, and about 55% in adults (Sieck & Fournier, 1991). Hence respiratory muscles of premature babies and young infants are more susceptible to fatigue with low oxidative capacity, resulting in earlier decompensation and ventilatory failure than older children. Mean cross sectional area of all fibre types increases postnatally which further improves contractility of the respiratory muscles with age. Chest wall muscle contraction helps to stabilize the compliant infant rib cage, minimizing inward displacement of the rib cage caused by diaphragmatic contraction.

Maximum inspiratory pressures exerted by infants and even children, are surprisingly high compared with adults (Cook, Mead, & Orzalesi, 1964). This is probably related to the small radius of curvature of the rib cage, diaphragm, and abdomen which according to the Laplace law, converts small tensions into relatively high pressures (American Thoracic Society/European Respiratory Society, 2002; West & Luks, 2016). However, despite relatively high maximal static inspiratory pressures, the inspiratory force reserve of respiratory muscles is reduced in infants with respect to adults because inspiratory pressure demand at rest is greater, with high minute ventilation and high metabolic demand in infants (Gaultier, 1995). In children, maximal pressure increases

with age and are greater in males than in females even prior to puberty (Gaultier, Perret, Boule, Buvry, & Girard, 1981; Gaultier & Zinman, 1983; Wagener, Hibbert, & Landau, 1984). By 11-12 years of age, the adult values are attained in both sexes (Laveneziana et al., 2019).

In summary, progressive changes in anatomy and subsequently respiratory mechanics and physiology occurs with increasing age during childhood in the respiratory system. Developmental changes in lung volume and pressures are already known (American Thoracic Society/European Respiratory Society, 2002; Gaultier & Zinman, 1983; Wagener et al., 1984). In combination with progressive changes in respiratory muscle characteristics and chest wall configurations and compliance, it is anticipated that respiratory muscle sEMG magnitude and activation profiles would also evolves during childhood. Therefore, it is not possible to extrapolate findings from adult studies of respiratory muscle EMG to children even if the underlying pathophysiology may be similar for the health conditions, confirming the importance of conducting inspiratory muscle sEMG research in children.

1.5 Influence of gender on respiratory system

Gender differences in spirometric measurements of lung functions are well recognised both in adults and children, with gender-specific normative equations applied for standard commercially available lung function equipment (Miller et al., 2005, Quanjer et al 2012). In infants, maximal expiratory flow measured from forced expiratory manoeuvres performed at the end of tidal breathing is higher in girls during the first 9

months of life (Hoo et al., 2002). Maximal expiratory flow has also shown to be higher in prepubertal girls in some studies (Leeder, Swan, Peat, Woolcook, & Blackburn, 1977). When maximal inspiratory and expiratory pressures are measured in children, it is noted that maximal respiratory pressures increase with age in children and are lower in girls than in boys (Gaultier & Zinman, 1983; Wagener et al., 1984). In healthy adults, parasternal sEMG was shown to be higher in females but there was no gender difference in diaphragm crural EMG (Jolley et al., 2009; MacBean, Hughes, Nicol, Reilly, & Rafferty, 2016). Gender is potentially another physiological factor that may affect interpretation of respiratory muscle sEMG in children other than age.

1.6 Assessing respiratory muscle function in children – routine clinical tests

The main function of respiratory muscle is to shorten and to develop force. Hence routine measurements of respiratory muscle function in children is usually focused on the measurement of changes in lung volumes and flow as the result of muscle shortening (i.e. contraction), estimating force as pressure changes, and through indices of gas exchange. When assessing respiratory muscle function in children, non-invasive, reproducible and easily available tests are preferred. Spirometry and measurement of static mouth pressures are the most widely applied tests of respiratory muscle function in a routine clinical respiratory laboratory. These tests are volitional and require full cooperation from the subjects hence children are expected to be at least 5 years of age to be able to perform acceptable and reproducible clinical lung function tests. Although invasive pressure assessments such as the measurement of sniff transdiaphragmatic pressure (sniffPdi) has

a narrower normal range and lower variability than the non-invasive maximal inspiratory mouth pressure, it requires the placement of oesophageal and gastric pressure transducers which is not well-accepted by children and their carers and the expertise is not routinely available at most clinical lung function labs (Chervin et al., 2003; Fauroux & Aubertin, 2007; Laveneziana et al., 2019). Assessment of thoracic and abdominal rib cage motion using respiratory inductance plethysmography (American Thoracic Society/European Respiratory Society, 2002) bands and monitoring of ventilatory parameters in children during sleep through overnight polysomnography (PSG) are other non-invasive tests utilised to assess respiratory muscle function in children.

1.6.1 Spirometry

Spirometry testing remains the most commonly used technique for assessing lung function in children and has been shown to be a sensitive marker in discriminating between health and disease in childhood, especially in conditions such as asthma and cystic fibrosis (Knottnerus, van Weel, & Muris, 2002; Miller et al., 2005; Nair, Daigle, DeCuir, Lapin, & Schramm, 2005). Spirometry measures how an individual inhales or exhales volumes of air as a function of time, by performing forced expiratory manoeuvres. The whole manoeuvre involves 3 phases: 1) maximal inspiration from the end of a normal breath (FRC); 2) a "blast" of forceful exhalation; and 3) continued complete exhalation to the end of the test (Miller et al., 2005). The primary signal measured are volume or flow, including forced vital capacity (FVC, the volume delivered during a forceful expiration from a maximal inspiration), forced expiratory volume in one second (FEV1, the volume delivered in the first second of a forced expiration from a position of full

inspiration), and the ratio FEV1/FVC. The lung function results obtained are acceptable if the two largest values of FVC and FEV1 are within 0.15L of each other, the manoeuvre performed are free from artefacts such as coughing and glottis closure, and a plateau in the volume-time curve is reached to ensure satisfactory exhalation (Miller et al., 2005).

Spirometry is useful and relatively easy to perform although it does have a few limitations. Forced expiratory manoeuvre are usually expected to be performed acceptably and reproducibly in a school-aged or older child (around 5 years of age) who is motivated and cooperative (Miller et al., 2005). The use of spirometry testing in younger children including infants to assess lung function remains mainly a research tool (Beydon et al., 2007). The technician involved also needs to be familiar in interacting with children, providing encouragement while giving detailed but simple instruction to the child (Miller et al., 2005). Spirometry testing is usually performed with the child breathing through a mouthpiece with a nose-clip in situ. Hence children who cannot tolerate a mouthpiece or have mouth-leak due to anatomical reasons will not be able to perform spirometry. Also, the manoeuvres need to be performed while the patient is relatively well, as factors such as fatigue, breathlessness, and cough, which are often present when the patient is acutely unwell, can impair the patient's ability to perform acceptable and maximal lung function. In comparison, a non-volitional technique such as measuring parasternal sEMG during tidal breathing may be more suitable for acutely ill patients who are not able to perform reproducible spirometry (Reilly, Jolley, Elston, Moxham, & Rafferty, 2012; Suh et al., 2015).

As discussed in section "1.4 Developmental changes and maturation of the respiratory system", lung function (based on FEV1) trajectory varies with age, increasing

from childhood until adulthood, followed by gradual decline in older adults (Agusti & Faner, 2019; Stanojevic et al., 2008).

1.6.2 Pressure measurement – respiratory muscle strength testing

The ability of respiratory muscles to generate force is usually estimated through measurements of maximal mouth pressures during quasi-static manoeuvres, reflecting global inspiratory and expiratory muscle strength or output. Maximal static mouth pressure is measured from the mouth whilst performing a maximal inspiratory (maximal inspiratory pressure, MIP; *Mueller manoeuvre*) or expiratory (maximal expiratory pressure, MEP; *Valsalva manoeuvre*) effort against an occlusion for at least 1-1.5 s, and the maximal pressure sustained for at least 1 s is recorded. The subject breathes in or out through a mouthpiece with an air leak orifice (1.5-2mm) to prevent the contribution of the facial muscles (American Thoracic Society/European Respiratory Society, 2002). Mouth pressure measured during these manoeuvres with the glottis open signifies the pressure developed by the respiratory muscles, plus the passive elastic recoil pressure of the whole respiratory system including those from the lung and the chest wall (American Thoracic Society/European Respiratory Society, 2002). The pressure measured varies with lung volume due to the force-length relationship of the respiratory muscles and the varying contribution of passive elastic recoil pressure of the respiratory system at different lung volumes hence it is important to standardise the volume at which the pressure is measured from (Gaultier & Zinman, 1983; Tobin & Laghi, 1998). At FRC, elastic recoil pressure of the respiratory system is zero hence mouth pressure would represent respiratory muscle pressure. Conventionally, MIP is measured from RV which

is easier to perform than from FRC, relating to the enhanced diaphragm's inspiratory action at RV as discussed in section "1.2.1.1 Diaphragm". Although MIP measured from RV is greater than from FRC, it is worth noting that at RV, the recoil pressure of the respiratory system can make a significant contribution to MIP of up to 30% (American Thoracic Society/European Respiratory Society, 2002; Gaultier & Zinman, 1983). As discussed in section "1.4 Developmental changes and maturation of the respiratory system", there is a progressive increase in maximal pressure with age, with adult values attainable by 11-12 years of age (Gaultier & Zinman, 1983; Laveneziana et al., 2019; Wagener et al., 1984). There is also a variation of maximal respiratory pressure between genders (Gaultier & Zinman, 1983; Wagener et al., 1984).

Static pressure measurements are non-invasive but volitional in nature, requiring full cooperation and motivation from the subject, similar to spirometry. Accurate mouth MIP and MEP measurements can only be obtained in children older than 6-8 years of age (American Thoracic Society/European Respiratory Society, 2002; Laveneziana et al., 2019). However, even trained adults may have difficulty doing this test reliably which can be harder for subjects who are naïve to lung function testing (Bigland-Ritchie, Furbush, Gandevia, & Thomas, 1992). Although there are normal values for MIP and MEP established from large series of children, there are wide variabilities in the values obtained (American Thoracic Society/European Respiratory Society, 2002; Fauroux & Aubertin, 2007; Laveneziana et al., 2019). When fewer than five manoeuvres were performed, the coefficient of variation (CV) was found to be 9% and was independent of manoeuvre, age, and sex (Gaultier & Zinman, 1983). A learning effect has also been demonstrated where the MIP and MEP obtained are significantly higher if multiple attempts were made, and that peak value are typically achieved in adults after 5-6 attempts

of MEP, and after 9 attempts for MIP (Fiz, Montserrat, Picado, Plaza, & Agusti-Vidal, 1989; Man et al., 2003; Wagener et al., 1984; Wen, Woo, & Keens, 1997). It has been suggested that five or more attempts of static pressure measurements should be made until two reproducible maximal values are obtained (American Thoracic Society/European Respiratory Society, 2002). However, in routine clinical practice, performing more than 5 maximal inspiratory and expiratory static efforts in young children is difficult to achieve due to lack of cooperation, undermotivation, and potential fatigue in the children. Another option recommended is to record the maximum value of three inspiratory or expiratory manoeuvres that vary by less than 10-20% (American Thoracic Society/European Respiratory Society, 2002; Fauroux & Aubertin, 2007; Laveneziana et al., 2019). However even when the pressures recorded are reproducible across attempts, it may not equate to maximality (Aldrich & Spiro, 1995). Moreover, other factors such as chest wall configuration and stabilisation during manoeuvres can contribute to the range of pressures observed even in healthy normal children although one study have suggested MIP and MEP obtained from seated healthy children is independent of the thoracoabdominal configuration assumed during the manoeuvre (Zinman & Gaultier, 1984). There is a clear need to develop a non-volitional, non-invasive, and simple to perform method of assessing respiratory muscle strength especially in children to overcome these challenges.

Measurement of sniff nasal inspiratory pressure (SNIP) is another method for assessing global inspiratory strength of respiratory muscles (American Thoracic Society/European Respiratory Society, 2002; Fauroux & Aubertin, 2007; Laveneziana et al., 2019). The nasal pressure is measured by a pressure sensor (plug) in a completely occluded nostril during a fast (< 500 ms) sharp maximal sniff from FRC whilst keeping the other nostril open (Laveneziana et al., 2019). This pressure closely

reflects oesophageal pressure, and thus inspiratory muscle strength (Fauroux & Aubertin, 2007; Steier et al., 2007; Stell, Polkey, Rees, Green, & Moxham, 2001). Usually 10 trials are sufficient to obtain a peak SNIP value although up to 20 trials may be necessary (Laveneziana et al., 2019; Lofaso et al., 2006). Normal values have been established for both children and adults (Fauroux & Aubertin, 2007; Stefanutti & Fitting, 1999; Uldry & Fitting, 1995). Values in healthy children aged 6-17 years are comparable to those obtained from healthy adults (Laveneziana et al., 2019; Stefanutti & Fitting, 1999; Uldry & Fitting, 1995). SNIP correlates with age, weight and height in boys but not in girls (Stefanutti & Fitting, 1999).

Similar to maximal static mouth pressures, SNIP is also effort dependent and requires good understanding and cooperation from the subject. Anecdotally, many children find sniffing an easier and more natural manoeuvre to perform than maximal static pressures. SNIP measurements in different populations has been shown to be reproducible, and less prone to learning effect compared to MIP (Lofaso et al., 2006; Maillard, Burdet, van Melle, & Fitting, 1998; Terzi, Corne, Mouadil, Lofaso, & Normand, 2010). However, the manoeuvre may be difficult for subjects with nasal obstruction (such as enlarged adenoids, nasal polyps, inflamed nasal turbinates) (Fitting, 2006). Also, the pressure measured may be an underestimation of oesophageal pressure in subjects with nasal obstruction or significant lung or airway disease, as a transnasal pressure of 10-15 cm H_2O is necessary to collapse the unplugged nostril valve to obtain an accurate approximation of oesophageal pressure swings (Fauroux & Aubertin, 2007; Fauroux, Aubertin, Cohen, Clement, & Lofaso, 2009; Fitting, 2006). The agreement between SNIP and MIP is variable and the two tests should be regarded as complementary but not interchangeable methods of assessing

global inspiratory muscle strength (Steier et al., 2007).

1.6.3 Thoracoabdominal motion – respiratory inductance plethysmography

As discussed in section "1.2 Respiratory muscle pump", our chest wall can be regarded as a two-compartment model as described by Konno and Mead, where the rib cage and the abdominal compartments are working in parallel as an integrated pump (American Thoracic Society/European Respiratory Society, 2002; Konno & Mead, 1967). Rib cage (RC) motion can be taken as an index of intercostal muscle action, while abdominal (AB) motion can be taken as an index of diaphragmatic descent into the abdominal cavity. Overall chest wall displacements can then be stipulated by knowing the RC and AB displacement. Respiratory inductive plethysmography (RIP) uses thin cloth bands with thin wires sewn-in placed around the rib cage (nipple line) and abdomen (around umbilicus) to assess RC and AB motion. Variations in electrical inductance reflect changes to the entire cross-sectional area enclosed by the RIP bands, displayed as sinusoidal shaped traces.

One of the outcomes assessed by RIP bands is the synchrony of RC and AB motion during tidal breathing, by measuring the phase angle, φ. Synchronous breathing is when outward movement of the rib cage during inspiration is completely in phase with the outward movement of the abdomen with $\varphi = 0°$. When there is increased work of breathing from any cause triggering the generation of negative intrathoracic pressure, the rib cage motion becomes out of phase with abdominal motion, with paradoxical motion between RC and AB defined as $\varphi = 180°$ (American Thoracic Society/European Respiratory Society, 2002). In neuromuscular disease, paradoxical

inward motion of the RC during inspiration would suggest intercostal muscle weakness, whereas paradoxical inward motion of the AB during inspiration indicates diaphragmatic weakness. In upper airway obstruction such as obstructive sleep apnoea (OSA), out of phase RC and AB motion can be detected on overnight sleep studies due to increased work of breathing resulting from upper airway obstruction (Vandenbussche, Overeem, van Dijk, Simons, & Pevernagie, 2015).

Another outcome measured by RIP bands is the relative contribution of the RC and AB motion to tidal volume. To assess this measure, the RIP bands need to have volume calibration procedures performed. Performing an "isovolume manoeuvre" is one of the methods used, where the subject is asked to voluntarily shifts volume between the RC and AB compartments by contracting and relaxing abdominal muscles with the glottis closed (American Thoracic Society/European Respiratory Society, 2002). Since the lung volume is constant with the glottis closed, the decrease in abdominal volume (i.e. the volume displaced by inward movement of the abdominal wall) would equal the increase in rib cage volume. Two isovolume manoeuvres performed at known lung volumes allows calibration of the RC and AB band tracings in terms of lung volume change. Volume calibration can also be performed by calibrating the combined RC and AB signal (representing total chest wall displacement and hence tidal volume) against a reference- volume device (pneumotachograph or spirometer), or by using specific statistical techniques and/or computer programs based on tidal breathing without isovolume measures (Carry, Baconnier, Eberhard, Cotte, & Benchetrit, 1997; Sackner et al., 1989).

Respiratory inductive plethysmography is useful as a non-invasive and non-volitional measure of chest wall motion that can be applied easily to assess breathing

awake or asleep, allows frequent measurement with minimal cooperation, obtainable even when the child is acutely unwell, and provides an indirect measure of respiratory muscle function and potentially tidal volume. However, chest wall motion is the result of integrated respiratory system output which can be influenced by not only the respiratory muscle function, but also lung mechanics and compliance of the chest wall. Asynchronous chest wall motion can be indicative of not only respiratory muscle weakness, but also fatigue, high chest wall compliance, abnormally low lung compliance or high lung resistance, upper airway obstruction, or a combination of more than one of the potential factors (American Thoracic Society/European Respiratory Society, 2002). Hence interpretation of abnormal chest wall motion needs to be performed with an understanding of the context or condition during which the abnormality occurs. Further, depending on the analysis method used to derive phase angles, phase angles may be difficult to interpret if breathing traces does not approximate a sinusoidal pattern (American Thoracic Society/European Respiratory Society, 2002; Prisk, Hammer, & Newth, 2002; Ulm et al., 2014).

Another disadvantage of the RIP bands is the requirement of volume calibration if quantitative assessment of lung volume is needed, which is difficult in children who have limited ability to perform the isovolume manoeuvres and specific software for calibration with tidal breathing are not readily available. Also, in infants especially preterm infants where the chest wall is highly compliant and paradoxical chest wall motions occurs easily, calibration may not be possible as the assumption of the two-compartment model of the chest wall would not be valid. Further, movement of the belts or changing body position can affect calibration. Hence using calibrated RIP to assess quantitative changes in tidal volume during sleep is not feasible as changes in sleeping

position throughout the night invalidates the calibration procedure (Tobin et al., 1983; Whyte et al., 1991). A non-volitional technique for assessing respiratory function that does not require complex calibration procedure is needed for use in children. Also, little is known about the relationship of RIP bands to other measures of lung function especially in young children (Beydon et al., 2007). There are no published data on repeatability of any of the outcomes assessed using RIP bands in infants or young children (Beydon et al., 2007).

1.6.4 Overnight sleep studies

Overnight sleep studies, or PSG, record multiple physiologic parameters (including sleep state, respiration, cardiac rhythm, muscle activity, gas exchange) continuously and simultaneously during sleep to characterize sleep and identify sleep disorders (Berry et al., 2013). By recording multiple physiologic parameters simultaneously, changes in sleep/wake state or alterations in one parameter can be correlated with other signals recorded. Polysomnography is the gold standard for diagnosing sleep-disordered breathing in children including OSA and nocturnal hypoventilation, and evaluation of treatment efficacy including titration of positive airway pressure (PAP) therapy for sleep-related breathing disorders (American Thoracic Society, 1996; Berry et al., 2013; Wise et al., 2011).

Physiological variables monitored during PSG routinely includes EEG and EOG to determine sleep stages, electrocardiogram (ECG) to monitor heart rhythm and heart rate, and chin EMG to help determine sleep stage. Respiratory parameters recorded usually includes movements of the chest wall and abdomen using uncalibrated RIP to assess

respiratory effort, detection of airflow at the nose and the mouth using nasal cannulae and oronasal thermistor, and assessment of gas exchange through oxygen saturation (O_2 sat) and transcutaneous carbon dioxide ($TcCO_2$) monitoring (American Thoracic Society, 1996; Berry et al., 2013; Berry et al., 2012). Electromyography of the diaphragm and intercostals were included as an alternative method for detecting respiratory effort in the 2007 American Academy of Sleep Medicine (AASM) scoring manual (Iber, Ancoli- Israel, Chesson, & Quan, 2007; Pamula, Campbell, Coussens, & al, 2011). However, due to the scant literature available on the use of surface EMG to detect respiratory effort, the 2012 AASM manual recommends only oesophageal manometry or calibrated/uncalibrated dual thoracoabdominal RIP belts as the endorsed methods for detecting respiratory effort in adults and children (Berry et al., 2013; Berry et al., 2012). Oesophageal manometry remains rarely used in clinical practice especially in children due to its invasiveness and patient discomfort, although it can provide quantitative assessment of respiratory effort (Berry et al., 2012; Chervin et al., 2003). Calibration of the RIP belt signals is usually not performed in routine clinical PSG (Berry et al., 2012; Sackner et al., 1989). Also, movement of the belt and change in posture during sleep renders the calibration ineffective (Whyte et al., 1991). Deflections in the summation channel of the uncalibrated RC and AB RIP signal only provide an estimation of the relative change in tidal volume compared to baseline breathing (Berry et al., 2012; Farre, Montserrat, & Navajas, 2004). Hence there are no quantitative measures for assessment of respiratory effort in routine clinical PSG respiratory variables. However, for clinical PSG, the main purpose of detecting respiratory effort is to differentiate between central respiratory events (absence in respiratory effort) and obstructive respiratory events (persistence of respiratory effort with reduced or absence of nasal airflow) hence

qualitative data from uncalibrated RIP belts, have up until now been deemed adequate (American Thoracic Society, 1996; Berry et al., 2012).

In patients with respiratory muscle dysfunction and /or weakness, episodic oxygen desaturation with or without carbon dioxide retention can be detected during sleep especially during REM sleep (American Thoracic Society/European Respiratory Society, 2002; Steier et al., 2008; Trucco et al., 2018; Wise et al., 2011). Nocturnal measurements are more sensitive for detection of abnormal pulmonary gas exchange than daytime blood gases (Trucco et al., 2018; White, Drinnan, Smithson, Griffiths, & Gibson, 1995). These desaturation events may appear to be "central" or "obstructive" in nature, or a mixture of both (American Thoracic Society/European Respiratory Society, 2002; Bersanini et al., 2012). The precise pattern of the respiratory events depends on the relative activation of the respiratory pump muscles and upper airway dilator muscles (White et al., 1995). Abnormal breathing during sleep in children with neuromuscular disorders may not be predicted by awake pulmonary function testing, arterial blood gases, or the degree of muscle involvement (Bersanini et al., 2012; Steier et al., 2008; Trucco et al., 2018). Hence overnight polysomnography is indicated in children with neuromuscular disease with reduced vital capacity on spirometry (VC < 60% predicted), non-ambulant, or have symptoms of OSA or hypoventilation (American Thoracic Society, 1996; Hull et al., 2012).

While overnight PSG can diagnose and further characterise breathing disorders during sleep including OSA and nocturnal hypoventilation, there are some limitations. Overnight PSG remains a resource- and labour- intensive investigation with limited availability of accredited paediatric sleep labs (American Thoracic Society/European Respiratory Society, 2002). As mentioned before, the current respiratory physiological

variables recorded during routine overnight PSG provide mainly qualitative measures of ventilation (other than O_2 sat and $TcCO_2$ monitoring). There is a need to develop an alternative quantitative measure of respiratory effort to oesophageal manometry that is non-invasive for assessing breathing during sleep in children especially with increasing recognition on the importance of assessing respiratory effort in the diagnosis of sleep-disordered breathing (Guilleminault, Pelayo, Leger, Clerk, & Bocian, 1996; Guilleminault, Stoohs, Clerk, Cetel, & Maistros, 1993; Vandenbussche et al., 2015).

1.7 Electrophysiological assessment of respiratory muscle function

As summarised in section "1.1 Introduction", and also section "1.3 Neural drive to the human respiratory muscles", human ventilation occurs through sequential steps starting from generation of electrical impulses from the respiratory neurons of the brainstem (American Thoracic Society/European Respiratory Society, 2002; Butler, 2007). The electrical impulses travel through motor nerves, transmit through neuromuscular junctions, and propagate throughout muscle membranes. Eventually calcium ions are released in the intracellular space of the muscle cells if a certain action potential threshold level is exceeded (Barrett, Barman, Brooks, & Yuan, 2019; West & Luks, 2016). Linked chemical process (electro-mechanical coupling) then produce a shortening of the contractile elements of the muscle cell, transforming electrical activity into pressure generation by the muscle of interest (Barrett et al., 2019; West & Luks, 2016). As described previously, the smallest function unit to describe the neural control of the muscular contraction process is a motor unit, consisting of a spinal motor neuron and its axon, and all the muscle fibres innervated by this motor neuron. Assessment of

the respiratory neuromotor function can be performed through two main types of test: stimulation tests, and EMG.

1.7.1 Phrenic nerve stimulation

Stimulation test measure the efficiency of neural and neuromuscular transmission and is the gold standard for quantifying the mechanical function of muscle. Unlike performing spirometry and static maximal inspiratory manoeuvres, the stimulation test does not require cooperation from the subject. Peripheral nerve, spinal, or cortical stimulation, either by externally applied electric or magnetic fields, can elicit relatively synchronized and supramaximal activation of motor units at reproducible and predictable levels (American Thoracic Society/European Respiratory Society, 2002). The most common stimulation test performed for the respiratory muscles is stimulation of the phrenic nerve to assess phrenic nerve / diaphragm EMG latencies (and therefore nerve conduction velocity or conduction time), diaphragm compound muscle action potential (CMAP), and twitch transdiaphragmatic pressure (TwPdi) (American Thoracic Society/European Respiratory Society, 2002; Fauroux, 2003; Kassim, Jolley, Moxham, Greenough, & Rafferty, 2011; Laveneziana et al., 2019; Nicot et al., 2006). Using these complex tests diaphragm muscle function and strength can be accurately assessed in virtually all patients including patients in intensive care unit who are unable to produce maximal volitional efforts.

The phrenic nerve can be stimulated electrically or by using magnets. Electrical stimulation of phrenic nerve occurs by using surface electrodes at the posterior border of the sternomastoid, or with implanted needle electrodes (American Thoracic

Society/European Respiratory Society, 2002). Needle stimulation of the phrenic nerve delivers more precise control of the stimuli directly to the phrenic nerve, however it is less comfortable during stimulation and exact location of the electrode is difficult to attain (American Thoracic Society/European Respiratory Society, 2002; Fauroux, 2003). Magnetic stimulation of the phrenic nerve can be achieved using a circular coil placed over the cervical phrenic nerve roots or a figure-of-eight coil placed bilaterally on the two phrenic nerves on the anterior part of the neck (Fauroux, 2003; Kassim et al., 2011). Discharge of the magnetic field from the coils create a pulsed magnetic field which causes current to flow to the phrenic nerve, which in turn causes the diaphragm to contract. Magnetic stimulation does not require contact with skin and is painless. However, compared to electrical stimulation, magnetic stimulation is expensive and relatively unselective in the nerves stimulated which can cause problems when measuring nerve latency (American Thoracic Society/European Respiratory Society, 2002). Also, the TwPdi elicited by magnetic stimulation is greater than the electrical TwPdi due to the activation of the accessory muscles within the magnetic field generated (Fauroux, 2003). It is also difficult to stimulate repetitively using magnetic stimulation.

The EMG signal elicited by supramaximal nerve stimulation (i.e. CMAP) provides different information to EMG signal recorded during spontaneous and voluntary contractions. The diaphragm CMAP is the composite electrical activity within the diaphragm when all the motor neurons innervated by the phrenic nerve is activated synchronously and maximally. It is recorded as one multipeaked summated action potential, with a higher signal-to-noise ratio than EMG recorded during spontaneous contractions (American Thoracic Society/European Respiratory Society, 2002). Diaphragm CMAP is usually detected using surface electrodes applied over the costal

margin of the diaphragm (Glerant et al., 2006; Verin et al., 2002), or using multipair oesophageal electrode catheters (Kassim et al., 2011). The time between triggering of the stimulus and detection of the elicited CMAP is recorded to determine phrenic nerve / diaphragm latencies. The TwPdi amplitude reflects the transformation of diaphragm force into pressure, which depends on diaphragm strength and contractile properties as well as chest and abdominal wall compliance. The TwPdi measured following bilateral supramaximal phrenic nerve stimulation would reflect the maximal strength of the whole diaphragm without the confounding effects of patient motivation or cooperation.

Normal phrenic nerve / diaphragm latencies are reported to average between 6-8 milliseconds in adults and 4.5-6.5 milliseconds in children (American Thoracic Society/European Respiratory Society, 2002; Fauroux, 2003). Due to the shorter length of the right phrenic nerve than the left, latency is slightly shorter on the right side. CMAP amplitudes, recorded from chest wall surface electrodes, average between 500-800 mV. Phrenic nerve/diaphragm latencies are abnormally slow in demyelinating polyneuropathies, such as Guillain-Barre syndrome. Absence of CMAP after nerve stimulation is an indication of paralysis with the pathology located proximal to or at the neuromuscular junction. A decrease in both CMAP amplitude and TwPdi suggests pathology involving neural or neuromuscular transmission defects, whereas a contractile defect of the muscle only manifests with decrease in TwPdi whilst CMAP amplitude is preserved (American Thoracic Society/European Respiratory Society, 2002).

Overall, phrenic nerve stimulation techniques require considerable technical expertise to perform and interpret, specialized equipment, time consuming to perform, and are not easily adapted for routine clinical use to assess diaphragm function. Simpler means of evaluation diaphragm function and electrical activity is preferred.

1.7.2 Electromyographic recording of respiratory muscles

Direct measurement of the output from the respiratory centre in the brainstem of humans is not currently possible. Measuring electrical activation of the respiratory muscle provide an estimate of the respiratory centre output provided the integrity of the motor nerves and neuromuscular junction is intact. Electromyography is the recording and analysis of electrical signal that muscles emanate as actional potentials propagate along muscle fibre membranes. The summation of the electrical activity recorded from all the innervated muscle fibres depolarizing synchronously within one motor unit (i.e. from activation of a single anterior horn cell) is termed motor unit action potential (MUAP). The size of the motor unit is directly related to the size of the MUAP. According to Henneman's size principle, smaller anterior horn cells and their motor units have lower excitability thresholds and are activated first at low force levels (Henneman, 1957). As the muscle force increases, each MUAP will start to obscure waveforms of other active units, creating an "interference pattern" consisting of superposed summation signal from all the MUAP generated by the asynchronously firing motor units. The interference pattern of EMG directly reflects the recruitment and firing frequency of the active motor units within the measured muscle during spontaneous and voluntary contraction.

Electromyography signals can be detected as the difference between the signal from an electrode placed on or in the muscle of interest (active electrode) and the signal from another electrode placed in an electrically silent zone (reference or ground electrode). This arrangement is usually referred to as "monopolar" electrode. Another arrangement is where two electrodes are placed on or in the muscle of interest with a common ground electrode, referred to as "bipolar" electrode.

Three methods are currently used to detect electrical signal from respiratory muscles: 1) the transcutaneous (or surface) method (sEMG), where sensors are placed on the skin overlying the muscle of interest; 2) the transoesophageal method (cathEMG), where sensors mounted on a catheter is positioned in the oesophagus to assess activity of the crural diaphragm; and 3) the intramuscular method (imEMG), in which needle or wire electrodes are introduce into the muscle of interest. Selection of the electrode system depends on individual technique's advantages and disadvantages, equipment and expertise available, patient acceptance and comfort, and also the context of the study (Table 1.1).

Table 1.1. Types of recording electrodes for respiratory muscle electromyogram

Type of electrode	Advantages	Disadvantages
Intramuscular electrodes	Less influenced by cross-talk Single motor unit recordings possible (limited to low levels of contraction)	Discomfort Difficult to place Expertise required Small pneumothorax risk Possible sampling error
Oesophageal catheter electrode (crural diaphragm)	Less influenced by body habitus Less cross-talk	Discomfort Unreliable in diaphragmatic hernia Unable to distinguish between left and right hemidiaphragm pathologies
Surface electrodes overlying chest wall	Non invasive Sample over multiple muscles simultaneously Large volume sampling Sample for long duration	Cross-talk Variable filtering

Adapted from American Thoracic Society statement on respiratory muscle testing (2002)

1.8 Equipment and setting for recording respiratory muscle EMG

1.8.1 Recording electrodes

1.8.1.1 Intramuscular (needle) electrodes

Intramuscular electrodes can record relatively selective EMG recordings from respiratory muscles to provide information on single motor unit activity (i.e. MUAP) (Butler et al., 1999; Butler, McKenzie, & Gandevia, 2001; De Troyer & Estenne, 1984; De Troyer et al., 1996; Gandevia et al., 1996; Gandevia & McKenzie, 1986; Saboisky et al., 2007). Wire or needle electrodes can be placed in the human diaphragm through the 7^{th} or 8^{th} intercostal space or through the abdominal wall under the costal margin assisted by real-time ultrasound to confirm electrode placement location (American Thoracic Society/European Respiratory Society, 2002; Hodges & Gandevia, 2000). Wire electrodes are often used in upper airway muscle studies such as the genioglossus or tensor palatini muscle during sleep (American Thoracic Society/European Respiratory Society, 2002; Carberry, Jordan, White, Wellman, & Eckert, 2016; Mezzanotte, Tangel, & White, 1996).

Intramuscular electrodes are optimal for analysis of action potentials when assessing changes associated with myopathy and for comparing single motor unit discharge frequencies among different respiratory muscles and clinical contexts (Gandevia et al., 1996; Gandevia & McKenzie, 1986). Cross-talk signals from muscles in the area adjacent to the muscle of interest is not completely eliminated, but is much less of an issue compared to surface electrodes (Hodges & Gandevia, 2000). Intramuscular electrodes are difficult to place due to the thinness of the respiratory

muscles (diaphragm ~ 3-4 mm), the proximity of overlying muscles, and the natural shortening and lengthening of the muscle with respiration (Hodges & Gandevia, 2000). Potentially imEMG is less representative of global respiratory muscle activity than recordings made using oesophageal or surface electrodes as only selective motor units are recorded using needle electrodes. Recording multiple MUAPs from multiple sites is needed to ensure that the sample of MUAPs studied is representative of the muscle as a whole. Also, single motor unit recordings may be limited to investigating respiratory muscle function at low levels of contraction e.g. quiet tidal breathing up to 40% of VC (Butler et al., 1999). There is a small risk of pneumothorax, especially when recording from the diaphragm and intercostal muscles (American Thoracic Society/European Respiratory Society, 2002; Hodges & Gandevia, 2000). There is also a risk of bleeding and bruising as with any techniques requiring skin penetration.

Although single motor unit recordings have been used successfully to assess the level of neural drive and also activation profiles of inspiratory muscles including the costal diaphragm, scalene, and parasternal intercostal muscles in adults, the invasive nature of intramuscular electrodes limit its use in children (Butler et al., 1999, 2001; Hudson et al., 2011a; Saboisky et al., 2007).

1.8.1.2 Catheter oesophageal electrodes

Oesophageal electrodes are metal electrodes mounted on a catheter inserted via the nose or the mouth and positioned at the level of the crural diaphragm to assess crural diaphragm EMG (cathEMGdi). Previous oesophageal catheters, with only 1-3 pairs of

electrodes and gastric balloons and weights on the proximal end to help secure the placement of the electrode, did not prevent diaphragm movement relative to the electrodes and resulted in artefactual measurements of cathEMGdi (Gandevia & McKenzie, 1986; Grassino, Whitelaw, & Milic-Emili, 1976; Kim, Druz, Danon, Machnach, & Sharp, 1978). Oesophageal catheters mounted with multiple pairs of electrodes with continuous optimization of diaphragm-electrode positioning by measuring data recorded by the electrode pair closest to the crural diaphragm at any point during the breathing cycle have led to reduction of electrode positioning artefacts during dynamic manoeuvres (Beck et al., 2001; Beck, Sinderby, Weinberg, & Grassino, 1995). Application of a double subtraction technique using the difference between signals obtained from each electrode pair caudal and cranial to the crural diaphragm further improve the signal-to-noise ratio of the cathEMGdi recorded (Sinderby et al., 1999; Sinderby, Beck, Lindstrom, & Grassino, 1997). Recent development of a single oesophageal electrode catheter mounted with two balloons for measurement of oesophageal (Poes) and gastric (Pgas) pressures allows the simultaneous assessment of cathEMGdi and Pdi. (Luo, Tang, et al., 2009; Luo, Wu, et al., 2008; Sinderby et al., 2001; Steier et al., 2009; Steier et al., 2010).

The advantage of oesophageal recordings of EMGdi are that they are not influenced by obesity and less susceptible to cross talk (American Thoracic Society/European Respiratory Society, 2002). Simultaneous measurement of cathEMGdi and Pdi allows assessment of the neuromechanical coupling of the diaphragm. However, the invasive nature of oesophageal catheter and associated patient discomfort, risks of regurgitation and aspiration, and vagally mediated bradycardia associated with their placement, continues to limit widespread clinical use of catheter mounted electrodes especially in children (American Thoracic Society/European Respiratory Society, 2002;

Chervin et al., 2003). Also, oesophageal recording is not able to separate the responses from the two hemidiaphragms during bilateral stimulation of the phrenic nerve, or when the subject is breathing volitionally, limiting its use in patients with hemidiaphragm pathology.

1.8.1.3 Surface (transcutaneous) electrodes

Surface electrodes have been used to measure activity of the costal diaphragm, intercostal, scalene, abdominal and other accessory respiratory muscles in both children and adults (Tabachnik, Muller, Bryan, et al., 1981; Tabachnik, Muller, Levison, & Bryan, 1981; Washino et al., 2017; Yokoba, Abe, Katagiri, Tomita, & Easton, 2003). After the skin is shaved, cleaned, and dried, electrodes are placed over or as close as possible to the muscle(s) of interest. Placement of the electrodes is determined by palpation and by the technician's knowledge of respiratory muscle anatomy.

The main advantage of surface electrodes is their non-invasive nature and hence well-tolerated by babies through to adults (Kraaijenga, de Waal, Hutten, de Jongh, & van Kaam, 2017; Kraaijenga, Hutten, de Jongh, & van Kaam, 2015a; Maarsingh, Oud, van Eykern, Hoekstra, & van Aalderen, 2006; Maarsingh et al., 2004; MacBean, Jolley, et al., 2016; Reilly et al., 2012; Steier, Jolley, Polkey, & Moxham, 2011; Tabachnik, Muller, Bryan, et al., 1981; Tabachnik, Muller, Levison, et al., 1981). They can also be applied easily with minimal discomfort other than redness of the skin. The surface electrodes can sample a large number of motor units, and simultaneously record from multiple muscles for prolonged periods if required, such as during sleep (Darian et al.,

1989; Hawkes, Nowicky, & McConnell, 2007; Lin, Guan, Wu, & Chen, 2018; Praud et al., 1988; Steier et al., 2011; Stoohs, Blum, Knaack, Butsch-von-der-Heydt, & Guilleminault, 2005; Tabachnik, Muller, Bryan, et al., 1981; Tabachnik, Muller, Levison, et al., 1981; Washino et al., 2017; Yokoba et al., 2003). Although the surface electrodes measures diaphragm EMG from the costal portion of the diaphragm, whereas the oesophageal catheter electrodes measures crural diaphragm EMG, close correlations between diaphragm EMG measured using the two different techniques in humans in both quiet and loaded breathing, awake and asleep have been demonstrated (Lin et al., 2018; Lozano-Garcia et al., 2018; Stoohs et al., 2005; van Lunteren, Haxhiu, Cherniack, & Goldman, 1985; Wu et al., 2017). Surface electrodes also has the potential to assess differences in left and right hemidiaphragm function by comparing recordings made from the left to the right costal diaphragm.

One of the disadvantages of the surface electrodes is the potential of cross-talk of signals from muscles in proximity to the one of interest which can make surface electrode recordings unreliable (American Thoracic Society/European Respiratory Society, 2002; Sinderby, Friberg, Comtois, & Grassino, 1996). An example is cross-talk from the sternocleidomastoid and platysma muscle when recording scalene EMG. Another example is crosstalk from the intercostal and abdominal muscles when recording costal diaphragm EMG, as both abdominal and intercostal muscles can be active during loaded breathing (Sinderby et al., 1996). Furthermore, respiratory muscles' EMG recorded using either catheter or surface electrodes are often contaminated by ECG spikes which can affect the analysis of EMG magnitude when the EMG is superimposed by the QRS complex. Another factor affecting surface EMG interpretation is the influence of muscle-to-electrode distance between individuals due to variations in body habitus (e.g. differing

amount of subcutaneous fat tissue, or chest wall deformity), producing a variable filtering effect (American Thoracic Society/European Respiratory Society, 2002; Beck et al., 1995). As highlighted by two recent systematic reviews on the use of inspiratory muscle sEMG in adults, the lack of consensus on multiple factors including the location of electrode placement, EMG recording and processing techniques, and on reporting methods to control for variable muscle-to-electrode distance between individuals, have made data comparison between studies difficult and pooling of data not feasible (Cabral et al., 2018; Dos Reis et al., 2019). These potential issues associated with recording of the sEMG signals will be discussed further in section "1.8.2 Electrode signal processing" and "1.8.3 Artefacts associated with respiratory muscle EMG signals". Methodological challenges need to be addressed in order to facilitate the clinical use and interpretation of inspiratory muscle sEMG.

1.8.2 Electrode signal processing

The raw EMG signals from the respiratory muscles are detected by the electrodes, and then amplified, filtered, digitized, and displayed on a screen for further analysis. The preferred amplifier design for detecting myoelectric signals is a differential amplifier, which amplifies the difference between two paired inputs and thereby eliminates or rejects signals, such as the 50 or 60 Hz power line artefacts that have common influences on both outputs. This process is known as "common mode rejection" (American Thoracic Society/European Respiratory Society, 2002; De Luca, 1997). Bandpass filtering is usually applied to help remove unwanted noise and artefacts from the EMG signal. A high pass filter passes signals with higher frequency than the set threshold and attenuates

signals with frequencies lower than the cut off; the converse is true for low pass filter. A high pass filter of 10-15Hz removes the EMG signal's direct current component and also potential motion artefacts (De Luca, Gilmore, Kuznetsov, & Roy, 2010). The low pass filter is usually set high to avoid distortion of the signal caused by undersampling. During tidal breathing, EMG of the diaphragm and intercostal muscles range between a tenth to a few microvolts in amplitude at a frequency between 20-250Hz (Hutten, Van Eykern, & Van Aalderen, 2010; Schweitzer, Fitzgerald, Bowden, & Lynne-Davies, 1979). Hence a bandwidth of 10 to 1000 Hz is usually used to record surface or oesophageal EMGs of the respiratory muscles (American Thoracic Society/European Respiratory Society, 2002). According to the Nyqvist theorem, only spectral components of frequencies lower than half the sampling frequency can be recorded in digitally sampled signals, therefore the sampling frequency is usually set at 2000 Hz (double the highest EMG frequency of interest) to ensure capture of the entire signal bandwidth (De Luca, 1997).

Surface EMG signal recorded during dynamic contractions without filtering or processing is stochastic and unpredictable at any instant in time, with rapid fluctuation between positive and negative values. Common forms of digital processing of the EMG signal includes obtaining the average of the random EMG values in a window which "slides" along the signal to smooth out the fluctuation in the EMG values, also known as "moving average window" (De Luca, 1997). Full-wave rectification of the EMG signal is also necessary which records the absolute value of each data point. Integrated EMG is another way of processing the EMG signal, where area under the rectified curve is determined for the entire activity or for pre-set time or amplitude values. Root-mean-square (RMS) of the respiratory muscle EMG has also been used in which the EMG signal recorded is squared to rectify the signals, the mean of a time-determinated window about

50-100ms calculated, then the square root value reported. RMS-EMG has been used widely in clinical respiratory muscle EMG research to quantify the amplitude of respiratory muscle EMG as it reflects the number and firing rate of the motor units recruited, increasing in value in relation to the level of contraction (Beck et al., 2001; Dos Reis et al., 2019; Sinderby, Beck, Spahija, Weinberg, & Grassino, 1998; Steier et al., 2011; Steier et al., 2008; Steier et al., 2009).

Depending on the clinical and/or research application, different signal processing techniques has been adopted to record and measure respiratory muscle EMG. It is known, that the window size used for signal integration, digital filter characteristics and the sampling frequency can directly interfere with the EMG data obtained (Dos Reis et al., 2019). The lack of standardisation and in some cases the lack of reporting of the signal processing methods used in different studies makes comparison between studies difficult and prevent meta-analysis of EMG research (Cabral et al., 2018; Dos Reis et al., 2019; Hutten, van Thuijl, van Bellegem, van Eykern, & van Aalderen, 2010). There is a need to ensure complete description of signal processing procedure is included in all future respiratory muscle EMG research.

1.8.3 Artefacts associated with respiratory muscle EMG signals

As discussed briefly in sections “1.8.1.2 Catheter oesophageal electrodes”and also “1.8.1.2 Surface (transcutaneous) electrodes”, respiratory muscle EMG signals can be affected by muscle-to-electrode distance. Increasing muscle-to-electrode distance causes decrease in the amplitude of the EMG signal recorded with relatively larger attenuation of high- than low-frequency power hence the relative distribution of power in the EMG

spectrum is also altered (American Thoracic Society/European Respiratory Society, 2002; Beck et al., 1995; De Luca, 1997). The distance between the electrodes can also affect the amplitude and frequency content of the EMG signal recorded hence interelectrode distance should be kept to a minimal distance without the electrode touching (De Luca, 1997; Verin et al., 2002). For surface EMG of the respiratory muscles, usually an interelectrode distance of 1-2 cm is recommended (Verin et al., 2002). If the interelectrode distance is too small, the detection surfaces may be shunted electrically if the surface of the skin become moist with sweat which is conductive. The electrical shunting decreases the signal amplitude recorded, decreases the signal to noise ratio, and may filter out the higher frequency components of the EMG signal (American Thoracic Society/European Respiratory Society, 2002; De Luca, 1997). Movement of the electrode or change in the pressure on the electrode can also cause amplitude artefacts in the low frequencies (mostly below 20-25 Hz) due to the redistribution of the charges in the interface between the electrodes and the recording surface (American Thoracic Society/European Respiratory Society, 2002). As mentioned in section "1.8.2 Electrode signal processing", a high pass filter is usually set at 10Hz to minimise motion artefacts, which also cause inevitable loss of some low frequency power from the EMG signal.

Another artefact associated with electrode placement position is the location of electrode in relation to the innervation zone of the muscle of interest. Electrodes placed over the innervation zone, a region with a high density of motor end places, would produce complex interference EMG pattern and potentially result in lower RMS-EMG amplitude due to signal cancellation and a higher frequency content (in mean power frequency) because of recordings of motor unit action potentials propagating away from the electrode (American Thoracic Society/European Respiratory Society, 2002; Cabral et

al., 2018; Dos Reis et al., 2019). However, a recent study has shown that submaximal contractions are less affected by signal cancellation when the electrodes are placed over the innervation zone compared to maximal contractions (Smith et al., 2015). Also the rates of fatigue induced changes in the RMS and mean power frequency are similar regardless of whether electrode placement was over the innervation zone or proximal or distal to it (Smith et al., 2015). One way to minimize this effect is to place the electrode in the middle of the muscle belly of interest, or to use multichannel or linear array electrodes to optimise signal analysis.

Powerline artefact is one of the most common problem with recording muscle EMG. The line frequency interference signal originating from the powerline and mains power equipment is typically a 50-60Hz signal depending on the power source. Properly earthed connections and using a differential amplifier with a high common mode rejection ratio (e.g. > 100 dB) as mentioned in section "1.8.2 Electrode signal processing" are useful methods to reduce the artefact. Connecting all recording instruments to the same ground point also helps. An isolated pre-amplifier with a notch filter of 50 Hz and a shielded electrode cable can minimize the artefact further (Hutten, Van Eykern, et al., 2010; Luo, Moxham, & Polkey, 2008). However, the EMG signal may be distorted if a notch filter is set at 50 Hz since the maximum power frequency of diaphragm and intercostal muscle EMG during tidal breathing is about 80 Hz (Hutten, Van Eykern, et al., 2010). Hence the use of notch filter is not recommended especially if the measurement of respiratory muscle amplitude is important for the clinical and/or research context (Merletti & Hermens, 2004; Stegeman & Hermens, 1998). Background noise, or signals of unidentifiable origin (including ancillary equipment such as pressure sensors, pumps, ventilators) may introduce noise in various frequency ranges, reducing the signal to noise

ratio (i.e. the ratio between the EMG signal compared to the background contamination noise signal, a measure of the quality of the amplified signal).

Cross-talk artefact from the ECG signal is an issue that can affect respiratory muscle EMG recordings made using oesophageal and surface electrodes. The much stronger intensity of the cardiac impulse compared to the respiratory muscle EMG signals can affect the amplitude and frequency parameters of respiratory muscle EMG. Diaphragm and intercostal muscle EMG frequency varies between 20-250Hz, whereas most of the ECG frequency is 0-100 Hz (Hutten, Van Eykern, et al., 2010; Schweitzer et al., 1979). It is difficult to eliminate the ECG effectively from the EMG signals using filters because of the overlap in the range of the two signals, although the P and T waves may be removed by setting a high-pass filter at 20 Hz (Bower, Sandercock, Rothman, Abbrecht, & Dantzker, 1984; Schweitzer et al., 1979). One method to remove ECG artefact is using gating techniques, where EMG with the superimposed QRS complex artefact is deleted for approximately 0.4 s around the QRS complex (Bartolo, Roberts, Dzwonczyk, & Goldman, 1996; Schweitzer et al., 1979; van Eykern, Maarsingh, & van Aalderen, 2001). However, segments of EMG activity of particular interest potentially may also be discarded, resulting in underestimation of the calculated EMG. A variation of the gating technique is to replace the EMG signal during the duration of the QRS complex with the average of the adjacent EMG activity or another predicted value (Hutten, Van Eykern, et al., 2010; Maarsingh, van Eykern, Sprikkelman, Hoekstra, & van Aalderen, 2000; Sinderby et al., 1998; Sinderby et al., 1999; Sprikkelman, Van Eykern, Lourens, Heymans, & Van Aalderen, 1998; van Eykern et al., 2001). A more complex but potentially more accurate method than the gating technique is subtraction of the ECG template from the EMG signal in the section contaminated by the ECG waveform

(Bartolo et al., 1996). This technique does not work if the ECG signal is fluctuating (Fox, Kosch, Feldman, & Stark, 1988). The easiest method is to analyse the EMG that falls between the QRS complexes, selecting only EMG data between 50-75% of the QRS complex interval for analysis (MacBean, Jolley, et al., 2016; Reilly et al., 2011; Steier et al., 2011; Steier et al., 2009; Steier et al., 2010). However for subjects with faster heart rate such as infants and young children, a greater proportion of the EMG activity will be contaminated by ECG and hence potentially not measurable.

Other sources of cross-talk in respiratory muscle EMG recordings (as discussed in section "1.8.1.2 Catheter oesophageal electrodes" and section "1.8.1.3 Surface (transcutaneous) electrodes") includes signals from oesophageal peristalsis when recording diaphragm EMG using oesophageal electrodes, and contamination from adjacent muscles such as abdominal or intercostal muscles when recording diaphragm EMG using surface electrodes (American Thoracic Society/European Respiratory Society, 2002; Luo, Moxham, et al., 2008; Sinderby et al., 1996).

1.8.4 Reporting of EMG signal

Different measures have been used to report the amplitude of EMG activity of the respiratory muscles, including RMS-EMG values and also values which are normalized to a reference value (Cabral et al., 2018; Dos Reis et al., 2019). Given that surface and catheter respiratory EMG values can be influenced by technical, anatomical (such as muscle-to-electrode distance) and physiological factors (such as lung volume), normalised EMG is preferred to allow comparison between individuals, between muscles, and also between occasions (Laveneziana et al., 2019; Sinderby et al., 1998). The choice

of methods and postures suitable to obtain the reference signal for normalization is important to ensure signal quality and comparisons although there is currently no standard on the preferred technique for normalisation (Cabral et al., 2018; Dos Reis et al., 2019).

One of the methods employed to normalise EMG is to use the resting or baseline EMG level before an intervention as the reference value and calculate a ratio of change in EMG activity from baseline (Dos Reis et al., 2019; Maarsingh et al., 2002; Maarsingh et al., 2004; Sprikkelman et al., 1998). Using individual subject's baseline EMG activity as a reference, the change in the respiratory muscle EMG activity is able to be compared between individuals and for assessing level of change within individuals. This method of normalization has been utilized in research involving children as it does not need cooperation or performance of specific manoeuvres from the children (Maarsingh et al., 2006; Maarsingh et al., 2002; Maarsingh et al., 2004; Sprikkelman et al., 1998). However, as some muscles are not activated during tidal breathing (e.g. accessory respiratory muscles or expiratory muscles), this reference value may not be suitable depending on the clinical or research question.

Normalizing EMG signal to the maximal EMG signal generated when performing a maximum manoeuvre has been shown to be a reproducible method for reporting diaphragm and parasternal intercostal muscle EMG activity for both older children and adults (Jolley et al., 2009; MacBean, Hughes, et al., 2016; MacBean, Jolley, et al., 2016; Reilly et al., 2011; Sinderby et al., 1998). However, performing reproducible maximum manoeuvres such as MIP and SNIP procedures may not be possible in young children (< 8 years of age), during exacerbation periods of disease, or where cooperation by the subject is not possible (e.g. if the subject is intubated and sedated or not able to obey instructions) (MacBean, Jolley, et al., 2016; Reilly et al., 2012). Also, as discussed in

section “1.6.2 Pressure measurements”, there is a learning effect associated with maximal manoeuvres and reproducibility does not guarantee maximality (Aldrich & Spiro, 1995; Fiz et al., 1989; Wen et al., 1997). Adult studies have shown different individuals achieves peak respiratory muscle EMG through different maximal manoeuvres (Jolley et al., 2009; Reilly et al., 2011; Sinderby et al., 1998). Although maximum or near maximal respiratory effort is achievable with maximal respiratory manoeuvres for diaphragm, these manoeuvres are not validated for other respiratory muscles (Dos Reis et al., 2019; Gandevia, McKenzie, & Plassman, 1990; Sinderby et al., 1998). In Gandevia et al’s study (1990), parasternal intercostals had the highest level of activation during trunk flexion whereas the scalene produced their highest activity during lateral flexion of the head. Due to the variations in the reference peak EMG values obtained during maximum manoeuvres, there can be greater discrepancies in the normalised value than the RMS-EMG value between- and within- occasions (MacBean, Hughes, et al., 2016; Reilly et al., 2011; Sinderby et al., 1998; Williams, Porter, Westbrook, Rafferty, & MacBean, 2019). There has been no study on the peak respiratory muscle EMG recorded when children perform different maximal inspiratory manoeuvres, and it is not known how many attempts of individual maximal inspiratory manoeuvres are required to achieve a reproducible peak respiratory muscle EMG. If respiratory muscle EMG should always be reported as a normalised value, then these questions needs to be addressed to ensure consistent and reliable reporting methods are used for future respiratory muscle EMG studies in children.

1.9 Clinical applications of respiratory muscle surface EMG measurement in children – a measure of neural respiratory drive

It is known that chronic respiratory diseases can lead to changes in the geometry of the rib cage and lung architecture which affect force-length relationship for respiratory muscles, causing mechanical disadvantage when respiratory muscle contracts. All of this can affect the movement of the rib cage, impairing pulmonary ventilation and the breathing pattern (Da Gama et al 201; Myrrha et al 2013; Ratnovsky et al 2008; Reilly et al 2011; Sieck et al 2013). Therefore, evaluating muscle activity through EMG can play a role in understanding ventilatory disorders and pulmonary alterations, helping to diagnose and monitor patients with respiratory disorders (Cabral et al., 2018; Dos Reis et al., 2019; Laveneziana et al., 2019).

As discussed earlier in section "1.7.2 Electromyographic recording of respiratory muscles", the interference pattern of respiratory muscle EMG recorded during spontaneous and volitional breathing reflects the degree of motor unit recruitment and /or motor unit firing rate, hence the NRD to the muscle. Increase in the NRD occurs when there is an increase in the load: capacity balance of the respiratory system. An example of the load on the respiratory system is the obstructive load on the upper airway from enlarged adenoids and tonsil in children during sleep causing increase in upper airway resistance. Another example is the obstructive load on the lower airways from bronchial constriction and hyperinflation associated with airway inflammation and bronchospasm in asthma. Chest wall disorders such as kyphoscoliosis and obesity can also increase the load on the respiratory muscle pump by reducing respiratory system compliance. The capacity of the respiratory muscle pump to offset the load on the respiratory system can

be reduced in certain circumstances, such as respiratory muscle weakness from neuromuscular disease such as muscular dystrophy or from critical illness experienced by patients in the intensive care unit. Lung pathologies causing hyperinflation can also reduce muscle capacity with the diaphragm contracting from an unfavourable length. When there is an overall imbalance of the load: capacity ratio of the respiratory system, an increase in ventilatory drive and hence NRD would occur as reflected by increase in the respiratory muscles' EMG.

Two recent reviews published have extensively discussed studies which have employed surface EMG as an outcome measure in adults with and without respiratory conditions (Cabral et al., 2018; Dos Reis et al., 2019). Clinical use of respiratory muscle EMG as an index of NRD has been explored extensively in conditions such as COPD in adults. Diaphragm crural EMG and parasternal intercostal sEMG has been used as an alternative to FEV1 as a marker of clinical deteriorations, risk of re-admissions, and indicator of long-term mortality risk (Jolley et al., 2009; Murphy et al., 2011; Patout et al., 2019; Suh et al., 2015). Assessment of respiratory muscle (including upper airway muscle) surface EMG has also been performed in neonates, infants, and children in a variety of clinical and experimental settings (Hutten et al., 2008; Kraaijenga et al., 2017; Kraaijenga et al., 2015a; Kraaijenga, Hutten, de Jongh, & van Kaam, 2015b). The use of sEMG to assess NRD in children is preferable as it is non-invasive and nonintrusive, and has potentials to be used as a non-volitional method of estimating lung function in children who are not able to perform clinical spirometry. Therefore, I will review current understanding on the clinical use of sEMG in children with various respiratory conditions to determine gaps in existing knowledge which require further research.

1.9.1 Sleep and Sleep-disordered breathing

As discussed in section "1.3.3 Control of breathing during sleep", previous studies have investigated respiratory muscle activation pattern during quiet tidal breathing in infants and adolescents without respiratory disorders asleep (J. Lopes et al., 1981; Muller et al., 1979; Tabachnik, Muller, Bryan, et al., 1981). In brief, phasic and tonic activity of the intercostal muscles is observed in infants during NREM sleep which is important for stabilizing the highly compliant chest wall in infants. (J. Lopes et al., 1981). In adolescents, similar to adults, increase in intercostal and diaphragm muscle activation compared to wakefulness was found during NREM sleep (Tabachnik, Muller, Bryan, et al., 1981). Marked decrease in intercostal muscle activity during REM was associated with increase in diaphragm activity. Upper airway muscles' sEMG (such as genioglossus) and abdominal muscle sEMG has been studied to further understanding of the role of respiratory muscle to obstructive events in neonates and children (Jeffries et al., 1984; Praud et al., 1988; Praud et al., 1989). Majority of the research performed using respiratory muscle sEMG in children have focused on investigating the respiratory mechanics associated with sleep in healthy infants or children with OSA. There is a deficiency of research on clinical uses of respiratory muscle sEMG in children even though diaphragm sEMG is one of the routine respiratory variables that are recorded during overnight clinical sleep studies.

Diaphragm sEMG currently is used as a qualitative index of respiratory effort during PSG as discussed in section "1.6.4 Overnight sleep studies". Persistent phasic chest wall (diaphragm) sEMG activity helps to identify obstructive respiratory events, with a high degree of agreement with RIP for classifying events as obstructive or central during sleep (Iber et al., 2007; Pamula et al., 2011; Berry, Ryals, Girdhar, & Wagner,

2016). Moreover, a significant number of events classified as central apnoeas by RIP were re-classified as obstructive events based on chest wall sEMG (Berry et al., 2016). Similarly, cathEMGdi was found to be a more reliable index of respiratory effort than RIP when differentiating between obstructive or central events in adults with OSA (Luo, Tang, et al., 2009). Diaphragm EMG may be a better measure of respiratory motor output than Poes as Poes can be affected by lung volume and airflow and the two measures are correlated but not interchangeable (Laveneziana et al., 2019; Luo, Tang, et al., 2009; Steier et al., 2010; Stoohs et al., 2005). It is worth noting that the AASM's recommended bandpass filter setting for EMG of 10-100 Hz with a sampling rate of 200-500 Hz is much narrower than the bandwidth of 10-1000 Hz and sampling rate of at least 2000 Hz suggested for respiratory muscle EMG (American Thoracic Society/European Respiratory Society, 2002; Iber et al., 2007). The EMG filter settings for PSG was suggested for recording submental and leg EMG for the purpose of evaluating muscle tone and twitches to assist with sleep stage scoring and the diagnosis of periodic limb movement (Penzel et al., 2007). The lack of a method to quantitatively assess surface EMG of the diaphragm (sEMGdi) using commercial sleep study software likely contributed to the lack of research using sEMGdi as a quantifiable index of NRD and respiratory effort during sleep in children. In conjunction, the impact of the smaller EMG filter range on PSG recordings on the magnitude of sEMGdi needs to be explored.

Another area that needs to be explored is the relationship between sEMGdi and Poes, the current gold standard for assessing respiratory effort during sleep (R.B. Berry et al., 2013). Diaphragm and intercostal EMG was not included in the 2013 AASM Manual for the Scoring of Sleep and Associated Events as an alternative index of respiratory effort to Poes and RIP due to a paucity of clinical studies

evaluating its accuracy compared to Poes (Berry et al., 2013; Berry et al., 2012). Diaphragm EMG (evaluated using surface and catheter-mounted electrodes) has been compared with but not validated against Poes during obstructive respiratory events in sleep (Luo, Tang, et al., 2009; Luo, Wu, et al., 2008; Steier et al., 2010; Stoohs et al., 2005). Good correlations were demonstrated between sEMGdi and Poes in one study highlighting the potential of using sEMGdi to assess NRD during sleep rather than cathEMGdi (Stoohs et al., 2005). There are no comparative studies between Poes with sEMG activities of other inspiratory muscles during sleep. There has been no study exploring the relationship between Poes and EMGdi during tidal unobstructed breathing in sleep. Upper airway resistance syndrome is a part of the spectrum of obstructive sleep-disordered breathing where increased respiratory effort without apnoea can cause significant disruption and daytime symptoms which usually requires the measurement of Poes for diagnosis (Berry et al., 2013; Berry et al., 2012; Guilleminault et al., 1993). This highlights the importance of developing a non-invasive alternative to assess respiratory effort during tidal breathing such as sEMG of the inspiratory muscles to enhance the diagnosis of children on the spectrum of obstructive sleep-disordered breathing.

A potential clinical use of respiratory muscle sEMG that should be explored is in the assessment of treatment efficacy in children receiving positive airway pressure (PAP) support for sleep-disordered breathing. Nasal continuous positive airway pressure (CPAP) is the treatment of choice for children who have residual OSA after surgical treatment (American Thoracic Society, 1994). Provision of continuous airway pressure in children and adults prevents upper airway collapse associated with OSA, and is associated with unloading of the inspiratory muscles and improvements in breathing pattern and gas exchange (Fauroux et al., 2001; Khirani et

al., 2013; Leboulanger et al., 2010; Luo, Qiu, et al., 2009). The titration of CPAP is usually based on the polysomnographic disappearance of apnoea, hypopnoea, and snoring as recommended by AASM (Kushida et al., 2008). However, evidence from both infants and adults have suggested the optimal pressure for minimising respiratory effort as measured by Poes is higher than the pressure required to eliminate snoring, apnoea/hypopnoea, or improvement of gas exchange parameters (Khirani et al., 2013; Montserrat et al., 1995; Sforza et al., 1995). In children, other causes of airway obstruction including laryngomalacia, bronchopulmonary dysplasia, cystic fibrosis, and bronchiolitis may benefit from PAP when awake due to improvements in breathing pattern, tidal volume, and unloading of the respiratory muscles associated with PAP (Essouri et al., 2011; Fauroux, Hart, & Lofaso, 2002; Fauroux et al., 2001; Khirani et al., 2013). In most of these studies, the degree of diaphragm or inspiratory muscle load were assessed through the measurements of cathEMGdi, Pdi and Poes swing, and/or the diaphragmatic or oesophageal pressure-time product (PTPdi, PTPoes), which requires the insertion of an invasive oesophageal catheter (Essouri et al., 2011; Fauroux et al., 2003; Fauroux et al., 2001; Khirani et al., 2013; Leboulanger et al., 2010; Luo, Qiu, et al., 2009). It has been shown in healthy adults that the relationship between PTPdi and cathEMGdi is linear in tidal breathing (Fauroux et al., 2003). Also a small study in adult (n=12) confirmed that PAP use was associated with improvement in phasic tidal inspiratory muscle sEMG magnitude (including sternomastoid, parasternal, and diaphragm muscle), and that changes in the inspiratory muscle sEMG corresponded with changes in intrathoracic pressure swings (Carrey, Gottfried, & Levy, 1990). Based on this study the AASM suggests the inclusion of inspiratory muscle sEMG as an indicator of improvement in work of breathing when titrating PAP for treating children and adults with alveolar hypoventilation despite the

lack of data on the impact of PAP on inspiratory muscle sEMG in children (Berry et al., 2010). These studies highlight the potential of using respiratory muscle sEMG as an alternative method to evaluate respiratory load and hence physiological changes associated with the use of PAP in children.

1.9.2 Obstructive disease of the lower airway

The measurement of NRD using respiratory muscle sEMG in children has been employed in disease states other than OSA (Hutten, Van Eykern, et al., 2010; Kraaijenga et al., 2017; Kraaijenga et al., 2015a; Maarsingh et al., 2006; Maarsingh et al., 2002; Maarsingh et al., 2000; Maarsingh et al., 2004; MacBean, Jolley, et al., 2016; Sprikkelman et al., 1998). Obstructive disease of the lower airways, such as asthma and cystic fibrosis (CF) are characterised by increased airway resistance, lung volume, and respiratory rate. High airway resistance and also lung hyperinflation increases the load on the respiratory system. In accordance with the length-tension characteristics of the muscle, the force exerted in response to a given stimulation decreases markedly when the muscle operates at non-optimal lengths at abnormally high lung volume, causing a decrease in muscle capacity. Overall, the increase in obstructive load and the potential decrease in muscle capacity causes an imbalance of the load: capacity ratio and hence an increase in the NRD of patients with obstructive airway disease.

1.9.2.1 Wheezy infants and preschool children

Wheezing is a common presenting symptom and sign of respiratory disease in infants and children, a musical sound produced by the oscillation of opposing walls of an airway narrowed almost to the point of closure (Forgacs, 1978). In children the most common cause of acute wheezing in infants is bronchiolitis, usually due to infection with viruses such as respiratory syncytial virus and rhinovirus (Ralston et al., 2014). The clinical assessment of severity of bronchiolitis is important to aid clinical decision-making and for evaluation of treatment efficacy. The inability of infants to perform volitional forced expiratory manoeuvres precludes the use of spirometry to help objectively assess the severity of airway obstruction. In Maarsingh et al's study (2006), sEMGdi and parasternal intercostal muscle sEMG (sEMGpara) correlated with a validated clinical symptoms score when infants and toddlers (2-37 months of age) were admitted to hospital due to wheezing illness. There was a significant decrease in diaphragm and intercostal EMG activities throughout the hospital admissions, in parallel with improvement in the clinical symptom score (Maarsingh et al., 2006). Parasternal intercostal sEMG was also shown to be elevated in wheezy preschool children (< 5 years of age) compared to healthy children (MacBean, Jolley, et al., 2016). Another use of this technique has been the performance of bronchial challenge testing in pre-school children using sEMGdi and sEMGpara as a surrogate measure of FEV1 (Maarsingh et al., 2004). A linear increase in sEMGdi and sEMGpara activity was observed with increasing histamine doses during the challenge with the return of respiratory muscle EMG back to baseline following administration of a bronchodilator. These studies support the use of respiratory muscle EMG as an alternative objective measurable outcome to assess airway

limitation and treatment efficacy for young wheezy children who are not able to perform reliable spirometry.

1.9.2.2 Asthma in school aged children (> 5 years of age)

Asthma is one of the most common chronic disease of childhood (Global Asthma Network, 2018). Demonstration of reversible airflow obstruction can help establish the diagnosis of asthma in children 6 years of age and older, facilitating the assessment of severity and treatment efficacy (National Asthma Council Australia, 2019). However for children under 6 years of age and also older children who are not cooperative and/or unable to follow instructions, the use of spirometry to assess airflow limitation is limited (MacBean, Jolley, et al., 2016). Even children as old as 13 years of age may not be able to perform reliable spirometry measurements (MacBean, Jolley, et al., 2016). Further, reproducible lung function testing is often not possible when the subject is unwell with an acute attack of asthma (National Asthma Council Australia, 2019).

Inspiratory muscle sEMG during tidal breathing in children has been demonstrated to be a potential alternative to spirometry in the assessment of asthmatic children both awake and asleep (Fokkema, Maarsingh, van Eykern, & van Aalderen, 2006; Maarsingh et al., 2002; Maarsingh et al., 2004; Sprikkelman et al., 1998; Steier et al., 2011; Tabachnik, Muller, Levison, et al., 1981). Respiratory muscle activity (including diaphragm, intercostal, and abdominal muscle sEMG) in children with asthma was shown to be different from healthy children, with a later peak of sEMG during the breathing cycle (Fokkema et al., 2006). Respiratory muscle EMG correlated with a fall in FEV1

during bronchial challenges, demonstrating a linear relationship between histamine doses and the increase in diaphragm and intercostal sEMG activity in school-aged asthmatic children (Maarsingh et al., 2002; Maarsingh et al., 2004). Respiratory muscle activity returned to pre-challenge value following the administration of a bronchodilator (Maarsingh et al., 2002; Sprikkelman et al., 1998). In asthmatic adolescents, the reduction in intercostal muscle activity during REM sleep is associated with marked paradoxical movement of the rib cage similar to those observed in newborn, and a significant decrease in mean inspiratory flows despite increased diaphragm activation is likely due to the unstable rib cage (Tabachnik, Muller, Levison, et al., 1981). Similar findings were demonstrated in adults, where sEMGpara during tidal breathing in sleep was highest in uncontrolled asthmatics followed by controlled asthmatics then healthy controls (Steier et al., 2011). Increase in the sEMGpara activity in early morning compared to evening in the asthmatics was associated with lower FEV1 performed at the same time (Steier et al., 2011). These studies indicate that respiratory sEMG activity is a sensitive objective tool for detecting airway limitation in children, allowing non-volitional assessment of airway response to bronchial challenge and bronchodilators in children. The possibility of long continuous periods of recording during tidal breathing also allows the opportunity to assess the level of asthma control during sleep.

1.9.2.3 Cystic Fibrosis

CF is the most common life-limiting autosomal recessive disease among Caucasian populations (Cystic Fibrosis Foundation, 2018). Typical respiratory manifestations of CF include a productive cough, hyperinflation and bronchiectatic

changes on chest imaging, and obstructive airway disease on pulmonary function testing (Hart et al., 2002). FEV1 and its rate of decline have been shown to be the best independent predictors of survival (Kerem, Reisman, Corey, Canny, & Levison, 1992; Milla & Warwick, 1998). It has been shown previously in children and young adults with advanced CF, that reduction in FEV1 was accompanied by a rise in respiratory rate, decrease in tidal volume, increased inspiratory muscle load (measured by oesophageal pressure time product, PTPoes), and impairment of gas exchange despite maintenance of global inspiratory muscle strength (assessed by sniff Poes) (Hart et al., 2002). On the contrary, in adult with CF, diaphragmatic strength (Pdi) decreased with declining FEV1 and also worsening nutritional status (Pradal et al., 1994). Furthermore, respiratory muscle strength (MIP) is significantly decreased in CF patients who are chronically infected with *Pseudomonas aeruginosa*, one of the most prevalent cause of chest infections in CF (Cystic Fibrosis Foundation, 2018; Dassios, Katelari, Doudounakis, & Dimitriou, 2014). Overall, with increasing elastic and resistive load (from mucus plugging causing airway obstruction, destruction of lung parenchyma by bronchiectasis as well as ongoing inflammation within the bronchial walls) in CF, and decrease in respiratory muscle capacity, it is expected that patients with CF would have higher NRD in order to maintain normal breathing and gas exchange.

Diaphragm EMG measured using oesophageal electrodes (cathEMGdi) and also sEMGpara has been measured in adults with cystic fibrosis (Reilly et al., 2012; Reilly et al., 2011; L. Smith et al., 2017). Patients with CF had significantly greater cathEMGdi and sEMGpara compared to healthy controls during tidal breathing (Reilly et al., 2011). The increase in cathEMGdi and sEMGpara was proportional to the degree of airway obstruction (assessed by FEV1), hyperinflation, and dynamic lung compliance (Reilly et

al., 2011). During incremental cycle exercise test, CF patients became progressively more hyperinflated, accompanied by increasing cathEMGdi activity in line with breathlessness. Neuromechanical dissociation occurred from 70% of total exercise time onwards, with plateau of PTPdi despite increasing cathEMGdi and sEMGpara (Reilly et al., 2011). When CF patients performed a different exercise test, the 10m incremental shuttle walk test, sEMGpara measured at rest prior to exercise correlated more with peak aerobic capacity (V_{O2} peak) than FEV1 highlighting sEMGpara assessing a different aspect of lung function than FEV1 (Smith et al., 2017). When sEMGpara was tracked in CF patients admitted to hospital with acute exacerbations, there was a significant reduction in sEMGpara between admission and discharge associated with improvement with FEV1 and VC (Reilly et al., 2012). These studies suggest the potential use of sEMGpara as a non-volitional measure of respiratory function in CF, complementing conventional lung function tests both in the acute setting and also for long-term monitoring. Currently there are no published data on respiratory muscle sEMG level in children with cystic fibrosis.

1.9.3 Intensive care unit and invasive ventilation

In the intensive care unit, cathEMGdi has been used in both paediatric and adult patients as a method to trigger and determine the level of assistance required during mechanical ventilation, i.e. “neutrally adjusted ventilatory assistance (NAVA)” (Baudin et al., 2015; Kallio et al., 2015; Sinderby et al., 1999). Use of NAVA in ventilated adults was shown to improve patient-ventilatory interactions, reduce trigger delays and total asynchrony events, reduce extra-diaphragmatic inspiratory muscle activity and improve ventilation of dependent lung regions with improved gas exchange (Carteaux

et al., 2016; Cecchini, Schmidt, Demoule, & Similowski, 2014; Piquilloud et al., 2012; Spahija et al., 2010; Terzi, Pelieu, et al., 2010). Similar benefits of using NAVA ventilators compared to pressure controlled ventilators with improved patient-ventilator interaction and synchrony, and decreased peak inspiratory pressure were also demonstrated in neonates and children (Beck et al., 2009; Bordessoule, Emeriaud, Morneau, Jouvet, & Beck, 2012; Breatnach, Conlon, Stack, Healy, & O'Hare, 2010; Stein & Howard, 2012). Normalising actual cathEMGdi to maximal cathEMGdi as a ratio can be used to estimate the patient's effort to breathe and allow intra-individual and inter-individual comparisons (Bellani et al., 2013; Muttini et al., 2015). Crural diaphragm EMG (cathEMGdi) has also been used to monitor and track NRD, predict success of weaning from ventilation, and assess efficacy of interventions such as nasal CPAP and high flow nasal prong oxygen in neonates (Oda, Parikka, Lehtonen, & Soukka, 2018; Singh, McNally, & Darnall, 2018; Stein, Hall, Davis, & White, 2013), and children (Pham, O'Malley, Mayfield, Martin, & Schibler, 2015).

Surface diaphragm EMG has also been used in the intensive care unit setting in both adults and children (Bellani et al., 2018; de Waal, Hutten, Kraaijenga, de Jongh, & van Kaam, 2017; de Waal, Kraaijenga, Hutten, de Jongh, & van Kaam, 2017; Kraaijenga et al., 2017; Kraaijenga et al., 2015a, 2015b). sEMGdi was found to have good correlation with cathEMGdi and Poes in ventilated adults (Bellani et al., 2018). sEMGdi was used to assess diaphragmatic activity in preterm infants weaning from respiratory support (Kraaijenga et al., 2017), transitioning from CPAP to high flow nasal prong oxygen (de Waal, Hutten, et al., 2017), assess clinical response to caffeine (Kraaijenga et al., 2015a), and as a method for monitoring respiratory rate and breathing in preterm infants (de Waal, Kraaijenga, et al., 2017; Kraaijenga et al., 2015b).

There is no data on the use of inspiratory muscle sEMG to estimate NRD in children admitted to intensive care.

1.10 Summary of current state of the art and research gaps identified

Surface respiratory muscle EMG measurement is a potential tool for assessing NRD in children across different age groups, ranging from neonates to adolescents (Kraaijenga et al., 2017; Kraaijenga et al., 2015a; Laveneziana et al., 2019; Maarsingh et al., 2006; Maarsingh et al., 2002; Maarsingh et al., 2000; Maarsingh et al., 2004; MacBean, Jolley, et al., 2016; Sprikkelman et al., 1998). It is non-invasive and tolerated well by children, making surface electrodes the preferred technique for recording respiratory muscle EMG compared to oesophageal or intramuscular electrodes. As discussed, surface respiratory muscle EMG has already been used in various clinical scenarios in paediatric research, ranging from neonates and children requiring respiratory support, infants and toddlers with viral induced wheeze, and school-aged children with asthma (Kraaijenga et al., 2017; Kraaijenga et al., 2015a; Maarsingh et al., 2006; Maarsingh et al., 2002; Maarsingh et al., 2000; Maarsingh et al., 2004; Sprikkelman et al., 1998). However, most of these studies adopted bilateral placement of surface electrodes as their method of recording sEMG which would be inappropriate for conditions where differential neural drive is expected between the left and right side such as in hemidiaphragm paralysis. In previous research involving adults and also in clinical sleep studies, right sided placement of electrodes has been used to minimise potential ECG artefacts (Cabral et al., 2018; Dos Reis et al., 2019; Berry et al 2010). Although diaphragm sEMG is already incorporated as a respiratory variable in paediatric clinical overnight sleep studies to help differentiate between

obstructive and central respiratory events, there are no studies on the use of sEMGdi as an objective index of respiratory effort in snoring children likely due to the lack of a readily available method to quantitatively measure sEMGdi recorded using commercial sleep study software. The development of a method to quantitatively assess sEMGdi would facilitate the exploration of NRD in the diagnosis and management of children with and without sleep-disordered breathing.

There are also gaps in the knowledge on the use of diaphragm and extra-diaphragmatic inspiratory muscles' sEMG as a complementary method of assessing lung function in children. Although in research studies, tidal inspiratory muscle sEMG (especially sEMGpara) has been shown to correlate with lung function parameters such as FEV1 in both children and adults with various respiratory conditions (Jolley et al., 2009; MacBean, Hughes, et al., 2016; Reilly et al., 2012; Reilly et al., 2011; Steier et al., 2011; Suh et al., 2015), the relationship between inspiratory muscle sEMG and lung function variables such as lung volume and mouth pressure has not been investigated in children. Although most studies have presented sEMG magnitude as a normalised value to allow minimisation of potential anatomical differences between individuals when interpreting sEMG, the ideal respiratory manoeuvre for obtaining the reference peak sEMG signal for normalization in children is not known. Recent adult studies have also suggested more variations in the sEMG recorded during maximum manoeuvre due to submaximal efforts than during spontaneous tidal breathing (MacBean, Hughes, et al., 2016; Williams et al., 2019), hence reporting non-normalized sEMG may be adequate if comparisons can be made to reference values recorded from healthy children. Currently there are limited data on inspiratory muscle sEMG magnitude in healthy children especially school-aged children (Maarsingh et al., 2000; MacBean, Jolley, et al., 2016).

Physiological factors which can affect interpretation of sEMG (such as age and anthropometric data) needs to be explored in children to determine what factors need to be accounted for when establishing healthy reference values of inspiratory muscle sEMG. There are also gaps in knowledge on the influence of body position on inspiratory muscle sEMG in children which need to be addressed since in certain circumstances children will be assessed in specific postures such as in supine position during sleep.

To address these gaps in knowledge in the recording and measurement of inspiratory muscle sEMG in children, in this thesis I aim to first explore whether it is technically possible to measure NRD quantitatively using a commercial polysomnography set up in a real life setting during routine clinical practice. I will then assess whether this technique is able to quantify NRD during sleep in children who have increased work of breathing and/or OSA. Furthermore, to validate this method, I will assess whether sEMGdi can reflect the unloading of diaphragm muscle when children with sleep-disordered breathing are treated with positive airway pressure support. I will also evaluate inspiratory muscle sEMG recorded from healthy children to determine the relationship between inspiratory muscle sEMG and routine lung function variables, and explore potential factors affecting inspiratory muscle sEMG interpretation including age, body mass index, gender, and posture. The findings from this research will help direct future studies of inspiratory muscle sEMG in children by broadening our current knowledge on the interpretation and clinical applications of inspiratory muscle sEMG in children.

1.11 Aims and hypothesis of the thesis

Aim 1 – To develop a reproducible method to quantitatively assess diaphragm surface EMG recorded using standard clinical polysomnography equipment and setting.

Hypothesis: Quantitative assessment of sEMGdi recorded during clinical overnight sleep studies is feasible, and the analysis method is reliable between occasions and between assessors.

Aim 2 – To determine whether tidal diaphragm surface EMG (as a marker of neural respiratory drive) during sleep is different between children with and without OSA.

Hypothesis: Diaphragm surface EMG is higher in children with OSA than children without.

Aim 3 – To determine whether neural respiratory drive (as measured by tidal diaphragm surface EMG) decreases in children treated with positive airway pressure support for sleep-disordered breathing.

Hypothesis: In children with sleep-disordered breathing (including OSA and nocturnal hypoventilation), sEMGdi decreases after commencement of positive airway pressure support.

Aim 4 – To explore the relationship between inspiratory muscle (scalene, parasternal intercostal, and diaphragm) sEMG and conventional lung function measures of lung volume and pressure in healthy children.

Hypothesis: There is a positive linear or curvilinear relationship between inspiratory muscle sEMG and lung volume and mouth pressure, similar to those reported in healthy adults.

Aim 5a – To measure tidal inspiratory muscle sEMG in healthy children, and to explore the relationship between inspiratory muscle sEMG and physiological factors such as age, gender, body mass index, and posture.

Hypothesis: Surface EMG can be recorded from multiple inspiratory muscles (scalene, parasternal intercostal, and diaphragm muscles) simultaneously during tidal breathing from healthy children. When expressing sEMG of inspiratory muscles as a value normalised to the peak sEMG obtained during a maximal inspiratory manoeuvre, the effect of anatomical factors such as age and body mass index on sEMG magnitude between individuals is minimised.

Aim 5b – To assess and compare the peak sEMG recorded from different inspiratory muscles (scalene, parasternal intercostal, and diaphragm muscle) when children perform various maximal inspiratory manoeuvres.

Hypothesis: Not all children will achieve the peak sEMG for normalisation by performing the same maximal inspiratory manoeuvre. Maximal sniff inhalation will produce the peak sEMG for most children as it is a natural and relatively easy manoeuvre for children to perform.

Chapter 2. Validation of a quantitative method to measure neural respiratory drive in children during sleep

Introduction

Surface EMG of the diaphragm muscle was endorsed as an adjuvant qualitative measure for assessing respiratory effort to assist in the classification of respiratory events as obstructive or central in the 2007 AASM Manual for the Scoring of Sleep and Associated events (Iber et al., 2007; Berry et al., 2012). Despite evidence of good correlation between oesophageal pressure and diaphragm EMG during obstructive breathing events in adults (Stoohs et al., 2005), there has been no study on the use of sEMGdi as an objective quantitative index of NRD in snoring children likely due to the deficiency of a reliable method to quantitatively assess sEMGdi using commercial sleep study equipment and set up. The Sleep Laboratory at Sydney Children's Hospital routinely records sEMGdi using the AASM prescribed settings during clinical overnight sleep studies (Iber et al., 2007). The aim of this chapter was to address this gap in knowledge by developing a reproducible method to quantitatively assess sEMGdi recorded during real-life clinical sleep studies (Aim 1). My hypothesis was that quantitative assessment of sEMGdi recorded during clinical overnight sleep studies is feasible and the analysis method is reliable between occasions and between assessors.

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Validation of a quantitative method to measure neural respiratory drive in children during sleep

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ABSTRACT

Aims: Quantitatively measure and validate analysis of neural respiratory drive (NRD) using a commercial polysomnography system in children during sleep.
Method: Surface electromyogram of the diaphragm (sEMGdi) recorded from primary snoring children were analysed. A subset was re-analysed to assess intra- and inter-investigator reproducibility. Effects of different band pass filter settings (20–100 Hz vs 10–1000 Hz) on sEMGdi amplitude were evaluated.
Results: Mean sEMGdi from 45 children aged 4.38 years (median; IQR 3.00–7.96) was 5.05 μV (SD 2.73). The sEMGdi had a high intra-subject intraclass correlation coefficient (ICC) of 0.88. sEMGdi analysis was reproducible with high ICC between occasions (0.99; 95% CI 0.98–0.99) and between investigators (0.98; 95% CI 0.97–0.99). There was also a high ICC (0.99, 95% CI 0.96–1.00) between the sEMGdi measured using different band-pass filter settings. Age and BMI were negative predictors of sEMGdi ($p < 0.0001$ and $p = 0.0004$ respectively).
Conclusion: NRD in children during sleep as assessed by sEMGdi can be quantified in a reliable and reproducible fashion.

1. Introduction

Assessment of neural respiratory drive (or respiratory effort) is an increasingly important aspect of evaluating sleep disordered breathing (SDB). Obstructive sleep apnea (OSA) is characterised by the persistence of respiratory effort during partial and/or complete upper airway obstruction (American Thoracic Society, 1996). Therefore, monitoring respiratory effort is essential in routine polysomnography to differentiate between an obstructive or central respiratory event (Berry et al., 2013). The recognition that partial airway obstruction can cause repeated respiratory effort related arousals (RERA) and significant daytime symptoms in the absence of apnea in upper airway resistance syndrome (UARS) further highlights the need for respiratory effort monitoring during sleep (Guilleminault et al., 1996, 1993). Esophageal pressure (Pes) is the current gold standard for quantitative measurement of respiratory effort in sleep, reflecting changes in intrathoracic pressure resulting from respiratory muscle contraction (Berry et al., 2013). However, its invasive nature and potential to disrupt sleep quality (Chervin and Aldrich, 1997) and distort pharyngeal dynamics (Woodson and Wooten, 1992) means Pes monitoring remains mainly a research tool, particularly in children. Instead, non-invasive technique such as dual thoracoabdominal respiratory inductance plethysmography (RIP) bands are preferable. When RIP bands are calibrated, quantitative measurements of tidal volume can be estimated based on the change in the entire cross-sectional area enclosed by the thoracic and abdominal bands (Carry et al., 1997). However, displacement of the RIP bands and changes in body position during sleep can alter calibration and the estimated volume, limiting its use as a quantitative marker of respiratory effort (Tobin et al., 1983; Whyte et al., 1991).

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Electromyography (EMG) of the diaphragm and other respiratory muscles is another method of detecting respiratory effort (Berry et al., 2013). When muscles such as the diaphragm contract, the electrical activity of multiple motor units summate to form a crescendo-decrescendo pattern which can be recorded indirectly using surface (transcutaneous) electrodes overlying the chest wall. The magnitude of the respiratory muscle EMG represents the neural respiratory drive (NRD) to that muscle, i.e. the balance between the load placed on the respiratory system and the muscle's force generating capacity (Ratnovsky et al., 2008). Compared to evaluating respiratory effort by changes resulting from respiratory muscle contraction such as Pes and lung volume, respiratory muscle EMG is a more direct assessment of NRD. Good correlations between the surface EMGdi and Pes magnitude for adults with OSA have been found (Stoohs et al., 2005). Further, the use of transcutaneous surface electrodes to record costal diaphragm EMG (sEMGdi) is non-invasive and well tolerated by neonates and children (Maarsingh et al., 2000; Prechtl et al., 1977).

Diaphragm and intercostal EMG currently are used as an adjunct to RIP bands as a qualitative sensor to differentiate between obstructive and central respiratory events in sleep studies (Berry et al., 2016). If sEMGdi recorded using a commercial sleep study software and setup is quantifiable, sEMGdi potentially can be used as a non-invasive surrogate for esophageal pressure measurements. However standard EMG filter settings recommended for routine polysomnography have a narrower range of filter settings (10–100 Hz) to those recommended for quantitative analysis of muscle EMG (10–1000 Hz) (ATS/ERS Committee, 2002; Berry et al., 2013), leading to potential loss of data in the high frequency range. The aim for this study was to investigate whether sEMGdi recorded using routine sleep study software and settings could be quantitatively analysed in a reproducible manner as a marker of NRD.

2. Methods

Sleep studies conducted in healthy children <18 years of age without any chronic medical comorbidities, including asthma or cardiac conditions, at Sydney Children's Hospital's (SCH) Sleep Laboratory during a 12-month period were retrospectively reviewed. Only children whose sleep studies were reported as 'normal' without a diagnosis of OSA (defined as an obstructive apnea hypopnea index (OAHI) ≥1/h) (Marcus et al., 1992; Uliel et al., 2004) were included in this study. This included non-snoring children who had routine sleep studies prior to a multiple sleep latency test (MSLT). Sleep studies with poor quality RIP band signals where assessment of breathing movement was not possible, and/or poor quality sEMGdi signals where phasic EMG could not be visualised were excluded.

Full polysomnography (PSG) was performed in all included children using Profusion Sleep® software (Compumedics, Melbourne, Australia). Sleep stages were identified by electroencephalography (C3/A2, C4/A1, O1/A2, and O2/A1), electrooculography, and submental EMG. In accordance with American Academy of Sleep Medicine's (AASM) guidelines (Berry et al., 2013), sleep was staged as light sleep (stage N1 and N2), deep sleep (stage N3), and rapid eye movement (REM) sleep. Electrocardiogram (ECG) was monitored from precordial leads. Oxygen saturation (SpO_2) was measured by oximeters (Masimo RadicalSET, Irvine, California) and airflow was detected by nasal prongs attached to a flow sensor and oronasal thermistor. Concurrent infrared video recording was used to record sounds and to visually monitor sleep. Uncalibrated RIP bands (Compumedics, Melbourne, Australia) placed around the chest and abdomen were used to detect chest wall and abdominal movements.

Sleep and respiratory events were scored according to standard terminology by pediatric sleep physicians (Berry et al., 2013, 2012). Obstructive apneas were defined as the persistence of respiratory effort (based on movement of the chest and abdomen on RIP bands and/or phasic bursts of sEMGdi) associated with the absence of airflow detection by either the nasal flow sensor and/or oronasal thermistor for at least two breaths. A hypopnea was scored when the peak airflow signal excursions dropped by ≥30% of pre-event baseline for at least two breaths accompanied by either ≥3% oxygen desaturation or an arousal.

2.1. Surface (chest wall) diaphragm EMG recording set up and analysis

To record sEMGdi, the active electrode (3 M Red dot®, USA) was placed on the chest in the right mid-clavicular line at the 8th intercostal space and the reference electrode below the active electrode on the rib at the costal margin. The two electrodes were set <2 cm apart without touching. The electrode cables were shielded to minimise line frequency interference. The settings on the configuration panel of Profusion Sleep® for recording sEMGdi were based on the recommended settings from the AASM manual (Berry et al., 2013; Penzel et al., 2007). EMG data were recorded with a sampling rate of 256 Hz and a range of 2 mV. The EMG signals were then amplified and band-pass filtered in the range of 22–100 Hz with a notch filter of 50 Hz set to minimise powerline artefacts.

Each participant's sleep study was reviewed using Profusion Sleep® software. Two excerpts of 10 consecutive tidal breaths with clear stable phasic respiratory effort (evident in both the sEMGdi and the thoracic and abdominal RIP band channels) with the child sleeping in one position without gross body movement artefact were selected from each of the three different sleep stages (light sleep (N1 and 2), deep sleep (N3), and REM sleep). A total of 6 excerpts (60 breaths) from each child were then exported to Spike2® (Cambridge Electronic Design, Cambridge, England) data acquisition and analysis system as European Data Format (EDF) files for offline manual waveform analysis. As demonstrated in Fig. 1a, the sEMGdi signal was phasic and coincided with inspiration as confirmed by the fluctuation in the thoracic RIP band signal to ensure the EMG signal captured originated from the diaphragm and not abdominal muscles. Diaphragm EMG signal contaminated by ECG QRS complex artefact was removed using a custom script (gated EMG). The gated EMG signal was converted to root-mean-square (RMS) with a time constant of 50 ms (Fig. 1a). This resulted in a RMS-sEMGdi signal almost free of ECG signal, although artefacts from P and T waves from the ECG may be seen (Fig. 1b). The peak and trough RMS-sEMGdi signals for every individual breath were manually identified. The difference between the two were used to determine the absolute amplitude of the RMS-sEMGdi signal per breath.

2.2. Data collection and analysis

Age, gender, height, weight, body mass index (BMI) centiles measured on the day of sleep study were recorded for each child. Mean RMS-sEMGdi per breath (referred throughout as sEMGdi) was calculated from the 60 breaths analysed for each child, and reported in μV.

For surface recordings of respiratory muscle activity, a band width of 10–1000 Hz was used customarily as the amplifier filter setting (ATS/ERS Committee, 2002). sEMGdi analysed using standard sleep study filter setting (22–100 Hz) was compared to routine respiratory muscle EMG band-pass filter setting (10–1000 Hz) by analysing 12 sleep study excerpts prospectively recorded on Profusion Sleep® using a sampling frequency of 2000 Hz, amplified and exported using a band-pass filter of 22–100 Hz then 10–1000 Hz.

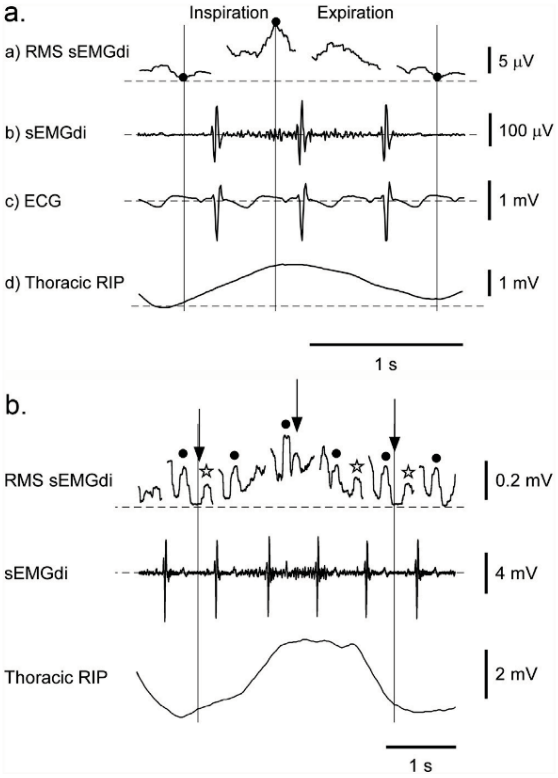

Fig. 1. Examples of surface diaphragm EMG recorded from children to demonstrate the method of analysing sEMGdi. Fig. 1a) Example of surface diaphragm EMG from one child; a) RMS-sEMGdi trace – gated EMG signals with ECG artefact removed converted to root mean square (RMS); Note the gaps in the sEMGdi signal from the removal of ECG QRS artefact (gated EMG), and the black dots denote where the analyzer marked as the peak and trough of the RMS-sEMGdi signal for this breath; b) sEMGdi channel – raw sEMGdi signal with ECG artefact from QRS complex still visible; c) ECG signal; d) Thoracic RIP band signal confirming period of inspiration and expiration. Fig. 1b) An example of residual ECG artefacts after removal of QRS complex from RMS-sEMGdi trace. P wave (stars) and T wave (black dots) are visible in the RMS-sEMGdi trace. This demonstrates the need to manually mark the peak and trough (arrows) of RMS-sEMGdi signal used to calculate the amplitude of RMS-sEMGdi per breath.
RMS: Root mean square; sEMGdi: surface electromyogram of diaphragm; RIP: respiratory inductance plethysmography; ECG: electrocardiogram.

To assess the reproducibility of the method of EMG analysis, identical excerpts from a subset of 20 children's sleep studies were analysed by the same investigator (SC) more than 6 months apart for intraobserver reproducibility, and by two investigators (SC and a medical student) for interobserver variability. The medical student underwent two 1-h sessions of training on the method of analysing sEMGdi by SC prior to independently analysing sleep studies.

The study was approved by Sydney Children Hospital Network's Human Research Ethics Committee (12/SCHN/450).

2.3. Statistical analysis

Statistical analysis of the data was performed using SPSS Version 22 (SPSS, Chicago, Illinois, USA) and graphs were drawn using Prism version 6 (GraphPad, California, USA). All normally distributed data were summarised as mean and standard deviation (SD). Non-normally distributed data were summarised as median and interquartile range (IQR). Intraclass correlation coefficient (ICC) of the sEMGdi measured within individual children was determined to assess the reliability of the sEMGdi analysis. After taking into account any variability in the sEMGdi between the 6 excerpts and within the 10 breaths per excerpt, the ICC for the sEMGdi measured within individual children was calculated as the ratio of the between participant variance to total of the between participant variance and error variance.

Reproducibility of sEMGdi analysis method between and within the same investigator was assessed by ICC and Bland-Altman analysis (Bland and Altman, 1986). The effect of different band-pass filtering was assessed using the same method. In general, an ICC >0.7 is considered adequate. sEMGdi and OAHI was examined using Spearman correlation coefficients and linear regression. The relationship between sEMGdi and physiological factors such as age and BMI centiles were assessed using linear regressions after logarithmic conversion of the sEMGdi values. A p value of <0.05 was considered statistically significant.

Based on previous studies' sample size we aimed to evaluate between 10 and 50 children's sleep studies (Maarsingh et al., 2000; Marcus et al., 1992; Stein et al., 2012). A post hoc analysis conducted confirmed that if the true mean sEMGdi in healthy snoring children was 5 μV, then a sample of 45 children could estimate the 95% confidence interval within 1 μV, with a resulting 95% confidence interval of 4–6 μV.

Table 1
Clinical and Sleep Study Characteristics and sEMGdi level in children without sleep disordered breathing.

	Healthy snorers
Number of patients	45
Male, N (%)	27 (60)
Age (years), Median (IQR)	4.38 (3.00–7.72)
Weight centile, Median (IQR)	68.84 (39.22–94.69)
BMI centile, Median (IQR)	75 (46.37–92)
OAHI (/h), Median (Range)	0.0 (0.0–0.9)
Minimal SaO_2 (%), Median (IQR)	93 (90–95)
Total sleep time(TST) (min), Mean (SD)	452.41 (63.84)
Time spent in light sleep (Stages N1 + N2) (% TST), Mean (SD)	49.88 (7.72)
Time spent in deep sleep (Stages N3) (% TST), Mean (SD)	28.36 (6.24)
Time spent in REM sleep, (% TST), Mean (SD)	21.79 (5.64)
Sleep efficiency, %, Mean (SD)	81.57 (0.66)
Arousal index (/h), Mean (SD)	7.60 (3.98)
sEMGdi (μV), Mean (SD)	5.05 (2.73)

OAHI, obstructive apnea hypopnoea index; sEMGdi, surface electromyography of diaphragm; TST, total sleep time; IQR, interquartile range; SD, standard deviation.

3. Results

Fifty- five slee0p studies from children without OSA were retrospectively reviewed and 10 studies were excluded, 7 due to poor sEMGdi signal quality and 3 due to poor quality traces from the uncalibrated RIP bands. The median (IQR) age of the 45 included children was 4.38 years (3–7.72). The demographics and sleep study data including sEMGdi of the cohort are summarised in Table 1. The majority (43/45, 95.6%) had a parental report of snoring. Two children had sleep studies as part of their diagnostic assessment for excessive daytime somnolence. Thirteen children (28.9%) were overweight with BMI centile ≥90th centile, including 9 children who were obese (BMI centile ≥95th centile). Phasic inspiratory activity of sEMGdi was observed in all 45 sleep studies with a mean sEMGdi (SD) of 5.05 (2.73) μV. There was a significant linear relationship between OAHI and sEMGdi (coefficient of beta 5.61, $p = 0.001$), and a moderate correlation between the two variables ($r_s = 0.49$, $p = 0.001$).

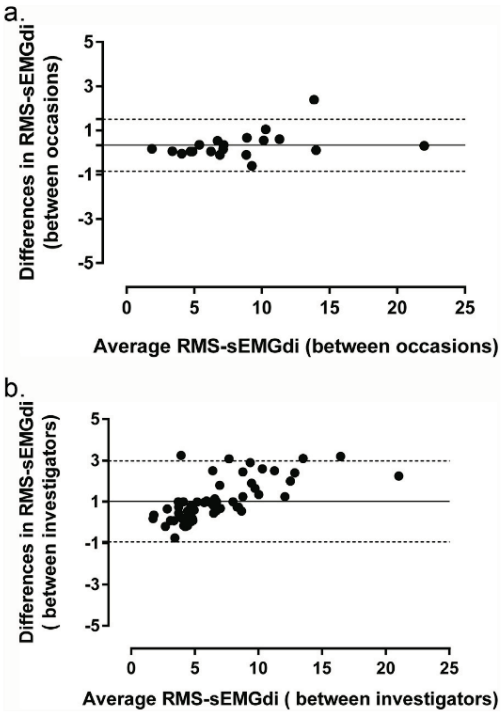

Fig. 2. Bland-Altman plots of the mean difference between sEMGdi vs mean average sEMGdi measured between two occasions by the same investigators (Fig. 2a) and between two investigators (Fig. 2b) in 20 children. The mean differences (solid line) and the 95% confidence interval (mean ± 1.96 SD) (dashed line) are shown.
(a) Mean difference 0.33 μV, 95% limits of agreement −0.85 to 1.51.
(b) Mean difference 1.02 μV, 95% limits of agreement −0.94 to 2.98.

3.1. Evaluation of analysis method

Our method of analysing sEMGdi was reliable, with a high intra-subject ICC (0.88) for the 60 measures of sEMGdi per breath in each child. To assess the consistency of the method of sEMGdi analysis, the ICC within the same and between different assessors were calculated. The inter-occasion ICC for the same investigator was high (0.99; 95% CI 0.98–0.99). The inter-rater ICC was also high (0.98; 95% CI 0.97–0.99). Fig. 2 shows the Bland Altman plots of the measurements between occasion (Fig. 2a) and between analysers (Fig. 2b). There was also good agreement (Fig. 3) between the sEMGdi measured using two different band-pass filter settings (20–100 Hz vs 10–1000 Hz) with a high ICC (0.99, 95% CI 0.96–1.00).

3.2. Relationship between physiological factors and sEMGdi in children during sleep

Univariate analysis confirmed age ($p < 0.0001$), and BMI centiles ($p = 0.0004$) were significant negative predictors of sEMGdi. Gender was not a significant predictor of sEMGdi ($p = 0.12$). Age and BMI centile remained as significant predictors of sEMGdi in the multiple linear regression analysis including all three predictors ($p < 0.0001$, $R^2 = 0.571$). For the variable of age, for 1 year increase in age, we expect to see a 9.24% (CI 5.72–12.54%) drop in sEMGdi. For the variable BMI centiles, with 1 unit increase in BMI centile we expect to see a 1.00% drop in sEMGdi (CI 0.50–1.69).

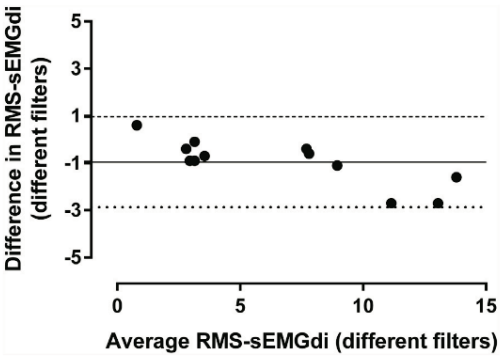

Fig. 3. Bland-Altman plot of the mean differences of sEMGdi vs mean average sEMGdi measured when two different range of band-pass filter settings were used (20–100 Hz compared to 10–1000 Hz). The mean differences (solid line) and the 95% confidence interval (mean ± 1.96 SD) (dashed line) are shown.
Mean difference −0.96 μV, 95% limits of agreement −2.87 to 0.96.

4. Discussion

Using our method of analysis, we quantified sEMGdi recorded in children using standard clinical polysomnography software and settings in a reliable and reproducible fashion. The sEMGdi measured had high ICC within individual children suggesting good reliability of our analysis method. The analysis method also demonstrated high intra- and inter-rater agreement indicating good reproducibility. Despite the narrower band pass filter setting recommended for routine polysomnography, there was a high ICC between the sEMGdi amplitude measured using the two different filter settings. Age and BMI centiles were negative predictors of sEMGdi in children without OSA during sleep. To our knowledge, this is the first study to investigate sEMGdi activity during sleep in healthy children without OSA.

Our primary aim was to develop a reproducible and reliable method to quantitatively analyse sEMGdi data recorded from children using commercial sleep study equipment and standard settings. Our approach of manually evaluating gated sEMGdi breath-by-breath ensured ECG artefacts (such as P and T wave artefacts) were not misinterpreted as EMG activity (Fig. 1b). The use of concurrent RIP band signals to determine inspiration confirmed the EMG activity measured was from the diaphragm during inspiration and not due to abdominal muscle cross talks. Further, the placement of active recording electrodes at the 8th intercostal space at the mid clavicular line with the reference electrode on the rib below (as in our study) is the location with the least contaminated sEMGdi signals (ATS/ERS Committee, 2002).

Before assessing the clinical utility of measuring sEMGdi in children during sleep, it is important to establish the validity and reliability of our method of analysing sEMGdi. In our study, mean sEMGdi in healthy children without OSA was 5.05 ± 2.73 μV. There was a highly significant correlation between repeated measurements of the sEMGdi within the same subjects with an ICC of 0.88, suggesting good reliability in our analysis method. Our technique of analysing sEMGdi was also reproducible with high correlation coefficient and good agreement between occasions, and between different analysers with different levels of expertise. Although a positive bias appears to be present in the Bland Altman plot between investigators (Fig. 2b) especially when the amplitude of sEMGdi was higher, the mean difference in sEMGdi was small (mean difference 1.02 μV, SD 1.00). Whether a 1 μV difference in sEMGdi is a clinically significant difference in NRD in children with and without disease will need to be determined in future studies.

Commercial computerized sleep study system such as Profusion Sleep® enables simultaneous recording of multiple physiological parameters including diaphragm EMG continuously as analog signals. To ensure accurate digital visualisation of the analog signals without losing essential details, appropriate filters and sampling rate needs to be set. For clinical polysomnography, a sampling rate between 200 and 500 Hz and a band pass filter setting of 10–100 Hz was recommended by AASM for EMG recordings (Berry et al., 2013). These criteria were chosen for sleep stage scoring and the qualitative evaluation of muscle tone, twitches and phasic activities (Penzel et al., 2007). As most muscle surface EMG signals are recorded between 30 and 500 Hz, ideally filter settings on the recording equipment should be set between 10 and 1000 Hz with a minimum sampling rate of 2000 Hz to ensure true muscle EMG activities are not lost (ATS/ERS Committee, 2002). We demonstrated that although the EMG filter settings in our routine sleep study recording (20–100 Hz) were limited in the high frequency range, there was a high correlation and good agreement between the sEMGdi measured using the two different band-pass filter settings. Nevertheless, our method requires off-line analysis by trained personnel, which can be time consuming. Automated algorithms and devices (Dipha; Inbiolab, Groningen, Netherlands) are in the pipeline for analysing surface EMG of respiratory muscles.

In our study, mean sEMGdi magnitude in healthy snoring children was 5.05 ± 2.73 μV. Interestingly, our results are comparable to those of Maarsingh's study (Maarsingh et al., 2000) in healthy children although their location of electrodes placement for assessing costal diaphragm was different to ours. They placed electrodes bilaterally at the costal margin at the nipple line instead of at and below the 8th intercostal space at the midclavicular line on the right, which may have resulted in greater ECG contamination in the EMG recorded (Maarsingh et al., 2000). A small study (n = 3) suggested mean peak crural EMGdi measured using an esophageal catheter in full term sleeping healthy neonates is 10 ± 4 μV (Stein et al., 2012). The difference between the magnitude of sEMGdi in our cohort and these healthy term neonates likely reflects the closer proximity of the esophageal electrodes to crural diaphragm muscles compared to surface chest wall electrodes overlying the costal diaphragm, and the influence of age on respiratory mechanics. Conversely, a small study in 5 adults demonstrated high correlations between EMGdi measured using surface chest-wall electrodes placed at a similar location to ours (lowest rib interspace in the midclavicular line on the right) and esophageal catheters (Sinderby et al., 1998). High correlation between surface EMGdi and Pes measured in adults with OSA in another study (Stoohs et al., 2005) further supports the use of surface EMG as a non-invasive method of assessing respiratory effort.

Our study found that with every one-year increase in age, sEMGdi decreased by 9.24% (CI 5.72–12.54%). The decrease in NRD of the diaphragm and other inspiratory muscles with increasing age in children is likely related to increased distance between muscle and surface electrodes with increased subcutaneous fat, decrease in chest wall compliance associated with increased mineralisation of the ribs, and changing orientation of the ribs with growth leading to improved mechanical efficiency of the respiratory muscles including diaphragm (Bryan and Wohl, 2011). In adults with OSA, EMGdi activity measured using esophageal electrodes was nearly 3 times greater than in healthy adults (Steier et al., 2010). Hence the minor decrease in sEMGdi magnitude with increasing age is unlikely to have a significant effect on the clinical interpretations of sEMGdi level in children. However, future studies on the level of sEMGdi activity in healthy non-snoring children and children with OSA across different age groups are needed to elucidate this further.

BMI was another negative predictor of sEMGdi. In our cohort of children without OSA, there was a decrease in sEMGdi by 1.00% (CI 0.50-1.69) with each unit increase in BMI centile. When esophageal electrodes were used to assess EMGdi in healthy adults, no significant correlation was found between EMGdi and height, weight, or BMI (Jolley et al., 2009). This suggests that the decrease in sEMGdi in our cohort with increasing BMI is likely due to the increased distance from the diaphragm to the chest wall skin surface with increasing subcutaneous fat. Studies in children assessing the relationship between EMGdi simultaneously measured using esophageal catheter and surface electrodes will be required to answer this question definitively, which is outside the scope of this study.

A limitation of our study was that sEMGdi was obtained mainly from children who were primary snorers (43/45) with an OAHI <1/h (Marcus et al., 1992; Uliel et al., 2004). Data on healthy non-snoring children was only available from two children who had polysomnography prior to MSLT as part of the assessment for narcolepsy. To obtain normative reference values of sEMGdi in children during sleep, healthy non-snoring children without any comorbidities should be assessed. As overnight polysomnography remains a clinical investigation reserved for children with suspected sleep disordered breathing and the study being retrospective, we were restricted in our patient choice for 'healthy' children. Habitual snoring is suggestive of increased obstructive upper airway load during sleep which can increase NRD (Deary et al., 2014). Hence the measured sEMGdi in healthy snoring children may be higher than those from healthy non-snoring children. This could also explain why the minimum oxygen saturation during sleep in our group (median 93%, IQR 90–95) was slightly lower than that previously reported from healthy children [95–96% (Marcus et al., 1992; Uliel et al., 2004)].

Another limitation of our study is that Pes measurement was not available. Physiological factors such as age and BMI centiles can influence interpretation of surface EMGdi magnitude as demonstrated in our cohort (Beck et al., 1995). One method to resolve individual anatomical differences is to measure EMGdi using esophageal electrodes, which is invasive and not used in routine sleep studies at our centre. An alternative technique is to express absolute sEMGdi as a percentage of a reference sEMGdi value such as those obtained during maximal inspiratory manoeuvres (such as inspiration to total lung capacity (TLC) or maximum inspiratory pressure (PImax)) (Maarsingh et al., 2006, 2004; Steier et al., 2011). This was not possible in our study due to its retrospective nature and because most children from our cohort were less than 6 years of age (68.8%) and thus unlikely to perform maximal inspiratory manoeuvres reproducibly. A recent study in healthy adults suggested normalising respiratory muscle (parasternal intercostal muscle) EMG to a maximal value conferred little advantage in the consistency of repeated measurements within- and between- occasions compared to expressing EMG as raw, non-normalised signal (MacBean et al., 2016a). Whether the same relationship exists for surface diaphragm EMG measurements in children will need to be determined in future studies.

In conclusion, the present study demonstrated a reliable and reproducible method to quantitively assess sEMGdi recorded during sleep study using commercial polysomnography equipment and settings. sEMGdi in healthy children without OSA was higher in younger children or children with lower BMI centiles. Overall, sEMGdi is a non-invasive, well-tolerated method for measuring NRD in children during sleep. Future studies in healthy non-snoring children, and children with disease will help delineate the clinical utility of sEMGdi as a non-invasive quantitative index of NRD.

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Conflicts of interest

None.

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Key messages

- sEMGdi recorded during clinical sleep study using commercial PSG equipment and settings can be quantitively measured reliably and reproducibly.
- Strong correlations exist between sEMGdi recorded using the narrower AASM prescribed band pass filter setting (22-100Hz) compared to the standard 10-1000Hz band pass filter settings recommended for assessing respiratory muscle EMG.
- In healthy snoring children, sEMGdi magnitude was negatively associated with age and BMI.

Contribution of this paper to the thesis

Development of a reproducible and reliable method to quantitatively assess sEMGdi recorded using a commercial sleep study set up highlights the translational potential of incorporating sEMGdi measurements as a routine objective respiratory variable when assessing children's breathing during sleep. Potential clinical uses of sEMGdi as a measure of respiratory load and hence NRD when assessing and treating children with sleep-disordered breathing will be evaluated in the next two chapters using this particular EMG analysis method.

Chapter 3. Quantitative assessment of neural respiratory drive in children during sleep using surface EMG

Introduction

A method for quantitative evaluation of sEMGdi recorded using commercial sleep study settings and equipment was validated in Chapter 2. This chapter explores the clinical utility of sEMGdi as a measure of NRD in children during sleep by comparing tidal sEMGdi recorded during unobstructed breathing in children with and without OSA (Aim 2). sEMGdi (also termed surface EMG of the chest wall, sEMGcw, in this chapter) is incorporated in the routine respiratory variables recorded during clinical overnight PSG at Sydney Children's Hospital (SCH). The sleep clinicians at SCH make subjective assessments of respiratory effort based on evidence of nasal airflow limitation, indication of paradoxical breathing with out-of-phase thoracic and abdominal RIP signals, bursts of abdominal sEMG reflecting active expiration, and also visual assessment of sEMGdi magnitude. Anecdotally, there is a subgroup of primary snorers who did not have OSA (as defined by OAHI < 1/h) but have subjective increased work of breathing during sleep. My hypothesis was that diaphragm surface EMG is higher in children with OSA, and also in children with subjective increased work of breathing (incWOB) than children without.

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Declaration

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RESEARCH PAPER

Quantitative assessment of nocturnal neural respiratory drive in children with and without obstructive sleep apnoea using surface EMG

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Abstract

Our aim was to investigate whether neural respiratory drive measured by non-normalized surface EMG recorded from the chest wall overlying the diaphragm (sEMGcw) differentiates children with and without obstructive sleep apnoea (OSA). Polysomnography data of children aged 0–18 years were divided into the following three groups: (i) primary snorers (PS); (ii) snoring children without OSA but with increased work of breathing (incWOB; subjective physician report of increased respiratory effort during sleep); and (iii) children with OSA [obstructive apnoea–hypopnoea index (OAHI) >1 h^{-1}]. Excerpts of sEMGcw obtained during tidal unobstructed breathing from light, deep and rapid eye movement sleep were exported for quantitative analysis. Overnight polysomnography data from 45 PS [median age 4.4 years (interquartile range 3.0–7.7 years), OAHI 0 h^{-1} (0.0–0.2 h^{-1})], 19 children with incWOB [age 2.8 years (2.4–5.7 years), OAHI 0.1 h^{-1} (0.0–0.4 h^{-1})] and 27 children with OSA [age 3.6 years (2.6–6.2 years), OAHI 3.7 h^{-1} (2.3–6.9 h^{-1})] were analysed. The sEMGcw was higher in those with OSA [8.47 μV (5.98–13.07 μV); $P < 0.0001$] and incWOB [8.97 μV (5.94–13.43 μV); $P < 0.001$] compared with PS [4.633 μV (2.98–6.76 μV)]. There was no significant difference in the sEMGcw between children with incWOB and OSA ($P = 0.78$). Log sEMGcw remained greater in children with OSA and incWOB compared with PS after age, body mass index centiles, sleep stages and sleep positions were included in the mixed linear models ($P < 0.0001$). The correlation between sEMGcw and OAHI in children without OSA was small ($r_s = 0.254$, $P = 0.04$). The sEMGcw is increased in children with OSA and incWOB compared with PS.

KEYWORDS
obstructive sleep apnoea, paediatrics, surface electromyography

1 | INTRODUCTION

There has been increasing research in the use of surface electromyography (sEMG) of the respiratory muscles to determine the neural respiratory drive (NRD) in both children and adults (de Waal, Hutten, Kraaijenga, de Jongh, & van Kaam, 2017; Maarsingh, van Eykern, Sprikkelman, Hoekstra, & van Aalderen, 2000; MacBean, Hughes, Nicol, Reilly, & Rafferty, 2016a; MacBean et al., 2016b). Measurement of respiratory muscle EMG, hence NRD, provides an index of the balance between the overall load on the respiratory system and the force-generating capacity of the respiratory muscles. Phasic changes of EMG of the diaphragm (EMGdi), our main muscle of inspiration, reflect the level and pattern of diaphragm activation by the phrenic nerve during respiration (American Thoracic Society/European Respiratory Society, 2002). The use of oesophageal catheter-mounted electrodes to measure crural EMGdi allows detection of EMGdi free from the interference of surrounding muscles (cross-talk) and varying muscle-to-electrode distance between individuals. However, owing to its invasive nature, this technique is not suitable for routine clinical use, especially in children (Chervin et al., 2003).

Transcutaneous EMG of the costal diaphragm measured using electrodes positioned on the skin of the lower lateral chest wall

overlying the diaphragm (sEMGcw) is advantageous because it is well tolerated from infancy to adulthood and can provide continuous monitoring of patients who are awake or asleep (Chuang et al., 2017; de Waal et al., 2017; Maarsingh et al., 2000). Good correlations have been demonstrated between EMGdi measured using oesophageal catheter and surface electrodes (Lin, Guan, Wu, & Chen, 2019; Stoohs, Blum, Knaack, Butsch-von-der-Heydt, & Guilleminault, 2005). To minimize the effect of interindividual differences in muscle-to-electrode distance, sEMGcw is typically expressed as a percentage of a maximal value, limiting its use to those who can perform consistent maximal inspiratory efforts (Lin et al., 2019). Recent studies in both children and adults have supported the reporting of raw respiratory muscle EMG (not normalized to a reference value; Kallio et al., 2015; Luo et al., 2008; MacBean et al., 2016a, b, 2017), demonstrating greater variability in normalized EMG compared with the raw signal both within and between occasions (MacBean et al., 2016a). However, further studies are required to ascertain the clinical utility of the non-normalized EMG to facilitate translational use of respiratory muscle EMG.

Surface EMG of the diaphragm is one of the parameters included in clinical overnight polysomnography (PSG) to assess respiratory effort in order to differentiate between obstructive or central respiratory events (Berry, Ryals, Girdhar, & Wagner, 2016; Iber, Ancoli-Israel, Chesson, & Quan, 2007). In obstructive sleep-disordered breathing (SDB), increased obstructive load on the respiratory muscles from upper airway obstruction and/or resistance results in increased NRD and respiratory effort, with reduced or absent nasal airflow. A diagnosis of obstructive sleep apnoea (OSA) is made in children if more than one obstructive event occurs per hour of sleep [obstructive apnoea hypopnoea index (OAHI) $>1\ h^{-1}$] during overnight PSG (Marcus et al., 1992). In clinical practice, pathologically increased respiratory effort during sleep [measured by oesophageal pressure (P_{oes}) deflection] is also indicative of obstructive SDB even if the increased P_{oes} is not associated with scorable breathing events and normal OAHI (Skiba, Goldstein, & Schotland, 2015). Measuring sEMGcw during tidal breathing in sleep is akin to assessing the level of P_{oes} between respiratory events without the use of an invasive oesophageal catheter (Chervin & Aldrich, 1997; Luo et al., 2008). Given that the peak incidence of OSA occurs between 2 and 5 years of age, when children are unable to perform reproducible volitional maximal inspiratory manoeuvres, the use of raw sEMGcw as an effort-independent marker of NRD would be ideal (Lumeng & Chervin, 2008).

At the Sleep Laboratory at Sydney Children's Hospital (SCH), sEMGcw is one of the clinical PSG parameters recorded routinely. It is the practice at our centre that sleep physicians document subjective assessment of respiratory effort during sleep in the formal sleep reports. These are based on a number of factors, which include video observation of the child's respiratory effort and visual evaluation of sEMGcw magnitude. We have previously demonstrated that quantitative evaluation of sEMGcw recorded in children using a clinical polysomnography set-up is achievable (Chuang et al., 2017). The aim of this study was to determine whether tidal non-normalized sEMGcw can differentiate children with OSA from those without. Our hypothesis is that non-normalized sEMGcw is higher in children with OSA than in children without.

New Findings

- **What is the central question of this study?**
  Recent studies have suggested potential utility of non-normalized respiratory muscle EMG as an index of neural respiratory drive (NRD). Whether NRD measured using non-normalized surface EMG of the lateral chest wall overlying the diaphragm (sEMGcw) recorded during nocturnal clinical polysomnography can differentiate children with and without obstructive sleep apnoea (OSA) is not known.
- **What is the main finding and its importance?**
  Non-normalized sEMGcw was increased in children with OSA and an additional group of snoring children without OSA but subjectively increased respiratory effort compared with primary snorers. The sEMGcw has potential clinical utility in evaluation of children with sleep-disordered breathing as an objective, non-invasive, non-volitional marker of NRD.

2 | METHODS

2.1 | Ethical approval

Ethical approval for the study was granted by the Sydney Children's Hospitals Network's Human Research Ethics Committee (reference no. 12/SCHN/450). A waiver for individual participants' consent was granted in accordance with the Australian National Statement on Ethical Conduct in Human Research [National Health and Medical Research Council, 2007 (updated 2018)] and the *Declaration of Helsinki* for the following reasons: (i) the majority of the children had been discharged from the care of the sleep physicians, hence it was impracticable to obtain the individual's consent; and (ii) the research involved no more than low risk to participants, with sufficient protection of the participants' privacy and confidentiality.

2.2 | Subjects and study design

Diagnostic overnight PSGs performed consecutively throughout 12 months at the sleep laboratory at SCH, a tertiary paediatric hospital, were evaluated retrospectively. Polysomnographs with poor-quality respiratory inductance plethymography (RIP) band signals where determination of phases of respiration (inspiration and expiration) was not possible, and/or poor quality sEMGcw signals where phasic sEMGcw could not be visualized were excluded. Children were assigned to one of the following three groups based on OAHI and the sleep physician's report: (i) primary snorers (PS) if their OAHI was $\leq 1\ h^{-1}$ without increased respiratory effort documented; (ii) the increased work of breathing (incWOB) group if snoring children had an OAHI $\leq 1\ h^{-1}$ and increased respiratory effort based on PSG features (as described below in section 2.3) documented in the sleep physicians' reports; or (iii) the OSA group if the OAHI was $>1\ h^{-1}$ on overnight

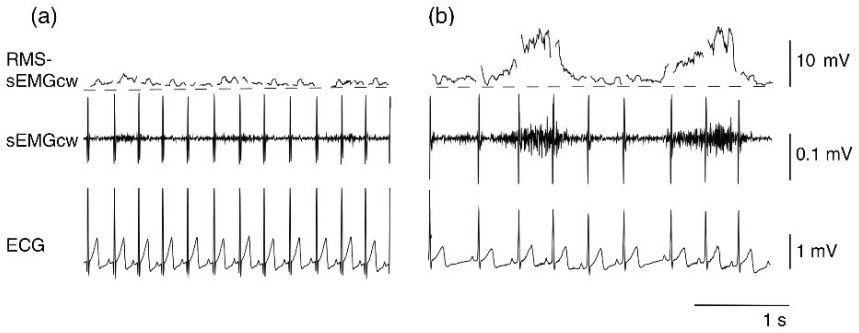

FIGURE 1 Examples of the surface electromyogram signals of the chest wall (overlying the diaphragm) (sEMGcw) from a primary snorer (a) and a child with obstructive sleep apnoea (b). The root mean square (RMS)-sEMGcw trace is the gated EMG signals with ECG artefact removed converted to root mean square. The absolute amplitude of the RMS-sEMGcw per breath was determined manually and averaged for each participant

PSG. Primary snorers with other co-morbidities (including craniofacial anomalies, neuromuscular disease, cardiac and chronic respiratory conditions including asthma) were excluded. For the incWOB and OSA groups, children with medical co-morbidities were included to reflect usual clinical practice. Age, sex, height, weight, body mass index (BMI) centiles (≥2 years old) or weight-for-length centiles (<2 years old) measured on the day of the sleep study were recorded. Children were considered to be overweight if the BMI or weight-for-length centile was >85th centile (Himes & Dietz, 1994).

2.3 | Overnight PSG

A full overnight PSG assessment was performed using a commercial computerized PSG system (Compumedics, Melbourne, Victoria, Australia). The following parameters were routinely recorded: EEG; electro-oculograms (EOGs); ECG; chin, diaphragm, abdominal and anterior tibial electromyogram (EMG); thoracic and abdominal movement (uncalibrated RIP; Compumedics), oxygen saturation (S_{pO_2}; Massimo RadicalSET, CA, USA), transcutaneous carbon dioxide ($TcCO_2$) monitoring (TCM4; Radiometer, Copenhagen, Denmark), nasal airflow through a nasal–oral thermistor and nasal cannulae, and infrared video recording. Body position was determined by a sensor attached to the torso and confirmed by video monitoring.

All sleep studies were manually scored by paediatric sleep physicians using standard criteria (Iber et al., 2007). Obstructive apnoea was defined as the absence of airflow with continued chest-wall and abdominal effort (based on chest and abdominal RIP bands signals and/or EMG signals) for at least two breaths (Iber et al., 2007). Hypopnoea was defined as a decrease in nasal flow of ≥50% accompanied by oxygen desaturation ≥3%, or arousal, or both (Iber et al., 2007). The OAHI was the total number of obstructive apnoeas, mixed apnoeas and obstructive hypopnoeas per hour of total sleep time (TST). The severity of OSA was defined based on the OAHI: mild OSA if OAHI ≥1 to 4.9 h^{-1}; moderate if OAHI 5–9.9 h^{-1}, and severe if OAHI ≥10 h^{-1} (Katz et al., 2002). Other PSG outcomes recorded included the oxygen desaturation index (ODI3; the number of times S_{pO_2} dropped by ≥3% per hour of TST) and gas exchange parameters (S_{pO_2} nadir and peak $TcCO_2$). The desaturation index is abnormal if ODI3 >2 episodes h^{-1} if <10 years old, or ODI3 >0.5 episodes h^{-1} if ≥10 years old (Kaditis, Kheirandish-Gozal, & Gozal, 2016).

The sleep physicians documented increased respiratory effort during sleep in the PSG report based on the repeated occurrence of one or more of the following criteria in at least two sleep cycles: visual determination of elevated sEMGcw; increased chest wall retraction and/or abdominal breathing on infrared video recording; evidence of paradoxical breathing with out-of-phase chest and abdominal RIP bands in children >2 years of age; and bursts of abdominal surface EMG suggesting active expiration, in association with nasal airflow limitation, tachypnoea and/or oxygen desaturation. The presence of at least one of these indicators of increased respiratory effort in the PSG recording of the children in the incWOB group was verified independently by one of the investigators (S.Y.C.).

2.4 | Recording and analysis of sEMGcw

The sEMGcw is routinely recorded in all clinical PSGs at SCH. The sEMGcw was measured as we have reported previously (Chuang et al., 2017). Briefly, surface electrodes (3M Red dot; 3M Australia, Sydney, NSW, Australia) were placed on the chest in the right mid-clavicular line at the eighth intercostal space and on the rib below at the costal margin to record EMG signals from the lateral chest wall overlying the costal diaphragm. The EMG signals were recorded using Profusion Sleep v.3 software (Compumedics), with a sampling rate of 256 Hz, amplitude range of 2 mV, and a bandpass filter of 20–100 Hz, in accordance with settings recommended by the American Academy of Sleep Medicine (AASM) (Iber et al., 2007; Penzel et al., 2007). In each participant, two excerpts of 60 s epochs exhibiting a stable breathing pattern in one sleeping position (supine, right lateral, left lateral or prone) from each of the three sleep stages [light sleep (N2), deep sleep (N3) and random eye movement (REM) sleep] were exported to Spike2 (Cambridge Electronic Design, Cambridge, UK) data acquisition and analysis system for offline manual waveform analysis. Segments of sEMGcw contaminated with electrical activity of the heart (QRS complex) were removed from sEMGcw traces using a customized script (Figure 1; Chuang et al., 2017). The gated EMG signals were converted to a root mean square (RMS) signal with a time constant

TABLE 1 Demographic data of primary snorers, children with increased work of breathing (incWOB) and children with obstructive sleep apnoea (OSA)

Characteristic	Primary snorers ($n = 45$)	incWOB ($n = 19$)	OSA ($n = 27$)	*P* value
Age (years)	4.38 (3.00–7.73)	2.83 (2.39–5.70)	3.60 (2.60–6.21)	0.2
Male [*n* (%)]	27 (60)	12 (63.16)	20 (74.07)	0.47
Overweight and obese [*n* (%)]	17 (37.78)	7 (36.84)	9 (33.33)	0.93
Body mass index (centiles)	75 (46.37–92.00)	78.48 (46.7–92.35)	77.78 (39.65–94.25)	0.92
Medical co-morbidities [*n* (%)]	0 (0)	7 (36.8)	3 (11.1)	<0.001

Data are presented as median (interquartile range) unless otherwise specified.

of 100 ms, moving average. For each individual excerpt, the absolute amplitude of the peak RMS-sEMGcw per breath was determined manually for 10 consecutive unobstructed breaths (i.e. no decrease in airflow of ≥30%, no drop in oxygen saturation ≥3% from baseline and no EEG evidence of arousal). For children with OSA, the selected breaths were at least five breaths before and after an obstructive event. For each participant, the final sEMGcw value presented was the averaged peak RMS-sEMGcw per breath over six excerpts (two excerpts from each of the three sleep stages); 60 breaths in total.

2.5 | Statistical analysis

Statistical analysis was performed using SPSS v.22 (SPSS, Chicago, IL, USA). Where data were not normally distributed, they are presented as the median [interquartile range (IQR)] and compared between groups using the Mann–Whitney *U* test or the Kruskal–Wallis rank sum test for continuous variables. All normally distributed data are summarized as the mean and SD and were compared using Student's unpaired *t* tests with Bonferroni correction for the number of tests used ($n = 3$) on each data set. For categorical variables, comparisons between groups were made using the χ^2 test. The within-subject coefficient of variation (CV) was calculated for sEMGcw. The association between sEMGcw and PSG data was examined using Spearman's correlation (r_s). The strength of the correlations was categorized as large (>0.5), moderate (>0.3–0.5), small (0.1–0.3) or insubstantial (<0.1) (Evans, 1996). Logarithmic transformation of sEMGcw was performed to normalize the data. The relationship between physiological factors such as age, BMI centile and sex on log sEMGcw was examined using linear regression. A mixed model, with random effects for person and time, was performed to examine the effect of potential confounding factors, including age, sex, BMI centiles, sleep stages (non-REM *versus* REM) and sleeping positions (supine *versus* non-supine) on the log sEMGcw of the three groups (primary snorers, incWOB and OSA). For the mixed-model analysis, the averaged peak sEMGcw per breath from each of the six excerpts of sleep analysed was included in the model as six repeated measurements for each child. A *P* value of <0.05 was considered statistically significant. Where Bonferroni corrections were made, significance was defined by a *P* value of ≤0.017.

2.6 | Sample size calculation

The sample size was calculated based on the magnitude of sEMGcw measured in healthy snoring children in our previous work (Chuang et al., 2017). Using a 1:2 ratio of cases (OSA or incWOB children) to controls (primary snorers), a minimal sample size of 17 children in each of the incWOB and OSA group and 33 primary snorers had 90% power to detect an effect size of one with a 0.05 two-sided significance level.

3 | RESULTS

One hundred and thirteen PSGs were reviewed, of which 22 studies (19.5%) were excluded owing to poor-quality signals. Ninety-one PSGs were analysed: 45 primary snorers; 19 children with incWOB; and 27 children with OSA. Demographic and polysomnographic data, including the sEMGcw magnitude, for the three groups are presented in Tables 1 and 2. There was no difference between the groups in the baseline characteristics, including their age, sex and BMI centiles (Table 1). In the incWOB group, seven children (36.8%) had medical co-morbidities: one had repaired cleft lip and palate; four had airway issues (unilateral congenital vocal cord palsy $n = 1$, subglottic stenosis $n = 1$, trisomy 21 and laryngomalacia $n = 1$, tracheostomy owing to congenital tracheal stenosis $n = 1$); one had Bardet Biedl syndrome; and one had an intramedullary spinal tumour. Three of the children with OSA (11.1%) had other medical co-morbidities (one had laryngomalacia, one had asthma and one had Prader–Willi syndrome).

Slightly more than half of the children with OSA (15/27, 55.6%) had mild OSA, seven children (25.9%) had moderate OSA, and five children (18.5%) had severe OSA (Katz et al., 2002). Children with OSA had a significantly greater number of obstructive and mixed apnoeas and hypopnoeas than children in the PS and incWOB groups (Table 2). There was no significant difference between the number of obstructive apnoeas and hypopnoeas between the PS and incWOB group.

Non-normalized sEMGcw was significantly higher in children with OSA [median 8.47 μV (IQR 5.98–13.07 μV)] compared with primary snorers [4.63 μV (3.04–6.65 μV); $P < 0.001$; Table 2; Figure 2]. The sEMGcw was also significantly increased in children in the incWOB group (median 8.97 μV (IQR 5.94–13.43 μV); $P < 0.001$] compared with the primary snorers, with no significant difference between the sEMGcw level in the OSA and incWOB groups ($P = 0.78$). The median within-subject CV for peak sEMGcw amplitude for the whole cohort ($n = 91$) was 33.08% (IQR 28.68–39.61%). There was no significant difference between the within-subject CV for peak sEMGcw between the three groups ($P = 0.97$; Table 2).

Linear regression analysis suggested that age ($\beta = -0.12$, 95% CI −0.16 to −0.08; $P < 0.0001$) and BMI centiles ($\beta = -0.010$, 95% CI

TABLE 2 Polysomnographic measures, including average peak sEMGcw per breath, during sleep of primary snorers, children with increased work of breathing (incWOB) and children with obstructive sleep apnoea (OSA)

Parameter	Primary snorers ($n = 45$)	incWOB ($n = 19$)	OSA ($n = 27$)	*P* value
OAHI (events h^{-1})	0 (0.0–0.2)*	(0.0–0.4)*	3.7 (2.3–6.9)	<0.0001
CAI (events h^{-1})	0.1 (0.0–0.4)	0.1 (0.0–0.3)	0.1 (0.0–0.2)	0.99
OAI (events h^{-1})	0 (0.0–0.0)*	0.0 (0.0–0.0)*	0.7 (0.3–1.3)	<0.0001
MAI (events h^{-1})	0.0 (0.0–0.0)*	0.0 (0.0–0.0)*	0.2 (0.0–0.6)	<0.0001
Hypopnoea index (events h^{-1})	0.0 (0.0–0.2)*	0.1 (0.0–0.1)*	2.4 (1.3–5.7)	<0.0001
ODI3	0 (0.0–0.3)‡	0(0.0–0.3)†	0.6 (0.0–1.5)	0.03
(episodes h^{-1})	Range (0–1.6)	Range (0–1.8)	Range (0–29.2)	
Abnormal ODI3 [*n* (%)]	0 (0)	0 (0)	6 (22.2)	0.001
Oxygen saturation nadir (%)	93 (90–95)	92 (87–93)	89 (86–92)‡	0.017
Peak $TcCO_2$ (mmHg)	46 (42–48)	47 (42–50)	48 (44–52)	0.08
TST (min)	452.4 ± 63.8	456.8 ± 59.2	451.7 ± 76.9	0.93
Stage N1 and N2 (%TST)	49.9 ± 7.7	49.8 ± 6.2	46.7 ± 9.1	0.16
Stage N3 (%TST)	28.4 ± 5.6	28.3 ± 5	31.1 ± 6.3	0.26
REM (%TST)	21.2 (18.8–25.0)	20.7 (18.0–25.0)	22.4 (19.0–29.7)	0.43
Sleep efficiency (%)	83.4 (77.7–89.0)	83 (72.9–89.5)	85 (75–91.3)	0.93
Arousal index (h^{-1})	7.7 (4.5–10.7)	9 (4.50–12.1)	8.7 (3–16.7)	0.48
sEMGcw (μV)	4.63 (3.04–6.65)	8.97 (5.94–13.43)§	8.47 (5.98-13.07)§	<0.0001
	Range (0.4–12.52)	Range (3.63–42.49)	Range (1.41–33.64)	
$CV\%_{sEMGcw}$	32.55 (28.33–39.69)	34.22 (26.47–40.92)	32.96 (29.04–41.41)	0.97

Data are presented as the mean ± SD for normally distributed data or the median (interquartile range) for skewed data. Abbreviations: CAI, central apnoea index; $CV\%_{sEMGcw}$, within-subject coefficient of variation for peak sEMGcw amplitude per breath; MAI, mixed apnoea index; OAHI, obstructive apnoea–hypopnoea index; OAI, obstructive apnoea index; ODI3, oxygen desaturation index 3% or greater; REM, random eye movement; sEMGcw, surface electromyogram signals of the chest wall (overlying the diaphragm); $TcCO_2$, transcutaneous carbon dioxide; and TST, total sleep time.

*$P < 0.0001$ when compared with the OSA group.

‡$P < 0.05$ when compared with the OSA group ($P = 0.016$ for PS *versus* OSA; $P = 0.029$ for incWOB *versus* OSA).

†$P < 0.005$ when compared with the primary snorers.

§$P < 0.0001$ when compared with the primary snorers.

−0.015 to −0.005; $P < 0.0001$) were significant negative predictors of log sEMGcw. Sex was not a significant predictor ($P = 0.21$). After adjusting for age and BMI as covariates and including sleep stages and sleep positions as potential interactions in the mixed model, log sEMGcw remained significantly higher in both the OSA and the incWOB group compared with primary snorers ($P < 0.0001$).

The sEMGcw was moderately correlated with OAHI ($r_s = 0.410$, $P < 0.001$), hypopnoea index ($r_s = 0.393$, $P < 0.001$) and oxygen saturation nadir ($r_s = -0.348$, $P < 0.001$) for all children (Table 3; Figure 3a; Evans, 1996). However, for children without OSA (primary snorers and incWOB group) the relationship between sEMGcw and OAHI was small ($r_s = 0.254$, $P = 0.04$), and the association between other PSG measures, such as hypopnoea index and desaturation index, became not significant. There was a wide range of sEMGcw (0.4–42.5 μV) at OAHI = 0 h^{-1} (Figure 3b).

4 | DISCUSSION

Using the non-normalized sEMGcw (overlying the diaphragm) as an objective non-invasive measure of NRD, hence respiratory effort, in snoring children during sleep, we have demonstrated that sEMGcw measured during unobstructed tidal breathing was significantly elevated in children with OSA compared with primary snorers. Furthermore, in children without OSA but with subjectively increased respiratory effort (incWOB group), sEMGcw was also significantly elevated compared with primary snorers. The sEMGcw remained significantly higher in children from the OSA and incWOB groups than in primary snorers after the effects of age, BMI centile, sleep stages and sleep positions were taken into account. The wide range of sEMGcw (0.4–42.5 μV) and the weak correlation between standard PSG measures and sEMGcw for children without OSA (primary snorers and incWOB children) highlights that sEMGcw evaluates a different aspect of breathing compared with OAHI, the current diagnostic index for SDB. Potentially, non-normalized sEMGcw would be a useful complementary technique for assessing SDB, especially in children where reproducible maximal inspiratory manoeuvres are not possible to obtain.

Traditionally, the diagnosis of OSA depends on the demonstration of recurrent obstructive events during sleep in children (OAHI >1 h^{-1}; Marcus et al., 1992). However, elevated respiratory effort without apnoea can cause sleep fragmentation and daytime symptoms as part

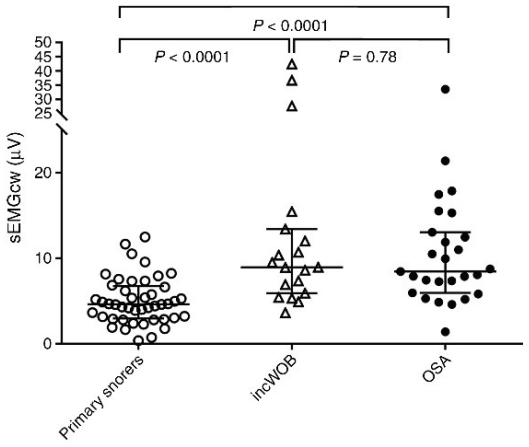

FIGURE 2 Magnitude of the sEMGcw in primary snorers (open circles), children with increased work of breathing (incWOB; open triangles) and children with obstructive sleep apnoea (OSA; filled triangles). The horizontal bars indicate the median and the 25th and 75th percentiles. Significant differences were found in the magnitude of the sEMGcw between primary snorers and both the incWOB and OSA groups. No difference was found between the incWOB and OSA groups

TABLE 3 Correlation of the sEMGcw magnitude with polysomnography measures for the whole cohort and for children without obstructive sleep apnoea (primary snorers and children with increased work of breathing, OAHI $\leq 1\,h^{-1}$)

Polysomnography variables	Spearman's rho (r_s) for all children ($n = 91$)	Spearman's rho (r_s) for children without OSA ($n = 64$)
OAHI	0.410***	0.254*
CAI	0.230*	0.251*
OAI	0.316***	0.098
MAI	0.342***	0.172
Hypopnoea index	0.393***	0.231
ODI3	0.228*	0.212
S_{pO_2} nadir	−0.348***	−0.326**
$TcCO_2$ peak	0.165	0.078
Arousal index	0.326**	0.277*
Sleep efficiency	0.094	0.094
TST	0.231*	0.272*

Abbreviations: CAI, central apnoea index; MAI, mixed apnoea index; OAHI, obstructive apnoea–hyponea index; OAI, obstructive apnoea index; ODI3, 3% oxygen desaturation index; OSA, obstructive sleep apnoea; S_{pO_2}, oxygen saturation; $TcCO_2$ transcutaneous carbon dioxide; and TST, total sleep time. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P \leq 0.001$ (P values from the Spearman's rank correlation coefficient between sEMGcw and the polysomnography variables listed).

of the obstructive SDB spectrum (Berry et al., 2013; Guilleminault, Stoohs, Clerk, Cetel, & Maistros, 1993). In the study by Skiba et al. (2015), of 105 adults with a normal PSG [based on apnoea–hypopnoea index (AHI)], 24 (22.9%) patients were diagnosed with SDB in a subsequent sleep study based on pathologically increased respiratory effort (measured by the magnitude of P_{oes} during tidal breathing) despite a normal AHI. The elevated level of sEMGcw during tidal unobstructed breathing in children with OSA compared with the primary snorers in our cohort suggests that sEMGcw was able to detect increased respiratory effort, similar to P_{oes}. We speculate that children with OSA had higher sEMGcw throughout sleep owing to elevated upper airway resistance even when there was no significant reduction (hypopnoea) or cessation (apnoea) in their nasal flow to be scored as an event. Furthermore, sEMGcw was sensitive enough to identify increased NRD in a group of children deemed by the sleep physicians to have increased respiratory effort (incWOB children) based on subjective assessments despite having a 'normal' OAHI. Future studies exploring the natural history and potential cardiovascular and neurocognitive morbidities in these children with increased NRD without significant apnoeic events is needed to determine whether they would benefit from clinical intervention.

Neural respiratory drive of the diaphragm and parasternal intercostal muscle during sleep has previously been evaluated mainly in adults (Berry et al., 2016; Gauda, Miller, Carlo, DiFiore, & Martin, 1989; Luo et al., 2008, 2009; Praud, D'Allest, Delaperche, Bobin, & Gaultier, 1988; Steier et al., 2008, 2010; Steier, Jolley, Polkey, & Moxham, 2011; Tabachnik, Muller, Bryan, & Levison, 1981a; Tabachnik, Muller, Levison, & Bryan, 1981b). Oesophageal catheter-mounted electrodes were the main technique used to assess EMGdi to minimize the potential of cross-talk signal from muscles surrounding the diaphragm (Luo et al., 2008, 2009; Steier et al., 2008, 2010). However, oesophageal catheters have major limitations, including increased arousals during sleep, reduced stage N2 and REM sleep and reduced total sleep time, and are poorly accepted by children and their carers (Chervin & Aldrich, 1997; Chervin et al., 2003; Luo et al., 2008). In a study assessing oesophageal pressure monitoring during sleep in children, only 54 out of 336 (16%) agreed to enrol in the study (Chervin et al., 2003). Another factor to consider is the interindividual difference in diaphragm-to-electrode distance and its effect on the magnitude of EMGdi. To overcome this effect, previous studies have normalized the EMGdi signal to a maximal value obtained through volitional inspiratory efforts (Steier et al., 2008, 2010). Given that the peak age of OSA in children is <5 years of age, this would not be possible in the majority of those with SDB, because children need to be at least school-aged (>5 years old) to perform acceptable and reproducible breathing manoeuvres (Lumeng & Chervin, 2008; MacBean et al., 2016b). Even when children are school-aged, reproducible volitional effort may still not be achievable (MacBean et al., 2016b). Recently, non-normalized respiratory muscle EMG has been reported as an outcome measure in adults and children with various clinical conditions, including patients ventilated on respiratory support (Kallio et al., 2015), wheezy preschool children (MacBean et al., 2016b), adults undergoing methacholine-induced bronchoconstrictions (MacBean et al., 2017) and adults with OSA (Luo et al., 2008). We have demonstrated in our cohort that non-normalized sEMGcw as an index of NRD was significantly higher in children with OSA and a group of children with subjectively increased respiratory effort compared with primary snorers. The NRD in children with OSA and increased WOB remained significantly higher than in primary

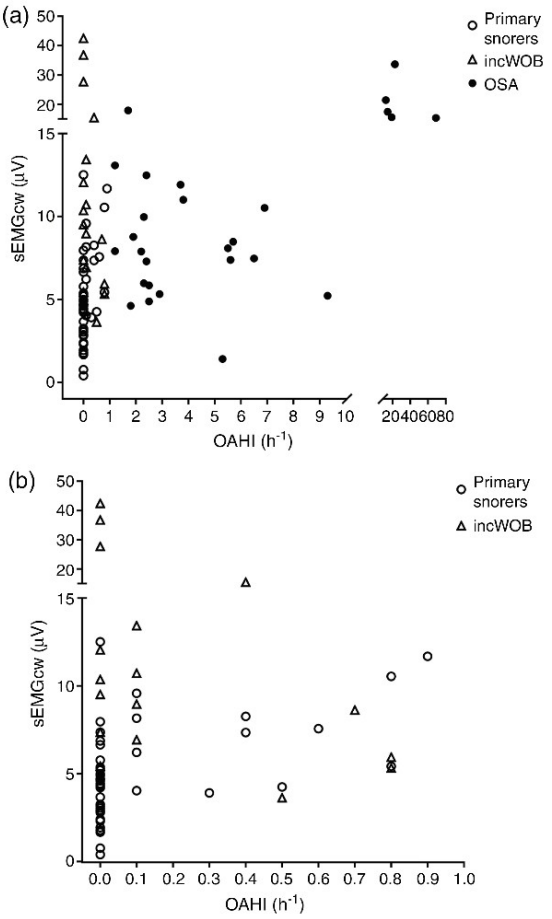

FIGURE 3 Scatterplots showing relationship between obstructive apnoea–hypopnoea index (OAHI) and sEMGcw (a, whole cohort; b, children without OSA). The distribution of sEMGcw was severely skewed when OAHI was ~0. Note the truncated *x*- and *y*-axis for panel a, and the truncated *y*-axis for panel b

snorers after taking into account the effect of individual anatomical factors, such as age and BMI, in the mixed model. Our data confirm the potential clinical utility of non-normalized sEMGcw to assess breathing during sleep in children.

Although P_{oes} is the gold standard recommended by the AASM to assess respiratory efforts (Berry et al., 2013), EMG of the diaphragm is a potentially more reliable method owing to the influences of airflow and lung volume on P_{oes} (Luo et al., 2008, 2009). Increasing lung volume alters the diaphragm length–tension relationship, resulting in a reduction in the pressure-generating capacity of the diaphragm for a given level of diaphragm activation, hence reduced P_{oes} (De Troyer & Wilson, 2009; Luo et al., 2008). Comparatively, EMGdi reflects the level and pattern of electrical activity to the diaphragm that is not artefactually affected by changes in lung volume (Beck, Sinderby, Lindstrom, & Grassino, 1998). Use of P_{oes} to assess respiratory effort in patients with OSA has limitations, because OSA is characterized by changes in lung volume and airflow. During obstructive apnoea events in adults, although a good relationship was found between P_{oes} and EMGdi measured using oesophageal catheter-mounted electrodes, P_{oes} peaked at the last breath during the apnoea, whereas EMGdi peaked at the first breath after the apnoea, when airflow resumed (Luo et al., 2008, 2009; Steier et al., 2010). We elected to study the sEMGcw in our cohort during unobstructed tidal breathing (evidenced by a lack of decrease in nasal airflow and oxygen saturation from baseline) to avoid the potential effect of fluctuating airflow and lung volume on the assessment of respiratory efforts.

The major strength of our study is the use of sEMGcw recorded by commercial polysomnographic equipment in a real-world setting, allowing potential introduction of sEMGcw measurements into clinical practice. Reliable sEMGcw recordings and analyses are achievable with standardized techniques (Chuang et al., 2017; Maarsingh et al., 2000). Based on our previous validation (Chuang et al., 2017), we were confident that the narrower EMG filter settings recommended by the AASM (Iber et al., 2007) were suitable for quantitative evaluation of sEMGcw, although a filter range of at least 20–450 Hz is the recommended setting for assessing respiratory muscle EMG (Stegeman & Hermens, 1998). Depending on the questions addressed, previous studies chose a wide variety of breathing periods during sleep for analysis [such as three or more breaths during five or more obstructive episodes (Luo et al., 2008; Steier et al., 2010; Stoohs et al., 2005), five to 18 breaths during tidal breathing (Luo et al., 2008; Steier et al., 2010), or 1–10 min during specific sleep stages (Steier et al., 2008, 2010)]. We chose to analyse 10 consecutive breaths, similar to previous studies on respiratory muscle EMG activity in children (Maarsingh et al., 2000; Maarsingh, van Eykern, Sprikkelman, & van Aalderen, 2004), and attempted to obtain a representative picture of each individual child's breathing pattern by analysing each sleep stage twice (60 breaths in total for each child). In line with previous reports of diaphragm electrical activity in infants (de Waal et al., 2017; Kassim, Jolley, Moxham, Greenough, & Rafferty, 2011), there were large individual variations in diaphragmatic activity, with a within-subject CV for peak sEMGcw ranging from 32.55 to 34.22% for the three groups of children. Only a few studies have evaluated sEMGcw in small cohorts of infants and children ($n = 7$–11) during sleep (Gauda et al., 1989; Praud, et al., 1988; Tabachnik et al., 1981a,b). Our study is the biggest study ($n = 91$) to date to evaluate the quantitative level of sEMGcw in snoring children.

There are some limitations to our study. As mentioned above, one of the main limitations of surface EMG recordings is the reduction in signal amplitude with increasing muscle-to-electrode distance (Beck, Sinderby, Weinberg, & Grassino, 1995). The increasing distance between the chest wall electrodes and the underlying diaphragm muscle with increasing subcutaneous fat explains why BMI centile was a negative predictor of sEMGcw in our cohort. Likewise, surface EMG signals can be difficult to detect in children who are overweight and/or obese, resulting in difficulties in distinguishing between EMG signals and baseline noise. The signal-to-noise ratios for all data traces from individual children were >1.7 except for two PS children who were overweight /obese with low sEMGcw (0.4 and 0.77 μV), highlighting the importance of interpreting low sEMBcw values with caution. Age was another negative predictor of sEMGcw, which is

likely to be related to developmental changes in chest wall mechanics and muscle-to-electrode distance (Bryan & Wohl, 1986). Surface EMG of the parasternal intercostal muscle, another obligatory inspiratory muscle, is an alternative site for assessing NRD in children during sleep with potentially less cross-talk from surrounding muscles than sEMGcw (MacBean et al., 2016b; Steier et al., 2011). Similar effects of age, height and weight on parasternal intercostal muscle EMG were previously observed in healthy children (MacBean et al., 2016b).

We did not have a control group of healthy children without a history of snoring; future work should include obtaining reference ranges of sEMGcw in healthy non-snoring children. We also excluded nearly 20% of the studies reviewed owing to the poor quality of the RIP and/or sEMGcw signals, similar to a previous adult study (Berry et al., 2016). We did not have a standardized calibration procedure to check the quality of sEMGcw signals before commencement of PSG recording. Given that these were clinical studies, and evaluated retrospectively, the sEMGcw electrodes were not replaced or recalibrated if the sEMGcw signal developed an artefact during sleep (possibly from sweating) if all other signals were satisfactory and the patient was asleep. However, studies with an artefact would have been excluded owing to poor-quality sEMGcw signals.

Another limitation is that the overlap between the sEMGcw values in PS and incWOB children might be attributable to group misclassification. This is not surprising, because the children were grouped based on the sleep physicians' subjective judgement of respiratory effort using a combination of PSG features (including visual interpretation of sEMGcw, nasal airflow, RIP band signals and the child's breathing on infrared video). The inclusion of subjective visual interpretation of elevated sEMGcw as one of the PSG features that sleep physicians used to determine the level of respiratory effort was also likely to biase the result towards a higher sEMGcw in the incWOB group. The sleep studies were scored based on the 2007 AASM manual (Iber et al., 2007). A change in the definition of hypopnoea from a decrease in nasal airflow of ≥50 to ≥30% meant that the OAHI values and subsequent group allocation for both the PS and incWOB children might be altered if re-scored based on the 2012 AASM manual's criteria (Nixon et al., 2014). However, given that the hypopnoea index and the ODI3 were both low in the PS and incWOB groups (median value 0–0.1, IQR 0–0.3), with only children in the OSA group having an abnormal ODI3 (Table 1), it is unlikely that the change in the definition of hypopnoea would have significantly affected the OAHI for the children without OSA.

In conclusion, our study has demonstrated that non-normalized sEMGcw, as an objective, non-invasive and non-volitional marker of NRD, is increased in children with OSA compared with primary snorers. Furthermore, sEMGcw was sensitive enough to detect the increased NRD in a group of children (incWOB) without OSA but subjectively increased respiratory effort compared with primary snorers. Our results support the view that non-normalized sEMGcw has potential clinical utility in the evaluation of children with sleep-disordered breathing, especially for those who might have increased upper airway resistance with obstructive symptoms, such as snoring, and daytime sleepiness without a definitive diagnosis of OSA. The sEMGcw is an accessible tool that can be adopted relatively easily into routine clinical PSG in children, which provides additional physiological information to standard PSG measurements. Future studies exploring the use of sEMGcw as a diagnostic tool for children on the spectrum of obstructive sleep-disordered breathing with increased NRD and as a potential indicator of the response to surgical and/or non-surgical treatment of sleep-disordered breathing, such as positive airway pressure support, would further validate the clinical role of sEMGcw in children.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

All the data were collected from the Sleep Laboratory at Sydney Children's Hospital, Sydney, NSW, Australia. S.Y.C., A.T., J.B. and A.J. contributed to the conception and design of the study. All authors contributed to acquisition, analysis or interpretation of data for the work. All authors were involved in drafting of the manuscript and/or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Key messages

- NRD as measured by tidal sEMGdi during unobstructed breathing was significantly higher not only in children with OSA but also in a group of children with subjective increased work of breathing compared to healthy snoring children.
- sEMGdi remained higher in children with OSA and children with increased work of breathing compared to healthy snorers after confounding factors including age, body mass index, sleep stages, and sleep positions were considered.
- The weak association between sEMGdi and OAHI in children without OSA suggests sEMGdi assesses a different aspect of breathing to OAHI.
- Tidal sEMGdi has potential clinical utility in the evaluation of children with obstructive sleep-disordered breathing as an objective, non-invasive, and non-volitional index of NRD.

Contribution of this paper to thesis

Using the sEMGdi analysis method validated in Chapter 2, I have demonstrated that sEMGdi was sensitive enough to differentiate the elevated NRD in children with OSA and increased work of breathing compared to primary healthy snorers. The increased NRD in children with increased work of breathing but "normal" OAHI highlights the deficiency of OAHI being the main diagnostic criteria of obstructive sleep apnoea in children. The higher sEMGdi and hence NRD to the diaphragm in children with increased work of breathing suggests there is an imbalance between the upper airway obstructive load and the capacity of the diaphragm muscle to overcome the respiratory load in these children. Provision of positive airway pressure

support such as CPAP is a well-recognised treatment for OSA in children and adults by maintaining airway patency. To further validate the clinical use of sEMGdi, whether sEMGdi can detect the unloading of the respiratory system when children with sleep-disordered breathing are treated with PAP support will be explored in the next chapter.

Chapter 4. Positive airway pressure support decreases respiratory load as assessed by diaphragm electromyography in children with sleep-disordered breathing

Introduction

In Chapters 2 and 3, I demonstrated that NRD as assessed by quantitative analysis of sEMGdi recorded during routine clinical overnight sleep studies was significantly higher in children with OSA and also children with incWOB compared to primary snorers (Chuang et al., 2017; Chuang et al., 2019). The clinical usefulness of sEMGdi is dependent on its ability to differentiate between health and disease, and severity of disease such as the different level of NRD in healthy snoring children compared to children with OSA on the upper airway obstructive breathing spectrum; in addition it should also be sensitive enough to reflect changes in NRD associated with treatment such as changes in sEMGdi before and after initiation of PAP support. There has been one study conducted in adults with ventilatory failure from restrictive (such as neuromuscular disease and chest wall deformities) and obstructive causes (such as COPD) which demonstrated a decrease in inspiratory muscle sEMG (sternomastoid, parasternal, and diaphragm muscles) during bilevel PAP (BPAP) support compared to spontaneous breathing (Carrey et al., 1990). Diaphragm EMG measured using oesophageal catheter-mounted electrodes also decreased when adults with severe OSA received CPAP support compared to when breathing spontaneously (Luo et al., 2009). In the AASM's clinical guideline for adjustment of PAP in children and adults with stable chronic alveolar hypoventilation syndromes, recording of inspiratory muscle sEMG activity during bilevel PAP titration studies was recommended to assess the

degree of inspiratory muscle rest with the use of PAP support in patients with neuromuscular disease and/or increased respiratory effort based on Carrey et al's study (Berry et al., 2010; Carrey et al., 1990). To my knowledge, there are no published data on the changes in inspiratory muscle sEMG associated with PAP use (either CPAP or BPAP) in children despite the publication of AASM's clinical guideline in 2010. In this chapter, I aimed to address this knowledge gap by assessing sEMGdi in children before and after provision of PAP support during split-night diagnostic-titration sleep studies using the analysis method I validated in Chapter 2 (Aim 3). I hypothesised that in children with sleep-disordered breathing (including OSA and nocturnal hypoventilation), sEMGdi would decrease after commencement of positive airway pressure support reflecting unloading of the diaphragm muscle.

Publication III This study has been submitted for consideration for publication:

Chuang SY, Teng A, Butler J, Gandevia S, Selvadurai H, Jaffe A. Positive airway pressure support decreases respiratory load as assessed by diaphragm electromyography in children with sleep-disordered breathing. *Pediatric Research*. 2019. (submitted)

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Signature

Positive airway pressure support decreases respiratory load as assessed by diaphragm electromyography in children with sleep-disordered breathing

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What is the key message of your article? (3-5 points)

- Respiratory load and hence respiratory effort is not routinely measured when titrating pressures on positive airway pressure support (PAP) devices for treatment of children with sleep-disordered breathing.
- Respiratory load as assessed by surface electromyography of the diaphragm (sEMGdi) decreased when children with obstructive and non-obstructive sleep-disordered breathing were treated with PAP support during split diagnostic-titration overnight sleep studies.
- sEMGdi is a novel non-invasive quantitative measure of respiratory load that can be incorporated as an additional respiratory variable to apnea hypopnea index (AHI) and gas exchange parameters when determining pressure on PAP.

What does it add to the existing literature? (3-5 points)

- Respiratory load is usually measured using an invasive eosphageal catheter to assess esophageal or transdiaphragmatic pressure swing and /or diaphragmatic and esophageal pressure time product, a method not easily tolerated and accepted by children.
- Surface electromyography of the respiratory muscles has been recommended by the American Association of Sleep Medicine as one of the indicators of adequate unloading of the respiratory muscles when titrating PAP for children and adults with chronic alveolar hypoventilation.
- This study is the first study in children to assess the changes in sEMGdi as a non-invasive index of respiratory load before and after commencement of PAP for treatment of children with sleep-disordered breathing.

- The lack of correlation between the degree of change in sEMGdi and routine PAP titration variables (including AHI, respiratory rate, and oxygen saturation nadir) before and after commencement suggests sEMGdi assesses a different aspect of breathing in children with sleep-disordered breathing.

What is the impact? (3-5 points)

- As demonstrated in a real-life heterogeneous clinical cohort, sleep-disordered breathing in children includes obstructive and non-obstructive causes. An additional indicator of treatment efficacy other than AHI is required especially for children with non-obstructive disordered breathing such as alveolar hypoventilation.
- Tidal sEMGdi is a non-invasive index of respiratory load which is well tolerated by children over long periods including sleep, making it a suitable complementary method to quantitatively evaluate breathing in children during sleep.
- Future studies comparing residual daytime and night time symptoms and compliance data in children treated with PAP therapy titrated to minimise AHI and/or respiratory load (assessed by sEMGdi) will help determine the clinical significance of providing optimal pressure to not only normalise AHI but also minimising respiratory load when treating children with sleep-disordered breathing.

Abstract

Background Surface electromyogram measures of the diaphragm (sEMGdi) reflects the respiratory load in children. This study evaluated the effect of treatment with positive airway pressure (PAP) on respiratory load in children with sleep-disordered breathing.

Method Twenty children who had split-night diagnostic and pressure titration sleep studies were analysed retrospectively. sEMGdi recorded during unobstructed breathing from random eye movement (REM) and non-REM (NREM) sleep before and after initiation of PAP were quantitatively measured.

Results Fourteen (70%) children had obstructive sleep apnea, 4 (20%) had nocturnal hypoventilation, and 2 (10%) had neither. sEMGdi decreased significantly post initiation of PAP (NREM sEMGdi from mean 7.93μV (SD 5.92) to 5.40μV (3.97), p = 0.01; REM sEMGdi 10.33μV (7.70) to 6.7 μV (5.81), p = 0.003). sEMGdi remained significantly lower when on PAP after the effects of age, body mass index, and sleep stages were accounted for (p = 0.019). Obstructive apnea hypopnea index (OAHI) also decreased (median 5.1/h (IQR 0.68-13.03) to 0/h (0-1.8), $p < 0.0001$). There was no correlation between the percentage changes in sEMGdi and OAHI before and after commencement of PAP (p=0.65).

Conclusion Respiratory load, as measured by sEMGdi, decreases following PAP support in children with obstructive and non-obstructive sleep-disordered breathing.

Keywords (3-10) Obstructive sleep apnea, sleep-disordered breathing, paediatrics, respiratory effort, polysomnography, diaphragm, surface electromyography

Introduction

Positive airway pressure support (PAP) devices, including nasal continuous positive airway pressure (CPAP) and bilevel positive airway pressure (BPAP) devices, are well-recognised treatments for children with sleep-related breathing disorders. For obstructive sleep-disordered breathing such as obstructive sleep apnea (OSA), PAP is manually titrated during overnight attended polysomnography (PSG) to determine the optimal pressure to maintain airway patency and overcome upper airway obstruction. The American Academy of Sleep Medicine (AASM) recommends increasing the pressure until obstructive breathing events, including apneas and hypopneas are minimised, as evidenced by a low apnea hypopnea index (AHI) and a normal oxygen saturation (1). However, respiratory effort and hence respiratory load is another parameter which should be considered but is often overlooked when determining the optimal pressure (1-3); insufficient level of PAP to minimise respiratory load despite normalisation of AHI in patients with OSA can cause residual upper airway resistance resulting in fragmented sleep and daytime somnolence (3-6). An accurate assessment of respiratory load is challenging when prescribing PAP for children with non-obstructive breathing disorders (such as alveolar hypoventilation resulting from bronchopulmonary dysplasia, neuromuscular and chest wall disorders) where the AHI is normal, and the titration of pressure is dependent on improvement of clinical observations such as decrease in respiratory rate, normalization of gas exchange and subjective improvement of chest wall retractions as evidence of unloading of respiratory muscles (7-10).

The respiratory load is usually assessed in clinical and research settings through the invasive measurement of esophageal pressure (Pes) swings, changes in transdiaphragmatic pressure (Pdi), and /or pressure time product of the diaphragm (PTPdi, an integration of Pdi swing over time) or esophageal pressure (PTPes) using an

esophageal catheter (1,2,7-11). In adults with OSA, it has been shown that higher positive airway pressure is needed to achieve the lowest Pes compared to the pressure required to minimise apnoea and hypopneas (3). Further, the degree of AHI does not reflect the level of PAP required to minimise Pes swings (2,3,6). Previous studies in children with obstructive and non-obstructive breathing disorders have also demonstrated that titrating PAP to minimise Pes results in greater improvement in respiratory load than titration based on clinical parameters such as normalization of gas exchange (8,10). However Pes is invasive and not well tolerated beyond infancy, limiting its inclusion in routine overnight sleep studies in children (1,12). Other limitations of measuring Pes during pressure titration studies include partial occlusion of one side of the nose by the esophageal catheter, distortion of pharyngeal dynamics, and difficulty obtaining a good nasal mask seal with the catheter in-situ (1,13). Comparatively, a non-invasive method of measuring respiratory load such as electromyogram of the diaphragm using transcutaneous surface electrodes (sEMGdi) is more suitable in children as it is well tolerated across all ages (14-16).

sEMGdi records the phasic inspiratory bursts of electrical activity to the diaphragm, reflecting the level and pattern of neural activation. Whereas sEMGdi represents the neural drive to the diaphragm, Pdi or Pes is the pressure generated by diaphragm contraction as a consequence of the neural drive (14,15). Increase in the neural respiratory drive (NRD) occurs when there is an increase in the load: capacity balance of the respiratory system, for example the balance between the obstructive load from increased upper airway resistance and the respiratory muscles' capacity to overcome the load such as occurs in children with OSA. Adult studies have demonstrated good correlation between EMGdi and Pes or Pdi (17-19). When adults with OSA are treated with CPAP, both EMGdi and Pdi swings decrease reflecting

improvement in diaphragm muscle load (19). In another study of adults with hypoventilation, PAP support was associated with decreased sEMGdi and also improvement in Pes swing compared to spontaneous breathing (9). Based on this one adult study, AASM recommended the evaluation of inspiratory muscle EMG as an indicator of respiratory muscle load when titrating PAP for adults and children with chronic alveolar hypoventilation although there are currently no data on the impact of CPAP or BPAP use during sleep on inspiratory muscle sEMG magnitude in children (7,9).

We have previously validated a method for assessing sEMGdi recorded using standard commercial sleep study set up and applied it to differentiate the level of respiratory load in children with OSA and increased work of breathing compared to healthy snorers (15,16). Our aim was to address the knowledge gap on the effect of PAP on the respiratory load in children with sleep-disordered breathing by evaluating changes in sEMGdi in response to PAP support. Our hypothesis was that PAP can reduce respiratory load (assessed using sEMGdi) in children with and without OSA.

Method

Ethics approval was granted by the Sydney Children Hospital Network's Human Research Ethics Committee (12/SCHN/450). A waiver for individual participants' consent was granted in accordance with the Australian National Statement on Ethical Conduct in Human Research as the research involved no more than low risk to participants, and sufficient protection on the participants' privacy and confidentiality was provided (20).

Subjects

This was a retrospective study of children aged 0-18 years who had undergone a split-night diagnostic and pressure titration sleep study for clinical indications at Sydney Children's Hospital over a two-year period. The inclusion criteria were: 1) the chest and abdominal respiratory inductance plethysmography (RIP) belt signals were of adequate technical quality to enable determination of periods of inspiration and 2) visible phasic inspiratory bursts of sEMGdi tracings during tidal unobstructed breathing (i.e. no evidence of decrease in airflow of ≥ 30% and no oxygen desaturation ≥3% from baseline) for the excerpts analysed.

Protocol

All studies commenced at the diagnostic part of the split-sleep studies. For children who were already prescribed PAP, therapy was discontinued for a 3-day washout period while under oxygen saturation (SpO_2) monitoring at home where feasible. PAP was commenced based on predetermined modified AASM criteria set by the patients' usual sleep clinicians (1,7), including one or more of the following: 1) at least 4 hours of diagnostic data recorded, including one period of rapid eye movement (REM) sleep; 2) repetitive obstructive events (such as apnoea, hypopnea, and snoring) present consistent with obstructive apnea-hypopnea index (OAHI) > 10/h based on technician assessment; 3) evidence of hypoventilation with repeated SpO_2< 90% and /or evidence of carbon dioxide retention (transcutaneous carbon dioxide ($TcCO_2$) consistently > 50 mmHg or increase of > 10 mmHg from awake); 4) remaining sleep study recording time adequate for > 3 hours of pressure titration. Once PAP was commenced, the pressure was titrated based on standard protocols (1,7). For CPAP,

therapy was started with a pressure of 4 cmH_2O and raised in 0.5 cmH_2O increments to eliminate obstructive breathing events. For BPAP, pressure levels commenced at inspiratory positive airway pressure (IPAP) of 8 cmH_2O and expiratory positive airway pressure (EPAP) of 4 cmH_2O or at the patient's usual pressure settings. Pressures were titrated by 1 cmH_2O increments to eliminate obstructive events and/or improve gas exchange. The PAP level was considered optimal if obstructive events (snoring, apnea and hypopnea) were minimised for ≥ 30 minutes in rapid eye movement (REM) and non-REM (NREM) sleep, gas exchange abnormalities improved (SpO_2 > 90% in room air and/or $TcCO_2$ < 50 mmHg) , and at least one titration period in REM sleep with the child in supine position was included (1,7).

Measurements

Standard full overnight polysomnograms were recorded and scored by pediatric sleep physicians in accordance with the AASM guideline using a computerised PSG system (Compumedics®, Melbourne, Australia) (21). Parameters recorded included: 8 channels of electroencephalogram (EEG); right and left electro-oculograms (EOG); electrocardiogram (ECG); chin, diaphragm, abdominal and anterior tibial electromyogram (EMG); thoracic and abdominal movement (uncalibrated RIP , Compumedics®, Melbourne, Australia), oxygen saturation (Masimo RadicalSET®, Irvine, CA, USA), $TcCO_2$ monitoring (SenTec, Fenton, MO, USA), combined nasal / oral thermistor and nasal airflow via nasal cannulae (for the diagnostic part of the split-night studies). Infrared video monitoring with microphone recording was routine. Age, gender, height, weight, body mass index (BMI) centiles (≥ 2 years old) or weight for

length centiles (< 2 years old) measured on the day of sleep study were recorded. Obesity was defined as BMI greater than or equal to 95th percentile for age and sex (22).

Diaphragm sEMG measurement and analysis

Diaphragm sEMG was routinely recorded using bipolar transcutaneous electrodes (3M Red dot®, USA) placed on the chest wall in the right mid-clavicular line at the 8th intercostal space and on the rib below at the costal margin in accordance with AASM guidelines as previously described (7,15-17). In brief, EMG signals were acquired at a sampling rate of 256 Hz, amplified and band pass filtered (20 to 100 Hz) in line with settings recommended by the AASM (23). Excerpts from REM and NREM (stages N2 or N3) sleep were analysed offline from both the diagnostic part of the study, and at the final pressure setting. Each excerpt consists of 10 stable consecutive tidal breaths (evidenced by consistent amplitude of RIP band traces to suggest stable tidal volume) in the supine sleeping position without evidence of obstruction (such as a decrease in airflow of ≥ 30% and a drop in oxygen saturation ≥3% from baseline) or arousal to ensure stability of sEMGdi measured (21)(Figure 1). A total of 40 breaths were analysed for each child (including 20 breaths when spontaneously breathing, 20 breaths while receiving PAP support at the final titrated pressure). A customized script was used to remove the portion of sEMGdi signal contaminated by the QRS complex of the heart's ECG activity (Figure 1). The gated EMG signals were rectified by converting to root-mean-square (RMS) sEMGdi signal with a time constant of 50ms and a moving window. The absolute amplitude of the RMS-sEMGdi per breath was manually determined and averaged for each excerpt.

Statistical analysis

Data are presented as means (standard deviation, SD) for normally distributed data, and median (interquartile range, IQR) for non-normally distributed data. Differences between measured variables during the diagnostic and the pressure titration part of the split-night study were made using paired t-tests for normally distributed data, or Wilcoxon matched pairs signed rank test for non-normally distributed data. A linear mixed model including sleep stages and PAP use as interactions was performed to examine the effects of factors including age, BMI centiles, sleep stages (REM vs NREM), and PAP use (no PAP vs PAP) on sEMGdi. Pearson or Spearman correlations were used to determine the relationship between percentage change of PSG variables such as OAHI and percentage change of sEMGdi before and after initiation of PAP. A p value < 0.05 was considered significant. Statistical analysis of the data was performed using SPSS Version 22 (SPSS, Chicago, Illinois, USA).

We based our power calculation on the improvement of respiratory load with PAP devices in children. In a small trial of 12 infants with severe upper airway obstruction or bronchopulmonary dysplasia, Pes swing decreased by 10 cmH_2O after commencement of CPAP (8). A sample size of 20 children was required to detect a similar level of improvement in respiratory load in our study with a two-sided significance level of 0.05 and power of 80%.

Results

Twenty-three split-night studies were reviewed and 3 studies were excluded due to poor quality of the sEMGdi tracings. Split-night studies from 20 children median age 2.8 years (IQR 0.3-13.8) with various underlying medical conditions were analysed

(Table 1, Figure 2). Eleven (55%) children were previously prescribed PAP however 6 (30%) were non-compliant and/or did not tolerate the use of PAP. Fourteen children (70%) had OSA (defined as OAHI >1/h) in the diagnostic part of the study, including 3 with mild OSA (OAHI >1 to 5/hour), 4 with moderate OSA (OAHI >5 to 10/hour), and 7 with severe OSA (OAHI > 10/hour) (Figure 2)(1). Six children did not have OSA in the diagnostic part of the study. Four of these six children were commenced on PAP due to hypoventilation with gas exchange abnormalities. The remaining two children were commenced on PAP after one period of REM sleep has been recorded as planned by their sleep physicians; one of these two patients was non-compliant with CPAP prescribed for residual OSA post adenotonsillectomy and the other child had repaired tracheoesophageal fistula (TEF) treated with nocturnal CPAP for breathing support when unwell with respiratory infections. The final pressure titrated was 5.5 cmH_2O (SD 0.83) for the 18 children on CPAP, and IPAP 11/ EPAP 6 cmH_2O for the two children on BPAP.

Overall, sEMGdi for the whole cohort decreased significantly in NREM sleep with a mean difference of 2.53 μV (95% CI 0.69-4.37, $p = 0.01$) post initiation of PAP (Table 2, Figures 2 and 3a). Similarly, there was a significant reduction in sEMGdi during REM sleep with a mean difference of 3.63 μV (95% CI 1.44-5.82, $p = 0.003$) after initiation of PAP (Table 2, Figures 2 and 3b). There was no significant difference between the change in sEMGdi during REM sleep or NREM sleep ($p = 0.19$). For the subset of 14 children with OSA, there was a significant decrease in the sEMGdi (mean difference 3μV (95% CI 0.54-5.46), $p = 0.02$) during both NREM sleep and REM sleep (mean difference 3.90μV (95% CI 1.32-6.47), $p =0.006$). The sEMGdi recorded from the 4 children with hypoventilation reduced from baseline when on BPAP although the sample was too small to perform valid statistical comparisons.

Reduction in sEMGdi during sleep was evident in 18 children (90%) in total, including 13 (72%) children with OSA, 4 (22%) children with hypoventilation (3 with obstructive hypoventilation, 1 with cystic fibrosis and alveolar hypoventilation), and 1 child (6%) who was non-compliant on CPAP prescribed for OSA post adenotonsillectomy but had normal OAHI and gas exchange indices during the diagnostic part of their split-sleep study (Figure 2). Linear mixed model analysis confirmed that after the effects of age, BMI centiles, and stages of sleep (REM vs NREM) were considered, there was a significant decrease in sEMGdi after initiation of PAP ($p = 0.019$). BMI centiles were not a significant predictor of sEMGdi in the mixed model ($p = 0.09$). Age was a significant factor affecting sEMGdi, with a decrease of 0.2 μV (95% CI 0.02-0.39; $p = 0.027$) per one-year increase in age.

Other respiratory variables also improved significantly after commencement of PAP, including OAHI, respiratory rate (RR), and SpO_2 nadir (lowest SpO_2 recorded) during the study period (diagnostic vs entire titration period) (Table 2). There was no correlation between the percentage changes in sEMGdi and percentage changes in OAHI, RR, and SpO_2 nadir before and after commencement of PAP ($p = 0.65$, $p= 0.27$, $p=0.67$ respectively). There was no correlation between the percentage change in sEMGdi and ΔCPAP pressure (i.e. change in pressure between beginning and end of the titration period) for the children with OSA ($p =0.87$).

Use of PAP was not associated with a decrease in sEMGdi in 2 children during sleep (Patients 2 and 16; Figure 2). Patient 2 was a 14 months old boy referred for investigation of snoring and witnessed apnea. He was initiated on CPAP unexpectedly due to recurrent obstructive events in the diagnostic sleep study (OAHI 13.2/h, SpO_2 nadir 71%, peak $TcCO_2$ 65 mmHg). After commencing on CPAP, his OAHI decreased to 0/h and gas exchange normalised but sEMGdi increased (from 7μV (spontaneously

breathing) to 10.6μV (on CPAP) during NREM sleep, and 12.8μV (spontaneous breathing) to 17.5μV (on CPAP) during REM sleep). Patient 16 was a 6 years old boy who had a history of TEF repaired and recurrent lower respiratory tract infections requiring multiple hospital admissions in the previous 12 months. CPAP was prescribed for overnight breathing support when unwell with respiratory symptoms. The split-sleep study was performed when the child had been well without any respiratory symptoms in the preceding 4 weeks to confirm that CPAP did not have a deleterious effect on his sleep. He did not have evidence of sleep-disordered breathing (OAHI 0/h, normal gas exchange, no evidence of snoring). His sEMGdi increased from 6.10μV (spontaneous breathing) to 9.86μV (on CPAP) during NREM sleep, 7.58μV (spontaneous breathing) to10.43 μV (on CPAP) during REM sleep.

Discussion

We have demonstrated that the respiratory load in the majority (90%) of children with sleep-disordered breathing decreased with PAP as objectively measured by sEMGdi. In addition to decreasing the sEMGdi in 13 children with OSA, PAP also decreased the sEMGdi in 4 children with hypoventilation, and 1 snoring child without OSA or hypoventilation. The reduction in sEMGdi after commencement of PAP remained statistically significant after the effects of sleep stages, age, and BMI centiles on sEMGdi were considered. There was no correlation between the degree of change in standard respiratory parameters reported (such as OAHI or gas exchange indices) and sEMGdi after commencement of PAP, suggesting sEMGdi, and hence respiratory load, is an independent variable when assessing breathing during sleep. sEMGdi may be helpful in identifying those patients in whom PAP level may be inadequate or

unhelpful, as demonstrated by the two children whose sEMGdi did not improve after PAP commencement. Our findings support the incorporation of sEMGdi as an index of respiratory load when assessing the efficacy of PAP in children with sleep-disordered breathing.

Inspiratory muscle EMG recorded using surface electrodes during tidal breathing at rest has been increasingly used in children to assess respiratory load in clinical research, including the change in obstructive lower airway load in children with wheeze and asthma treated with bronchodilators and also in children in intensive care when extubated to CPAP (14,16,24). We have previously shown that sEMGdi recorded using commercial sleep study equipment and setting can be reliably measured quantitatively, and that sEMGdi was sensitive enough to differentiate the obstructive upper airway load between children with OSA and increased work of breathing compared to healthy snorers (15,16). Using the same method of EMG analysis, we showed that sEMGdi reduced in the majority (90%) of our cohort after initiation of PAP support, validating the utility of sEMGdi as a non-invasive marker of respiratory load in children. The significant decrease in sEMGdi in the children with OSA and obstructive hypoventilation after initiation of PAP support in the split-sleep studies reflects the unloading of the diaphragm muscle once upper airway patency was maintained. Since currently the main goal of pressure titration in children with obstructive sleep-disordered breathing is to eliminate obstructive respiratory events including apneas and hypopneas (1,7), it was not surprising that the OAHI decreased significantly from 5.1 events/hour (0.68-13.03) to 0 events/hour (0-1.8). However, using Pes, it has been demonstrated that the optimal pressure titrated for an individual may be up to 5 cm H_2O above the pressure set to eliminate apnea and hypopnea and that failure to minimise respiratory effort may result in persistently elevated upper airway resistance, daytime

sleepiness, and suboptimal compliance with PAP use (1-6). This may partly explain the lack of improvement in sEMGdi in patient 2 where unplanned CPAP was commenced for severe OSA, suggesting residual upper airway obstructive load despite the normalisation of his OAHI and gas exchange. Both lung hyperinflation associated with CPAP use, and patient discomfort due to unexpected initiation of CPAP, can cause increased respiratory muscle load and hence patient 2's higher sEMGdi when on CPAP. Future studies comparing minimising sEMGdi as an additional titration goal in pressure titration compared to normalising OAHI alone for obstructive sleep-disordered breathing is needed to explore the clinical significance of reducing respiratory load in this cohort of children.

With the increasing use of PAP in children without OSA, including children with neuromuscular and other musculoskeletal disorders associated with chest wall restriction, and chronic lung diseases including bronchopulmonary dysplasia and cystic fibrosis, another objective index other than OAHI is required to demonstrate the efficacy of PAP (8,10,11,25,26). Transdiaphragmatic pressure, Pes swings, PTPdi and PTPes have been used to assess the degree of unloading of the diaphragm and/or inspiratory muscles when PAP is provided to children with bronchopulmonary dysplasia, bronchiolitis, and other causes of hypoventilation (8,10,11). Titrating CPAP to reduce respiratory load as measured by Pdi, Pes, PTPdi, and/or PTPes results in higher CPAP levels compared to titrating to other clinical parameters such as normalisation of respiratory rate and SpO_2 and/or chest wall retractions in children with non-obstructive breathing conditions when awake or asleep (8,10). However these measures of respiratory load are invasive and hence its use is limited to research settings in children. A small study in adults with ventilatory failure (n=12) previously demonstrated progressive reduction in diaphragm, parasternal and sternocleidomastoid

surface EMG accompanying improvement in Pes swing when treated with PAP support (9). Based on this study, the AASM suggests absence or reduction of inspiratory muscle EMG activity compared to baseline should be included with other parameters such as increase in tidal volume and decrease in respiratory rate as evidence of adequate PAP support when treating patients with chronic alveolar hypoventilation syndrome including obstructive hypoventilation and neuromuscular disease (7). In our cohort of children, sEMGdi objectively demonstrated the improvement in respiratory muscle load in children with obstructive hypoventilation (n=3) and alveolar hypoventilation from cystic fibrosis (n=1). Our data also highlight the potential for sEMGdi as a suitable objective non- invasive index to evaluate effects of PAP on respiratory muscle load in children without OSA which needs to be confirmed in bigger studies.

The results suggest that, in addition to demonstrating successful effects of PAP, sEMGdi can help identify those children for whom it is ineffective. In a small infant study (n=7), lack of improvement in Pes swing and PTPes was used as an indicator for discontinuing treatment with BPAP in infants with hypoventilation (10). For patient 16 in our cohort with repaired TEF and using CPAP as respiratory support at home when clinically unwell, the use of PAP was not associated with improvement in any of the respiratory parameters recorded (including OAHI, gas exchange, and sEMGdi) during sleep. This was not surprising given that the patient did not have a history of sleep-disordered breathing and the sleep study was performed when the child was well. The use of CPAP in healthy adults can induce acute lung hyperinflation and increase in respiratory muscle load as measured by increase in scalene muscle EMG during inspiration and transversus abdominis muscle during expiration, which may be the cause of the increase in sEMGdi in this child (27). The use of PAP is not without risks (28-30). In children, other than common complications such as local skin irritations or

ulcerations and drying of the nasopharynx, there are also the potential risks of increasing otological complications, gastric distensions, and midfacial hypoplasia (28,29,31). Previous studies have demonstrated between 10-30% of children discontinue PAP therapy due to intolerance or side effects (29,30), which is reflected in 30% of the children in our cohort where part of the indication for the split-night study was non-compliance with prescribed PAP therapy. Hence it is important to commence PAP only in children in whom benefits can be objectively demonstrated, either through improvement in OAHI, gas exchange parameters, and/or potentially sEMGdi as an index of respiratory load.

Other non-invasive alternative to Pes for assessing respiratory load during a clinical sleep study set up in children is RIP tracings (1,21). With RIP, variations in the electrical inductance of a wire sewn in the bands allows evaluation of the changes in the cross-sectional area of the rib cage and abdomen enclosed within the band. However, RIP signals must be calibrated for accurate assessment of flow which either requires performance of isovolume manoeuvres (i.e. a period of breathing effort with no airflow) which is difficult for children or use of computer algorithms based on tidal breathing which is not readily available (32). Also, changes in sleeping position throughout the night can invalidate the calibration procedure (33). Comparatively our method of analysing sEMGdi quantitatively potentially allows for a more objective and standardised method to evaluate changes in respiratory load associated with PAP use. Future work in validating Pes against sEMGdi in children would help confirm the use of sEMGdi as a non-invasive technique for assessing respiratory load in children during sleep.

Our study has some limitations. The inclusion of children who only had split-night sleep studies have contributed to the heterogeneity and complexity of the cohort

with a higher proportion of children with coexisting medical conditions compared to previous studies (25,26,29,34). The sleep physicians' decision to utilize split night studies as opposed to full night diagnostic PSG followed by a full night titration study was dependent on the clinical indication for PSG in each child and availability of laboratory resources. The children in our cohort had heterogeneous underlying disorders with varied indications for a split-night sleep study reflecting real-life clinical paediatric sleep medicine practice. This included children with clinical suspicion of significantly abnormal breathing during sleep (obstructive or non-obstructive) warranting urgent treatment with PAP, and /or uncertainty regarding the benefit of PAP (such as non-compliance with PAP therapy prescribed). Similar to the typical pediatric populations requiring PAP therapy (25,29,34), the majority of the children (14/20, 70%) had OSA, and 30% (6/20) had previous surgery to treat obstructive breathing. Thirty percent of the children had already been prescribed PAP but were non-compliant. A higher proportion of children in our cohort (75%) had coexisting medical conditions such as obesity, craniofacial anomalies, syndromal diagnoses, and chronic respiratory conditions than previous studies highlighting the complexity of our cohort (29,30,34). Regardless of the potential impact of the varying underlying medical conditions and treatments received for each child, we were able to demonstrate unequivocally the beneficial effect of PAP on reducing the sEMGdi and hence improving respiratory load in the majority of the children.

Another potential limitation to our study is the shorter pressure titration period in a split-night diagnostic-titration study compared to a full-night pressure titration study. The AASM has suggested that an 'optimal' titration study for children with OSA reduces OAHI to less than 5 events/hour and maintain a minimum $SpO_2 > 90\%$ at the selected pressure (1). In our cohort, OAHI decreased significantly from 5.1 events/hour (0.68-13.03) to 0 events/hour (0-1.8), and the average SpO2 was greater than 90% at the

final pressure for all the children, hence we believe that our titration period was adequate for the current recommended goals of titration (1,7). The lack of a standardized calibration procedure to check the quality of the sEMGdi signal prior to the commencement and also at the end of PSG recording is another limitation. The included studies are clinical studies which were evaluated retrospectively, hence sEMGdi were not replaced if the sEMGdi signal developed an artefact during sleep if all the other routine PSG signals were satisfactory and the child was asleep. However, studies with significant sEMGdi artefact would have been excluded due to poor quality sEMGdi signals.

The strength of our study is the utilisation of split-night sleep studies to investigate the effects of PAP on respiratory load in children during sleep. One of the criticisms of using surface electrodes to assess respiratory muscle activity is the effect of differing electrode placements between occasions on the magnitude of EMG activity recorded (32). Another challenge affecting interpretation of respiratory muscle surface EMG is the anatomical variations between individuals causing a filtering effect on sEMGdi magnitude due to interindividual differences in diaphragm-to-electrode distance unless the sEMGdi is normalised to a reference value (32). By assessing the changes in sEMGdi before and after commencement of PAP within the same child during a split-night sleep study, we ensured that these physiological and methodological factors affecting sEMGdi were minimised. In our cohort, although age remained a negative predictor of sEMGdi likely due to changes in chest wall configuration and respiratory mechanics with age (35), the reduction in sEMGdi after commencement of PAP remained significant in the linear mixed model. Another strength of our study was that this was a real-life pragmatic study; all the split-sleep studies were clinically indicated studies recorded using standard commercial polysomnography equipment and

settings. Although ideally a filter range of at least 20-450 Hz is employed to assess respiratory muscle EMG (36), we were confident from our previous research that the narrower filter setting based on AASM's recommendation (21) was still appropriate for quantitative evaluation of sEMGdi (15). In our study, quantitative analysis of the sEMGdi signals required manual offline analysis. However the development of commercial equipment to measure respiratory muscle EMG in real-time such as Dipha-16 (Inbiolab BV, Groningen, Netherlands) (37) and neurally adjusted ventilatory assist ventilators (NAVA®, Maquet, Germany) (38) indicates the possibility of incorporating quantitative sEMGdi assessment in commercial polysomnography systems.

In summary, this study has shown that sEMGdi can be an objective and non-invasive index used in combination with measurement of other current respiratory parameters such as OAHI and gas exchange abnormalities to evaluate the efficacy of PAP support in children with obstructive and non-obstructive sleep-disordered breathing. It can help identify children for whom PAP is not beneficial and hence avoid potential complications associated with PAP use. Future research will help consolidate the role of sEMGdi in PAP titration studies in children.

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Table 1 - Patient characteristics

N	20
Age, year (median, IQR)	2.8 (0.3-13.8)
Males, n (%)	14 (70%)
Weight centiles (mean, SD)	60.37 (34.04)
BMI centiles (mean, SD)	64.12 (28.72)
Previous adenotonsillectomy, n (%)	6 (30%)
Prescribed use of PAP, n (%) Non-compliant with PAP, n (%)	11 (55%) 6 (30%)
Other diagnoses*, n Obese Genetic syndrome (e.g. Down syndrome) Craniofacial syndrome (e.g. Robin sequence, choanal stenosis) Pulmonary disease (e.g. cystic fibrosis, tracheoesophageal fistula)	 5 4 3 3

*Note that some children had multiple diagnoses

BMI, body mass index; IQR, interquartile range; PAP, positive airway pressure; SD, standard deviation.

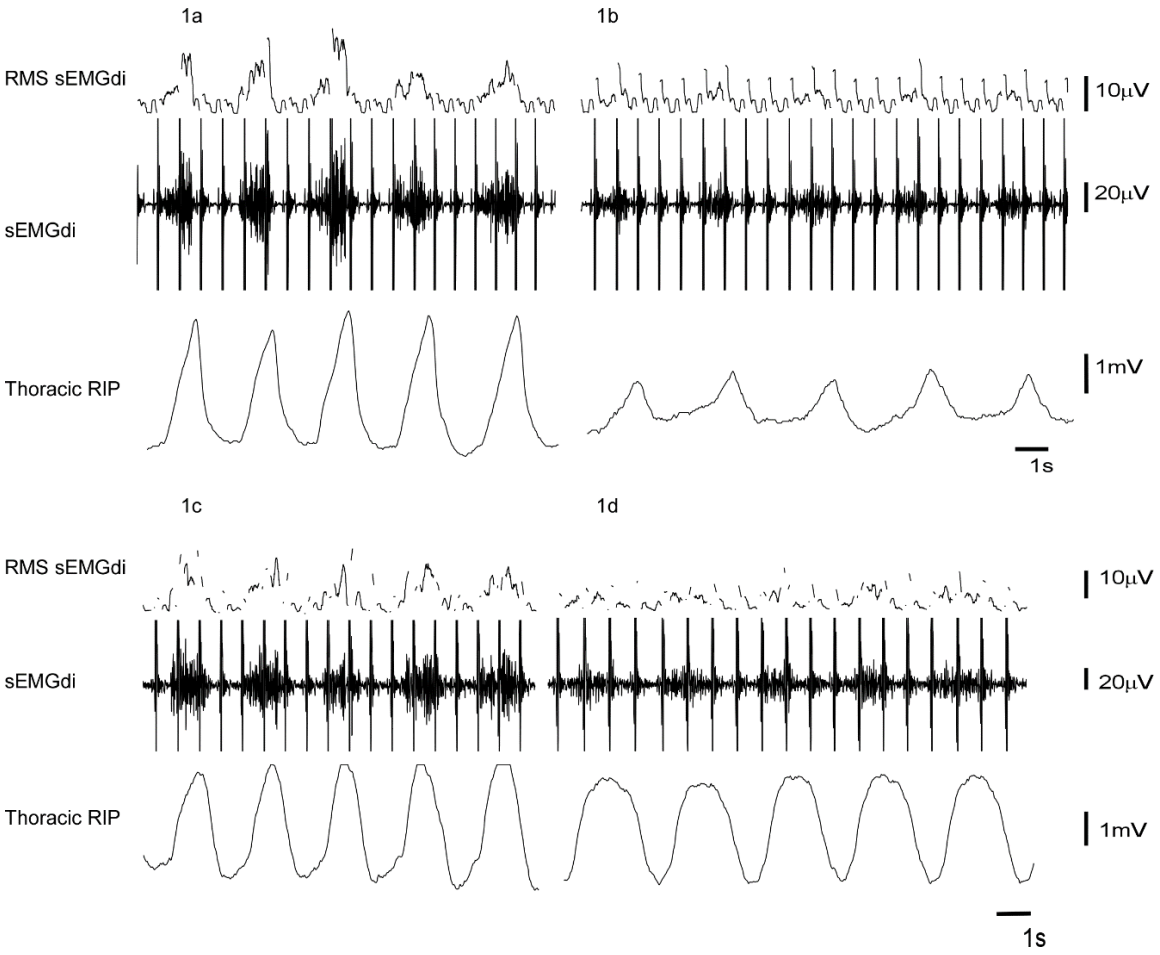
Table 2 –Sleep study and physiological parameters before and after commencement of positive airway pressure support during sleep

	Spontaneous breathing	**Post PAP**	***P value***
sEMGdi in NREM (μV)	7.93 (5.92)	5.40 (3.97)	**0.010**
sEMGdi in REM (μV)	10.33 (7.70)	6.77 (5.81)	**0.003**
OAHI (events/h)	5.1 (0.68-13.03)	0 (0-1.8)	**<0.0001**
CAI (events / h)	0.1 (0-0.7)	0 (0-1.3)	0.15
RR (breaths / h)	25.84 (9.76)	21.38 (6.25)	**0.001**
SpO_2 nadir (%)	83 (80-86.75)	91 (87-92)	**0.001**
Peak TcCO2 (mmHg)	53.32 (7.74)	51.78 (5.47)	0.38

Data are presented as mean (standard deviation) for normally distributed data, or median (interquartile range) for skewed data. CAI, central apnea index; PAP, positive airway pressure support; NREM, non-rapid eye movement sleep; OAHI, obstructive apnea hypopnea index; REM, rapid eye movement sleep; RR, respiratory rate; SpO_2, oxygen saturation; TcCO2, transcutaneous carbon dioxide level; sEMGdi, surface electromyogram of the diaphragm.

Figure legends

Figure 1 - Examples of the surface diaphragm electromyogram (sEMGdi) recorded before and after commencement of positive airway pressure support



Excerpts of recordings taken from a child with obstructive sleep apnea (Figure 1a and 1b) and a child with cystic fibrosis complicated by nocturnal alveolar hypoventilation (Figure 1c and 1d) before (Figure 1a, figure 1c) and after (Figure 1b, figure 1d) commencement of positive airway pressure support during the split-night sleep study. The RMS-sEMGdi trace is the gated EMG signals with most of the ECG QRS artefact removed converted to root mean square. The thoracic respiratory inductance plethysmography (RIP) signals were used to determine period of inspiration. ECG,

electrocardiogram; EMG, electromyography; RIP, uncalibrated respiratory inductance plethysmography; RMS, root mean square; sEMGdi, surface electromyogram of the diaphragm.

Figure 2 - Overview of the study cohort results from the diagnostic component of the split-night sleep study sEMGdi – surface electromyogram of the diaphragm; OAHI – obstructive apnea hypopnea index; OSA – obstructive sleep apnea; PAP – positive airway pressure; TEF – tracheoesophageal fistula.

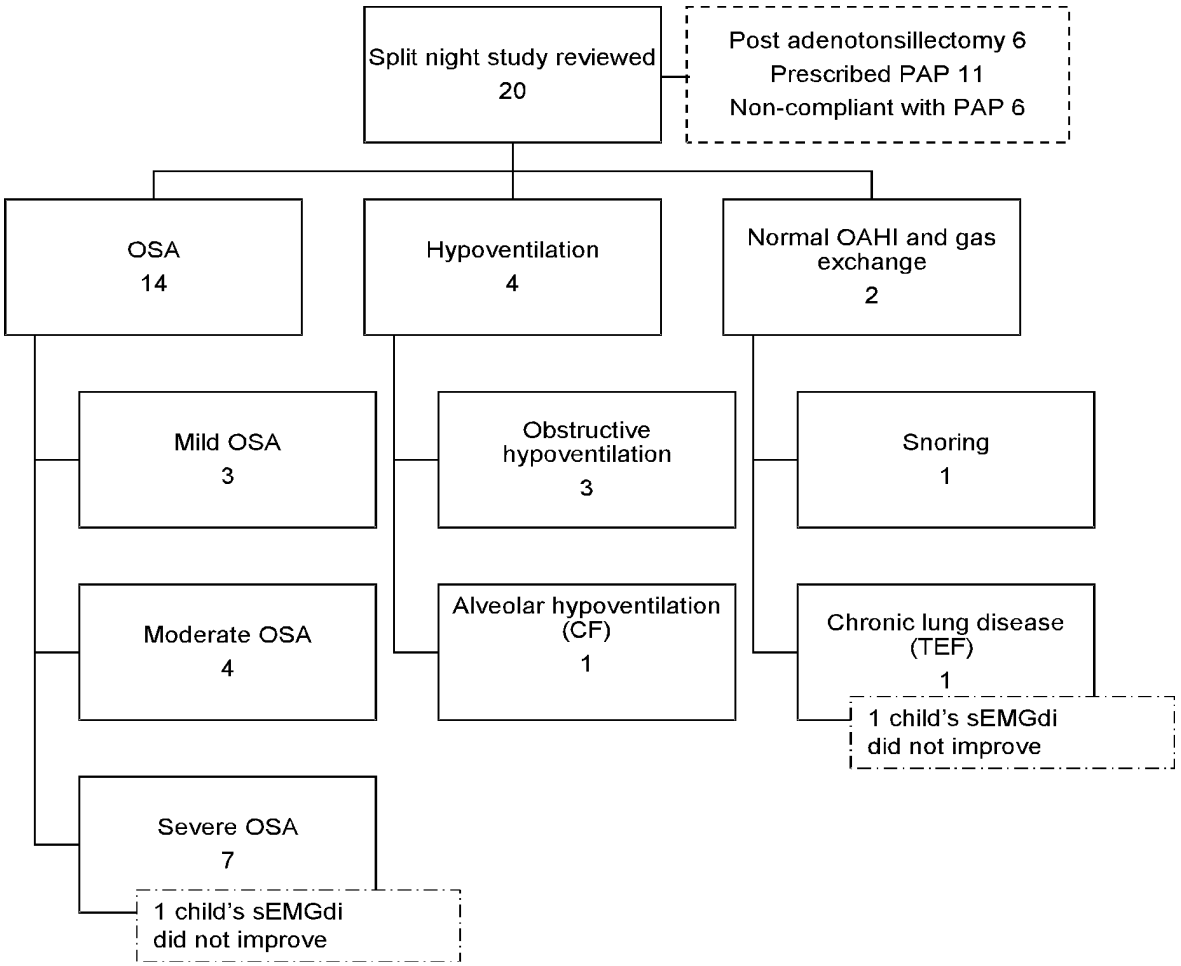
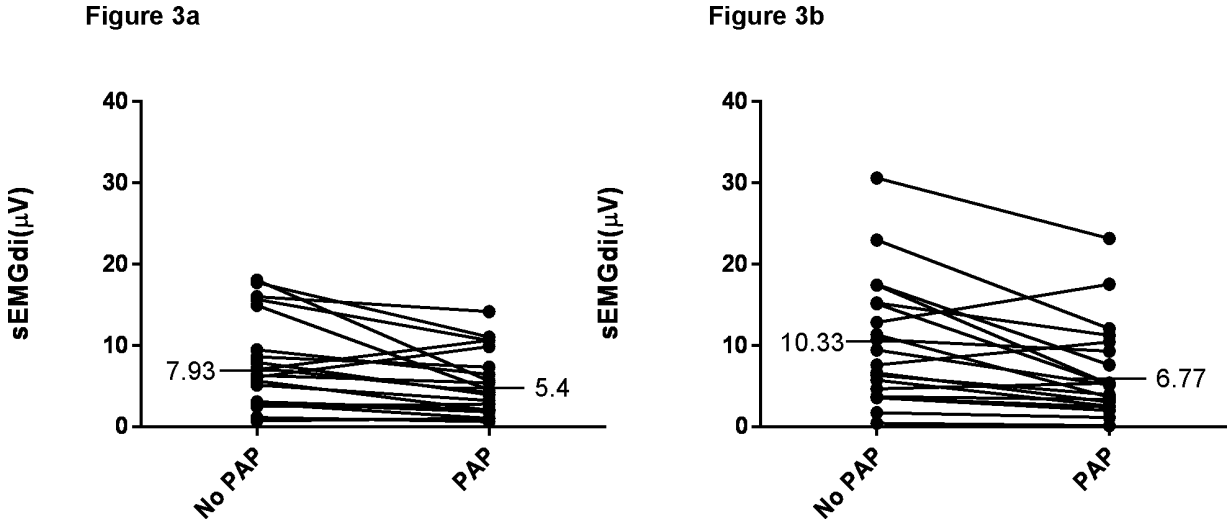


Figure 3 - sEMGdi magnitude before and after initiation of positive airway pressure support during NREM (Figure 3a) and REM (Figure 3b) sleep. NREM – non-random eye movement; PAP – positive airway pressure support; REM – random eye movement; sEMGdi - surface electromyography of diaphragm.



Key messages

- NRD as assessed by tidal surface electromyography of the diaphragm (sEMGdi) decreased when children with obstructive and non-obstructive sleep-disordered breathing were treated with PAP support during split diagnostic-titration overnight sleep studies.
- The sEMGdi remained significantly lower while on PAP support compared to spontaneous breathing after factors potentially influencing sEMGdi magnitude including age, BMI, and sleep stages were accounted for.
- The decrease in sEMGdi and hence NRD reflects the unloading of the diaphragm muscle when children with sleep-disordered breathing were treated with PAP support.
- sEMGdi should be considered as an additional titration variable to AHI and gas exchange parameters when determining optimal pressure for treating children with obstructive and non-obstructive sleep-disordered breathing.

Contribution of this paper to the thesis

This chapter provides further evidence that our method of assessing sEMGdi recorded during routine clinical sleep studies in children using commercial equipment and set up is valid, and that sEMGdi is a useful quantitative measure of NRD in children with sleep-disordered breathing. My studies in Chapters 2, 3, and 4 are the first studies to use sEMGdi recorded during tidal unobstructed breathing to quantify NRD in children with sleep-disordered breathing during spontaneous breathing, and when receiving treatment for sleep-disordered breathing. To further my understanding of the use of inspiratory muscle sEMG as an assessment of NRD in children, I needed to explore the

contribution of extra-diaphragmatic muscles to breathing in children and also how NRD relates to conventional measures of respiratory output, lung volume and pressure.

Chapter 5. Exploring the relationship between respiratory muscle sEMG and lung volume and pressure

Introduction

In Chapters 3 and 4, I studied the diaphragm EMG recorded during routine overnight sleep studies using the analysis method validated in Chapter 2 to assess NRD in children during sleep. In Chapter 3, sEMGdi was able to measure the higher NRD in children with OSA and increased work of breathing compared to primary snorers (Chuang et al., 2019). Moreover, in Chapter 4, sEMGdi decreased after initiation of PAP support in children with sleep-disordered breathing, reflecting unloading of the respiratory system and reduced NRD during sleep. Although the diaphragm is the main inspiratory muscle in humans, it is also supported by other obligatory inspiratory muscles such as the scalene in the neck, and parasternal intercostal muscles in the rib cage (Cabral et al., 2018; De Troyer & Estenne, 1984; Dos Reis et al., 2019; Gandevia, Leeper, McKenzie, & De Troyer, 1996; Gandevia, McKenzie, & Plassman, 1990; Saboisky, Gorman, De Troyer, Gandevia, & Butler, 2007). Assessment of NRD to inspiratory muscles other than diaphragm would contribute to further our understanding of ventilation, especially in certain respiratory disease such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (Gandevia et al., 1996; Jolley et al., 2015; Jolley et al., 2009; MacBean, Jolley, et al., 2016; MacBean et al., 2017; Reilly, Jolley, Elston, Moxham, & Rafferty, 2012; Reilly et al., 2011; Steier, Jolley, Polkey, & Moxham, 2011; Steier et al., 2009; Tabachnik, Muller, Levison, & Bryan, 1981); or in different loaded breathing conditions such as during sleep or hypercapnia (Reilly et al., 2013; Steier et al., 2011; Steier et al., 2010); and between postures (Butler,

McKenzie, & Gandevia, 2001; Gandevia & McKenzie, 1986; MacBean, Hughes, Nicol, Reilly, & Rafferty, 2016; Reilly et al., 2013; Steier et al., 2009; Tabachnik, Muller, Bryan, & Levison, 1981; Tabachnik, Muller, Levison, et al., 1981; Williams, Porter, Westbrook, Rafferty, & MacBean, 2019). In order to utilise sEMG of inspiratory muscles as a quantitative index of NRD in children it is important to 1) understand the contribution of different inspiratory muscles to NRD, and 2) explore how sEMG relates to conventional measures of lung function such as changes in lung volume and pressure as spirometry remains the clinical method of choice in evaluating lung function in health and in disease.

Electrical activation of the respiratory muscles by the brainstem respiratory centres leads to muscle shortening as reflected by changes in lung volume, and force generation which is usually estimated as pressure. Thus, quantitative characterization of respiratory muscle function usually relies on measurements of lung volume, mouth or airway pressures, and the rates of change of these variables. In recent years, there has been an increasing use of inspiratory muscle EMG (from muscles such as the diaphragm and the parasternal intercostal muscle) in paediatric and adult research as a complementary method to spirometry when assessing lung function (Jolley et al., 2009; MacBean, Jolley, et al., 2016; MacBean et al., 2017; Reilly et al., 2012; Reilly et al., 2011; Steier et al., 2010). An example is the assessment of airflow limitation and airway responsiveness in asthmatic children and adults using sEMGdi and/or sEMGpara compared to FEV1 (Maarsingh et al., 2002; MacBean et al., 2017; Sprikkelman, Van Eykern, Lourens, Heymans, & Van Aalderen, 1998). In both adults and children, sEMGdi and/or sEMGpara during tidal breathing increased while FEV1 decreased during bronchial provocation challenge, with a measurable improvement of both sEMG and FEV1 after treatment with bronchodilators (Maarsingh et al., 2002; MacBean,

Jolley, et al., 2016; MacBean et al., 2017; Sprikkelman et al., 1998). Further, increase in sEMG associated with lower airway obstruction were able to be quantified in toddlers and young pre-school children who were not able to perform routine spirometry (Maarsingh, Oud, van Eykern, Hoekstra, & van Aalderen, 2006; Maarsingh, van Eykern, Sprikkelman, & van Aalderen, 2004; MacBean, Jolley, et al., 2016). These studies highlight the interplay between clinical lung function testing and respiratory muscle EMG activity, where an increase in airway obstructive load to the respiratory system caused by bronchoconstriction leads to an increase in NRD (as reflected by the increase in sEMGdi and sEMGpara) during tidal breathing.

To use sEMG of the inspiratory muscles to assess breathing in children with lung conditions, we need to first understand the relationship between inspiratory muscle EMG and conventional lung function measures of volume and pressure in healthy children. Studies in adults have demonstrated a linear or a curvilinear relationship between EMG activity of the inspiratory muscles and lung volume or airway pressure (Beck, Sinderby, Lindstrom, & Grassino, 1998b; Yokoba, Abe, Katagiri, Tomita, & Easton, 2003). With progressive activation of the respiratory muscles with increasing EMG, a corresponding increase in respiratory muscle output such as mouth pressure is expected especially under isometric conditions (Beck et al., 1998b; Ng & Stokes, 1992; Raper, Thompson, Shapiro, & Patterson, 1966; Washino, Kanehisa, & Yoshitake, 2017; Yokoba et al., 2003). To my knowledge, no previous study has investigated the relationship between EMG activity of the inspiratory muscles and respiratory output in healthy children.

Another aspect of respiratory function assessable using surface EMG but not obtainable through conventional lung function testing, is the respiratory muscle recruitment pattern under different breathing conditions. As discussed in Sections 1.3.1

Control of breathing during quiet breathing, and 1.3.2 Control of breathing during 'voluntary' respiratory contractions from Chapter 1, distribution of neural drive to inspiratory muscles during different breathing manoeuvres varies depending on the individual muscle's role in achieving efficient ventilation (De Troyer & Boriek, 2011; De Troyer, Legrand, Gevenois, & Wilson, 1998; De Troyer, Legrand, & Wilson, 1996). The recruitment pattern of multiple inspiratory muscles during spontaneous and voluntary contraction in children has not been reported previously. With developmental changes occurring in the chest wall and the lung in growing children, it is likely that the recruitment pattern of inspiratory muscles differs compared to those of adults depending on the breathing strategies adopted. To appreciate the potential changes in ventilation strategies in children with respiratory conditions such as lung hyperinflation from asthma, we need to understand the respiratory muscle recruitment pattern in healthy children first.

In this chapter, I aimed to increase the understanding of using sEMG of inspiratory muscles in children as an index of NRD by answering the following questions: 1) what is the relationship between inspiratory muscle EMG and conventional lung function measures of lung volume and mouth pressure in healthy children (Aim 4); 2) what is the relative activity and pattern of recruitment of the three major obligatory inspiratory muscles: diaphragm, parasternal intercostal, and scalene muscles in healthy children when performing maximal inspiratory manoeuvres; 3) does the relative activity of the three inspiratory muscles change if the same inspiratory manoeuvre are performed in different body positions (sitting vs supine)? I hypothesised that similar to adults, a linear or curvilinear relationship exists in children between inspiratory muscle sEMG and lung volume and mouth pressure. Further, similar to adults, the diaphragm will have higher NRD than scalene and parasternal muscles when

children perform maximal inspiratory manoeuvres (Nava, Ambrosino, Crotti, Fracchia, & Rampulla, 1993; Reilly et al., 2013; Segizbaeva, Pogodin, & Aleksandrova, 2013). Lastly, I hypothesised that posture would not affect inspiratory muscle activity when children performed maximal inhalation manoeuvres based on an adult study of intramuscular EMGdi and EMGpara recorded during quiet breathing (Butler et al., 2001).

Method

Participants

The study protocol was approved by the Sydney Children's Hospitals Network's Human Research Ethics Committee (LNR/14/SCHN/424). All enrolled children's parents or carers provided informed written consent.

Young healthy children > 6 years of age with no history of cardiorespiratory or neuromuscular disease were recruited from friends and family of hospital staff, and from the community through flyers within Sydney Children's Hospital (SCH). Any children with a history of acute respiratory tract infection (including cough, runny nose) within the last 4 weeks were excluded. All enrolled children's height, weight, and body mass index (BMI) were measured and recorded on the day of testing. Standard spirometry with forced expiratory volume manoeuvres was performed in all children using portable spirometer (Easy On-PC Spirometer, NDD Medical, Zurich, Switzerland) to assess FEV1 and FVC. Results were expressed as z-scores of published reference values (Quanjer et al., 2012).

Experimental set-up

Respiratory muscle activities from scalene (sEMGsc), parasternal intercostal muscle (sEMGpara), and diaphragm (sEMGdi) were recorded using surface electrodes (3M Red dot©, USA). Prior to placement of the surface electrodes, the skin was thoroughly cleaned with abrasive gel to optimize skin impedance (Nuprep™ abrasive skin gel; Weaver &Co., Aurora, CO, USA). For the scalene muscle, the active electrode was placed in the posterior triangle of the neck, posterior to the border of the sternocleidomastoid muscle at the level of the cricoid cartilage with the location confirmed by palpation during voluntary sniffs (Hudson et al., 2016). The reference electrode was placed on the clavicle at a site indicated by an imaginary line along the long axis of the scalene muscle. sEMGpara recordings were made by placing the active electrode on the right 2nd intercostal space approximately 2-3 cm from the mid-point of the sternum, and the reference electrode placed on the sternum at the same horizontal level. Diaphragm EMG recordings were made from electrodes placed on the right 8th intercostal space at the nipple line (between midclavicular and anterior axillary line) with the reference electrode at the rib directly below the active electrode without touching (Verin et al., 2002). A ground electrode was applied over the right shoulder.

The sEMG signals were amplified and bandpass filtered between 10 Hz and 1000 Hz (Cambridge Electronic Design (CED) data acquisition and analysis interface, Cambridge, UK) with analogue to digital sampling at 2000 Hz. Respiratory flow and mouth pressure were measured by children breathing through a mouthpiece connected in series to a calibrated pneumotachograph (Hans Rudolph, MO, USA) and a manually activated shutter. The flow and pressure signals were sampled at 100 Hz. Lung volume was obtained by digital integration of the flow signal by the acquisition software. All signals (EMG and respiratory flow, volume and pressure signals) were recorded

simultaneously and displayed in real time on a computer running the Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Study protocol

All participants attended the SCH respiratory laboratory on one occasion. The children were asked to sit in a chair with handrests and back support. sEMG activities were measured simultaneously when the children performed the maximal inspiratory manoeuvres. After demonstration and practice with an experienced paediatric respiratory scientist, all children performed a total of four maximal volitional manoeuvres. Two of the four manoeuvres were performed as graded inspiratory ramps in order to determine the activation profile of the inspiratory muscles sEMG with increasing lung volume or mouth pressure: 1) inspiratory capacity by asking the children to breathe in gradually from the end of a normal breath (FRC) to TLC over 5 seconds (dynamic TLC ramp); 2) maximal static inspiratory effort against an occluded airway by asking the children to "suck in" as strong as they can for a brief period (1-3 seconds) from FRC against a manually closed shutter (short standard MIP recorded, (shMIP)); 3) children were asked to "suck in" gradually from FRC against a closed shutter to their maximal effort over 5 seconds (static MIP ramp), and 4) maximal sniff inhalation by asking the children to perform a short sharp sniff from FRC with one nostril occluded by a nasal olive (SNIP recorded) (MacBean, Jolley, et al., 2016; Reilly et al., 2011; Sinderby, Beck, Spahija, Weinberg, & Grassino, 1998). The decision to perform the MIP manoeuvre, not only as a gradual MIP ramp, but also as a short maximal inspiratory effort (as per routine respiratory muscle strength testing), was to ensure the maximum peak sEMG would be obtained for the purpose of

normalising sEMG values (described in further details below). Higher mouth pressure is expected with short inspiratory efforts resulting in potentially higher inspiratory muscle EMG than a graded MIP ramp (American Thoracic Society/European Respiratory Society, 2002).

A nose clip was worn for all the manoeuvres except for sniff inhalations. All manoeuvres were performed after a short period of relaxed tidal breathing of at least 5 resting breaths. Each manoeuvre was repeated at least three times with at least one-minute rest between each attempt until at least two reproducible efforts with less than 20% variance in the maximum pressure recorded or less than 5% variance in the maximum volume recorded (American Thoracic Society/European Respiratory Society, 2002; Miller et al., 2005). To assess the impact of posture, all manoeuvres were repeated with the children in a supine posture, facing up to the ceiling while lying flat on a firm mattress with their hands by the side of their body.

Data analysis

Analysis was performed with custom-written software that measures the maximum and minimum value of all signals (lung volume, mouth pressure or nasal pressure, sEMGsc, sEMGpara, sEMGdi) during the inspiratory phase of the respiratory cycle. (Figure 5.1, Figure 5.2) This was a breath-by-breath analysis with user verification (by visual inspection) for every signal. Cursors were placed manually at the start (i.e. the point of deflection from the baseline volume or pressure trace at the end of a normal expiration to suggest onset of inspiration) and the end (i.e. maximal volume or pressure) of inspiration for each attempt of the TLC ramp and MIP ramp. Out of the three or more attempts for the static task (MIP ramp), the highest MIP produced was

selected as the reference MIP value (Figure 5.2). sEMG signals were converted to root mean square (RMS) using a time constant of 50 ms. To determine the profile of inspiratory activation of scalene, parasternal and diaphragm muscle throughout the static task, mouth pressure (presented as absolute values) and the scalene (RMS-sEMGsc), parasternal (RMS-sEMGpara), and diaphragm (RMS-sEMGdi) EMG signals were manually determined at 11 time points for each attempt at the task for individual children, i.e. at 0% (baseline), then successive 10%-time intervals to 100% of the reference peak MIP (Figure 5.2). The term RMS-sEMG will be used interchangeably with sEMG for the rest of the chapter. The same process was repeated for the dynamic TLC ramp using the highest peak inspired lung volume (TLC) out of the three best attempts at the manoeuvre as the reference TLC value (Figure 5.1).

Figure 5.1 Analysis of dynamic inspiratory TLC ramps.

An example of the data recorded from one of the participants. Participants were asked to perform a gradual inspiration from functional residual capacity (FRC) to total lung capacity (TLC) over 5 seconds. Lung volume expressed as a percentage of TLC are expressed as timepoints on the x-axis. From top to bottom, panels show lung volume, raw and RMS-EMG from scalene (sEMGsc), parasternal intercostal (sEMGpara), and diaphragm (sEMGdi) muscles respectively. Cursors were placed at baseline (0%) and maximal lung volume (100%) of the ramp, respectively. Lung volume, scalene (RMS-sEMGsc), parasternal intercostal (RMS-sEMGpara), and diaphragm (RMS-sEMGdi) RMS EMG were then determined at 10%-time intervals to give a profile of muscle activation across the task. In this particular example, at 10% and 40% of TLC ramp duration, sEMGpara and sEMGdi were contaminated by the ECG artefact hence the values for sEMGpara and sEMGdi at both these timepoints were substituted with sEMG value closest to the time interval without ECG artefact or omitted if no substitute value without ECG artefact was available.

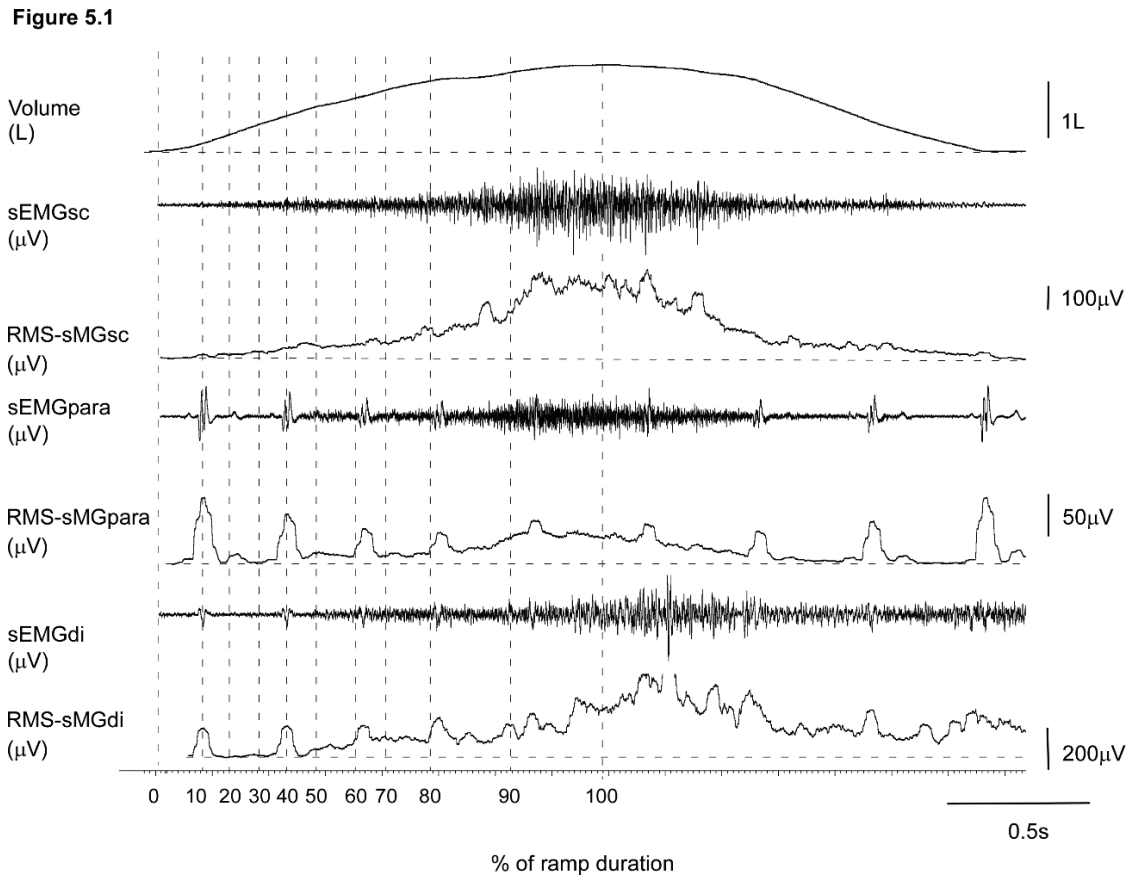
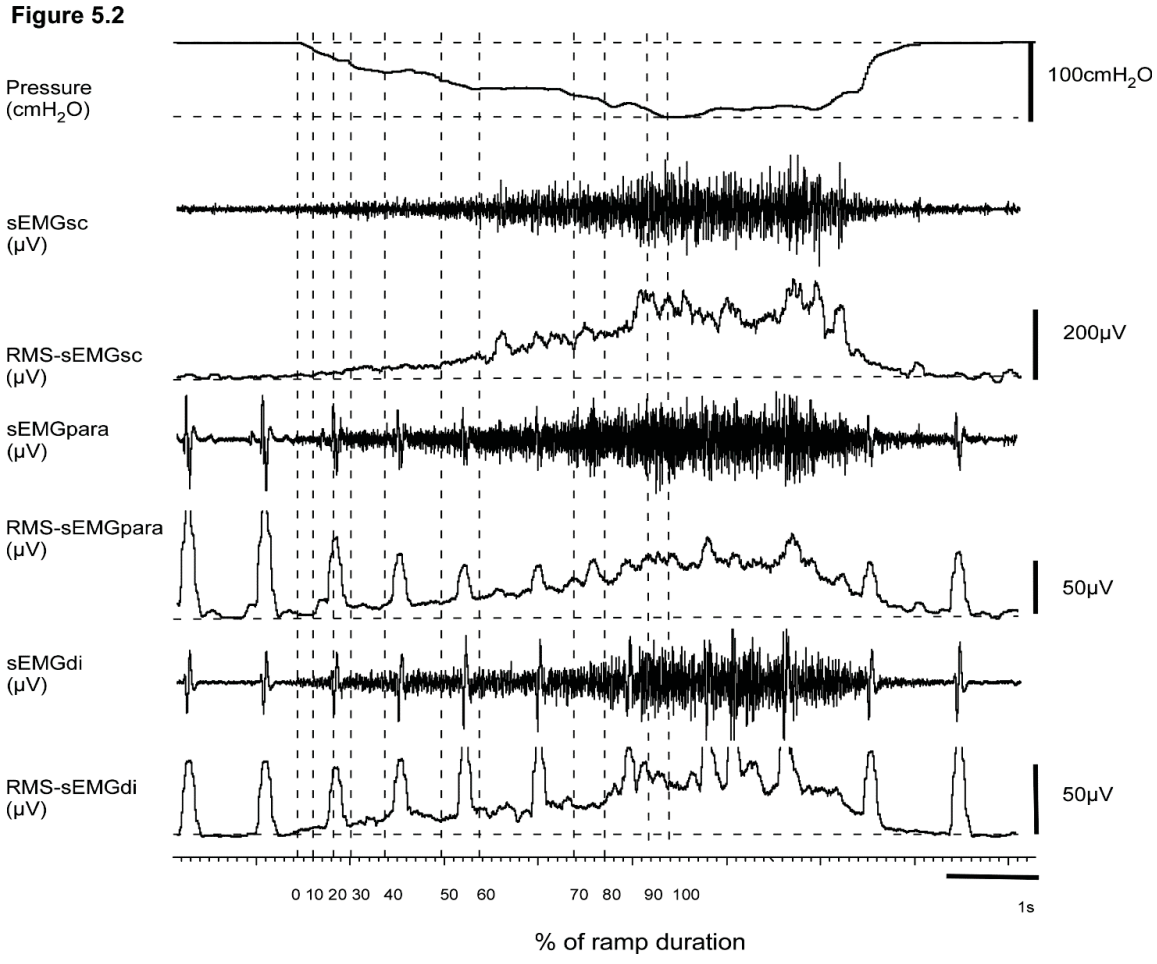


Figure 5.2 Analysis of static inspiratory MIP ramps.

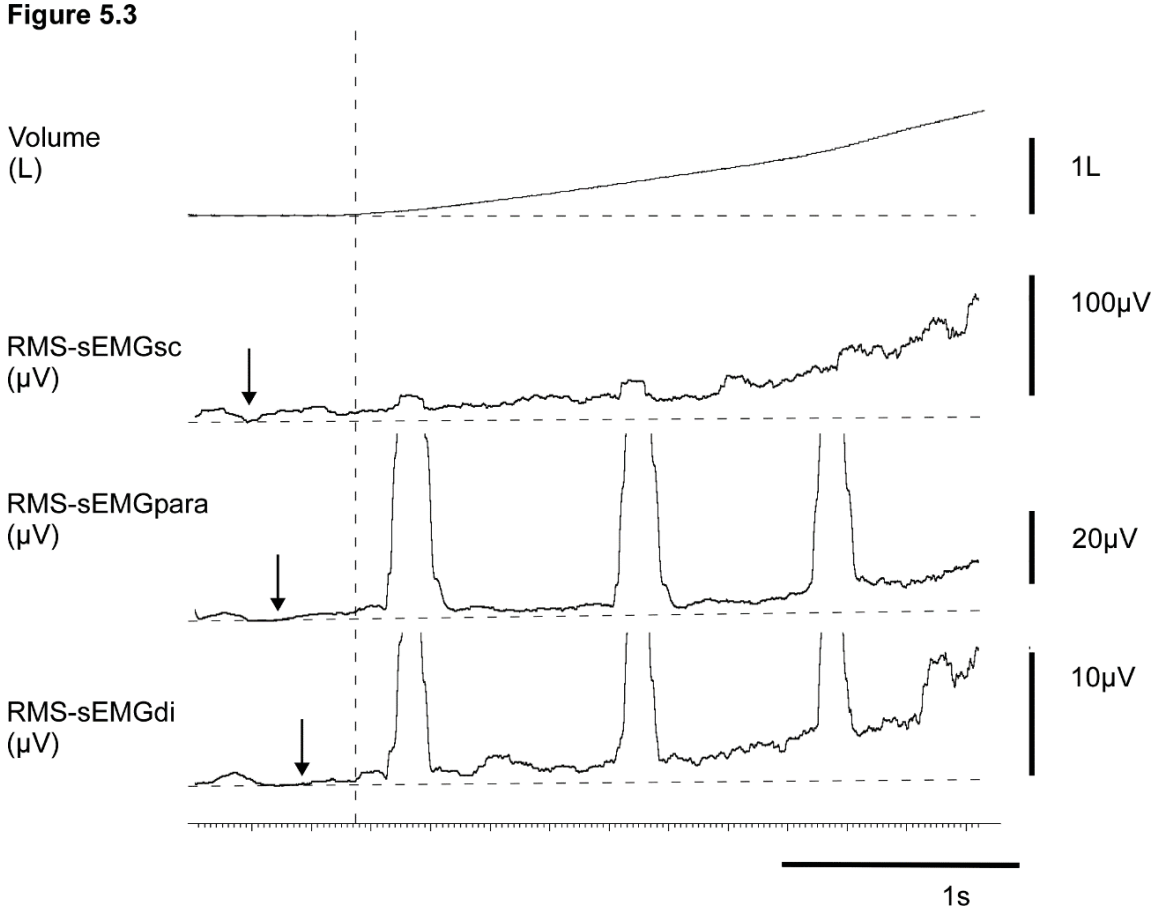
Participants were asked to perform a gradual 'sucking in' effort from functional residual capacity (FRC) to maximal inspiratory pressure (MIP) over 5 seconds. Mouth pressure (Pmo) expressed as a percentage of maximal inspiratory pressure (MIP) are expressed as timepoints on the x-axis. From top to bottom, panels show mouth pressure, raw and RMS- EMG from scalene (sEMGsc), parasternal intercostal (sEMGpara), and diaphragm (sEMGdi) muscles respectively. Cursors were placed at baseline (0%) and maximal mouth pressure (100%) of the ramp, respectively. Mouth pressure, scalene (RMS-sEMGsc), parasternal intercostal (RMS-sEMGpara), and diaphragm (RMS-sEMGdi) RMS EMG were then determined at 10%-time intervals to give a profile of muscle activation across the task. Negative pressure is represented as a downward inflection from baseline in the pressure trace, and the magnitude is presented as absolute numbers. In this particular example, at 20% of MIP ramp duration, sEMGpara and sEMGdi are contaminated by the ECG artefact hence the values for sEMGpara and sEMGdi at both these timepoints were substituted with sEMG value closest to the time interval without ECG artefact or omitted if no substitute value without ECG artefact is available.



The sEMG magnitude at each time point for each of the three muscles was manually selected. Baseline sEMG (near 0 μV at complete relaxation at the end of expiration) was subtracted from the sEMG value selected at each time point. If the sEMG signal fell within the QRS complex of the ECG, the sEMG value closest to the time interval without ECG artefact was used instead or omitted if no substitute value was available. For the time interval 90%MIP or 90% TLC, and 100% MIP or 100% TLC, sEMG value for the inspiratory muscle may not be measured for all three attempts at the task for each individual if the child was not able to produce the same reference peak MIP or TLC for all three attempts. Onset time (To) of inspiratory muscle sEMG was defined as the time at which individual respiratory muscle sEMG activity increased above tonic or baseline level at the end of expiration of the preceding breath based on visual inspection, relative to the onset of inspiratory volume signal (for TLC ramp) or mouth pressure signal (for MIP ramp) (i.e. 0% of ramp) (Figure 5.3). Therefore a negative value for the onset time denotes onset of sEMG activity prior to the onset of inspiratory volume or pressure.

Figure 5.3 Analysis of scalene, parasternal intercostal, and diaphragm sEMG onset time during dynamic ramp.

A representative recording from a participant during the dynamic TLC ramp is shown below. From top to bottom, the panels show lung volume, scalene, parasternal intercostal, and diaphragm RMS-sEMG. A vertical dashed line is placed at the onset of inspiration (i.e. the point at which the volume trace deflects away from baseline at the end of expiration). Horizontal dashed lines are placed at the baseline level at the end of expiration for each of the traces. The downward arrows are placed at the onset of scalene, parasternal intercostal, and diaphragm sEMG (based on visual determination of increase in RMS-sEMG from baseline level). In this particular example, the onset of all three muscles' RMS-sEMG commenced prior to the onset of inspiration.



To compare the three different muscles (scalene, parasternal, and diaphragm) across different attempts and different individuals, RMS-sEMG values at each time point were normalised to the maximal sEMG (sEMG%max) achieved across any of the four maximal inspiratory manoeuvres (TLC ramp, shMIP, MIP ramp, SNIP). The averaged value of sEMG%max for each of the three muscles (sEMGsc%max;

sEMGpara%max; sEMGdi%max) at each of the 11 time points over the three best attempts at the static ramps and the dynamic task were used for analysis.

Statistics

Normality of the data was assessed using normality plots of continuous variables and also by using the Shapiro-Wilk normality test. Data are expressed as mean (SD) if normally distributed, and median (IQR) for non-normal data. Comparison between the sEMG%max at three different time points (0%, 50%, and 100% of static or dynamic task duration) was made using a linear mixed model including age, gender, BMI z score, posture (sitting vs supine) and muscle groups (scalene vs parasternal vs diaphragm muscles) as fixed effects terms, and random effect for subject. Logarithmic transformation was performed to normalise the outcome variable sEMG%max (LnEMG%max). The onset of the respiratory muscle's sEMG relative to the onset of inspiration for each inspiratory manoeuvre (TLC or MIP ramp) performed in each posture (sitting or supine) was compared using repeated measure ANOVA for the 3 muscles, and paired t-tests for each muscle group comparison. Statistical analysis of the data was performed using SPSS version 25.0 (IBM Corp. Armonk, NY.). The criteria for statistical significance was taken as $P < 0.05$.

Sample size

Due to the exploratory and observational nature of the study, a priori sample size calculation was not undertaken. The final sample size of 24 children represented a convenience sample.

Results

Twenty-four healthy children, with a mean (SD) age of 11.15 (3.14) years, 50% males, were recruited. Thirteen children (54.17%) were adolescents, defined as 10-19 years of age (World Health Organisation., 2015). All included children were naïve to respiratory measurements with no prior experience of spirometry and respiratory muscle sEMG testing. The anthropometric characteristics and baseline spirometry results are summarised in table 5.1a (Table 5.1b for full descriptions of the baseline demographics of all participants). Two children (8.33%) were obese with a BMI z score > 1.64 (Cole, Faith, Pietrobelli, & Heo, 2005). All children had essentially normal lung function on spirometry, with a z score ≥ -1.64 (Quanjer et al., 2012). One participant had slightly lower FEV1/FVC z-score than lower limit of normal despite having a normal FEV1 z-score (participant no. 20, FEV1/FVC z score = -1.72, FEV1 z score = -0.04) (Table 1b).

Table 5.1a: Summary demographic and anthropometric data for all participants.

	N=24
Age, year	11.15 years (3.14) (Range 6.8-17.2)
Male (%)	12 (50%)
Height z score	0.50 (1.04)
BMI z score	0.10 (0.84)
FVC, z score	0.15 (0.68)
FEV1, z score	0.11 (0.75)
FEV1/FVC, z score	-0.08 (0.89)

Data are presented as mean (SD), unless otherwise stated. BMI: body mass index: FEV1: forced expiratory volume in one second; FVC: forced vital capacity;

Table 5.1b: Demographic and anthropometric data for individual participants.

Subject	Age (years)	Gender	BMI z score	Height z score	FVC Z score	FEV1 z score	FEV1/ FVC z score
1	7.2	M	-0.37	1.15	0.59	1.59	1.77
2	12.6	M	0.09	1.42	-0.56	-0.59	-0.19
3	9.8	F	-1.41	1.48	-0.56	-0.12	0.84
4	12.4	M	-0.32	2.62	0.01	-0.04	-0.19
5	8.0	M	0.84	1.12	1.26	0.17	-1.57
6	9.5	F	1.69	0.62	0.65	0.13	-1
7	9.6	F	1.83	0.31	-0.5	-0.78	-0.5
8	10.6	M	0.69	-0.19	-0.81	-0.83	-0.1
9	10.2	M	-0.99	0.57	0.16	0.6	0.67
10	9.1	F	-0.65	-0.25	1.02	1.15	0.02
11	15.4	F	-0.37	-1.27	-0.65	-0.05	1.62
12	16.6	F	-0.49	-0.46	0.41	0.07	-0.7
13	16.2	F	0.88	-1.41	1.04	0.84	-0.49
14	15.8	M	0.43	0.29	-0.25	-0.97	-1.19
15	9.8	F	-0.11	0.98	1.08	1.07	-0.19
16	12.2	M	0.24	-1.97	0.92	1	0.09
17	7.28	F	-0.4	1.62	1.19	1.12	-0.33
18	17.2	M	-0.54	1.05	-0.57	-0.06	0.91
19	12.2	F	-0.81	-0.07	0.05	-0.42	-0.84
20	10.6	M	0.39	0.59	-0.04	-1.14	-1.72
21	11.1	F	0.71	1	-0.27	-0.3	0.07
22	7.7	F	1.21	1.15	-0.77	-0.5	0.55
23	9.7	M	0.51	1.42	0.18	0.69	0.75
24	6.8	M	-0.71	1.48	0.06	-0.01	-0.23

For gender, M denotes male, F represents female. BMI, body mass index. FEV1: forced expiratory volume in one second; FVC: forced vital capacity.

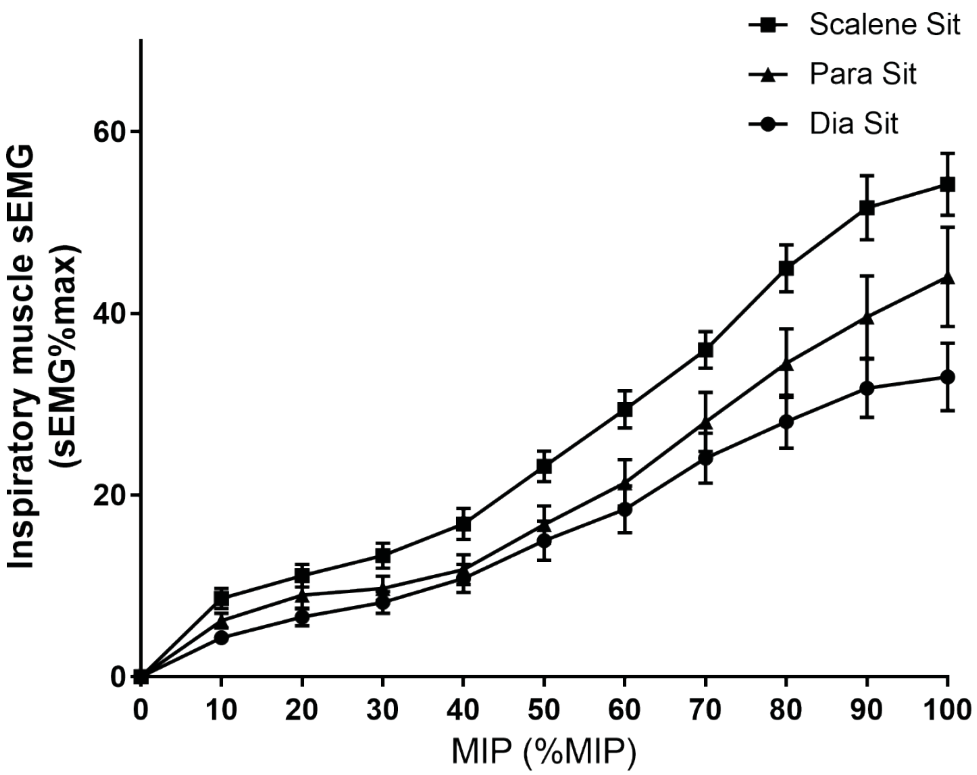
Static tasks – MIP ramp

The activation of scalene, parasternal, and diaphragm muscles during graded inspiration against occlusion from a participant is shown in Figure 5.2 as an example. As the mouth pressure became increasingly negative, there was a gradual increase in scalene, parasternal, and diaphragm sEMG. As demonstrated in Figure 5.4, the relationship between mouth pressure and sEMG activity from scalene, parasternal, and diaphragm muscles is almost linear. There was no significant group difference between the inspiratory muscles in the mean onset time of sEMG in both the sitting and supine

position when performing the MIP ramp ($p = 0.052$ for sitting, $p = 0.692$ for supine, Table 5.2).

Figure 5.4: Profile of activation of scalene, parasternal, and diaphragm muscle during a maximal inspiratory pressure ramp (static task) when sitting and supine. Mean +/- S.E.M data from the 24 participants during 5 s inspiratory mouth pressure ramps from FRC shown.

Mouth pressures (Pmo) expressed as a percentage of maximal inspiratory pressure (MIP) are expressed as timepoints on the x-axis. The top panel is the data recorded with the children in the sitting posture, and the bottom panel with the children in the supine posture. sEMG%max, the sEMG value normalised to a maximal value obtained from one of the maximal manoeuvres; Dia, diaphragm; Para, parasternal intercostal muscle.


Scalene Sit
Para Sit
Dia Sit
Inspiratory muscle sEMG
(sEMG%max)
0
20
40
60
0
10
20
30
40
50
60
70
80
90
100
MIP (%MIP)

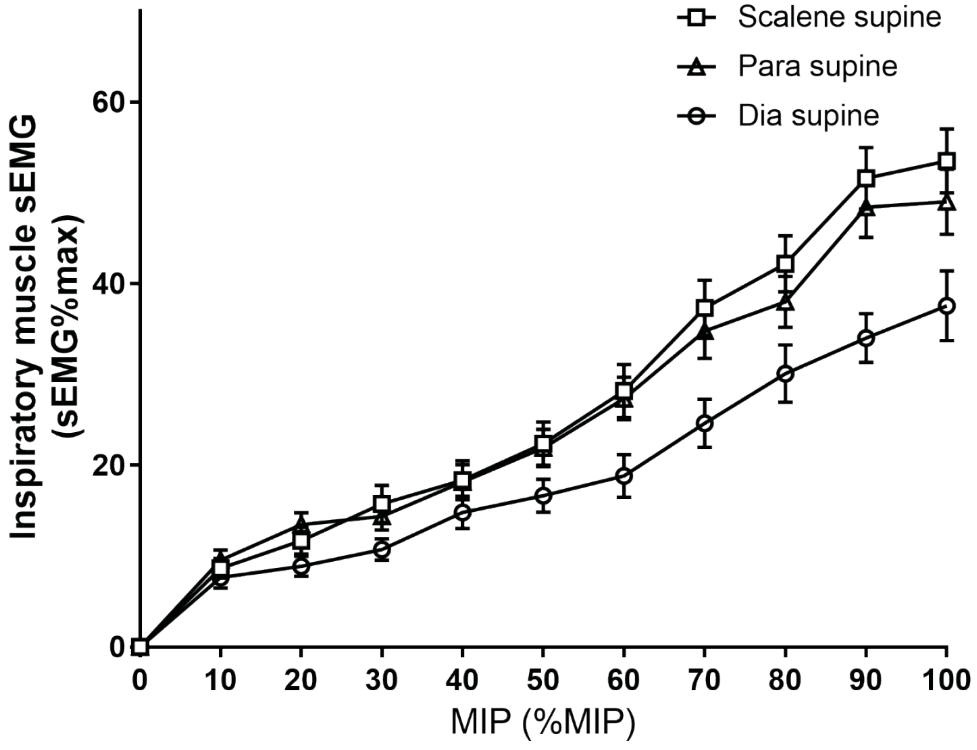

Scalene supine
Para supine
Dia supine
Inspiratory muscle sEMG
(sEMG%max)
0
20
40
60
0
10
20
30
40
50
60
70
80
90
100
MIP (%MIP)

Table 5.2 The onset time of sEMG activity in scalene, parasternal, and diaphragm muscles relative to onset of inspiration (based on the time of deflection from baseline in the lung volume or mouth pressure trace) during voluntary isovolumetric inspiratory ramps to maximal inspiratory pressure over 5 seconds (MIP ramp) and dynamic inspiration ramps to total lung capacity over 5 seconds (TLC ramp).

	sEMG onset time relative to onset of inspiration (s)			
Manoeuvre	Scalene	Parasternal	Diaphragm	*P* value
MIP ramp				
Sitting	-0.25 (0.42)	0.01 (0.44)	0.06 (0.64)	0.052
Supine	-0.18 (0.20)	-0.11 (0.36)	-0.16 (0.48)	0.692
TLC ramp				
Sitting	-0.19 (0.32)	0.17 (0.54)*	0.05 (0.36)*	0.003
Supine	-0.15 (0.28)[#]	0.04 (0.46)	-0.18 (0.41)[#]	0.037

Data are presented as mean (SD). Negative onset time represents onset of sEMG prior to onset of inspiration. sEMG, surface electromyography, MIP, maximal inspiratory pressure TLC, total lung capacity.

*When performing the TLC ramp manoeuvre sitting, there were significant differences between scalene and parasternal sEMG onset time (p=0.005); scalene and diaphragm sEMG onset time (p = 0.013). No significant difference between diaphragm and parasternal sEMG onset time (p =0.15)

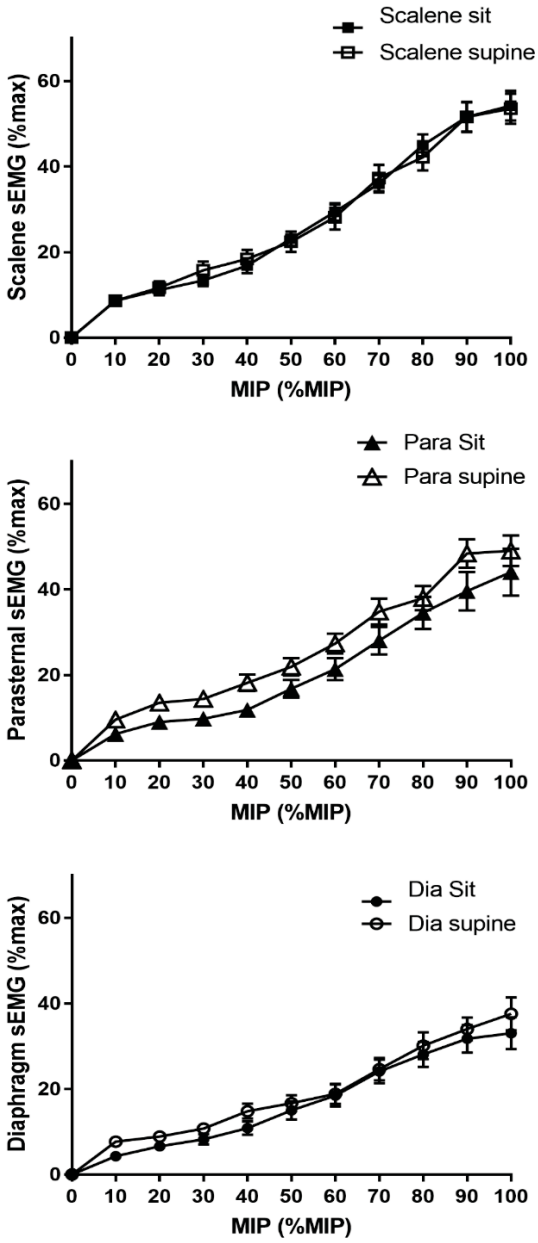
[#] When performing the TLC ramp manoeuvre supine, there were significant differences between parasternal and scalene sEMG onset time (p = 0.024), parasternal and diaphragm sEMG onset time (p=0.03). No significant difference between diaphragm and scalene sEMG onset time (p = 0.74).

In the linear mixed model, postures (p = 0.041), muscle groups (p< 0.0001), and timepoint (0%, 50%, 100% of MIP ramp duration) (p < 0.0001) were significant predictors of LnEMG%max during the MIP ramp. Age (p = 0.118), BMI (p = 0.871), and gender (p =0.822) were not independent predictors of LnEMG%max. sEMG%max increased significantly from 0%, 50%, then 100% of peak pressure (p< 0.0001) as graphically presented in Figure 5.4. sEMG%max recorded when supine was significantly higher than when sitting (p = 0.041) (Figure 5.5) At 100% MIP, mean difference (SD) in sEMG%max between supine and sitting is -2.20% (20.21) for scalene, 5.84% (29.59) for parasternal intercostal muscle, 4.14% (23.91) for diaphragm. Scalene (p =0.001) and parasternal (p <0.0001) sEMG%max were significantly greater

than diaphragm sEMG%max. There was no difference between scalene and parasternal sEMG%max (p =0.89).

Figure 5.5 Scalene, parasternal intercostal, and diaphragm sEMG during maximal inspiratory pressure ramps in the sitting and supine postures.

Surface EMG presented as a normalised value, sEMG%max. Data presented as mean +/- SEM. Mouth pressure (Pmo) expressed as a percentage of maximal inspiratory pressure (MIP) are expressed as timepoints on the x-axis. MIP, maximal inspiratory pressure.

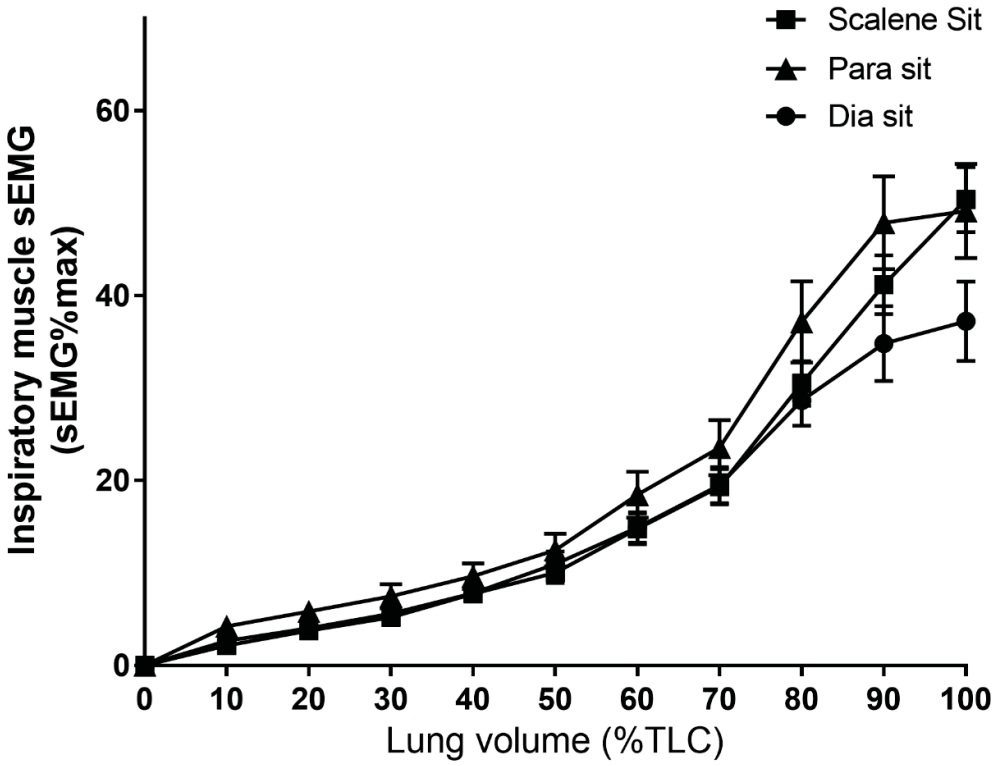


Dynamic task - TLC ramp

In the dynamic inspiratory ramp, scalene, parasternal and diaphragm activation had a similar stereotypical profile to the static tasks with a curvilinear relationship between changes in lung volume and respiratory muscle sEMG. (Figure 5.2, example from a participant; Figure 5.6 for the group result). As the lung volume increased, scalene, parasternal and diaphragm sEMG%max also increased.

Figure 5.6 Activity in scalene, parasternal, and diaphragm muscle during dynamic tasks (total lung capacity ramp).

Mean +/- S.E.M data from the 24 participants during 5 s inspiratory lung volume ramps from FRC to TLC. Lung volume expressed as a percentage of TLC represents timepoints on the x-axis.


Scalene Sit
Para sit
Dia sit
Inspiratory muscle sEMG (sEMG%max)
0
20
40
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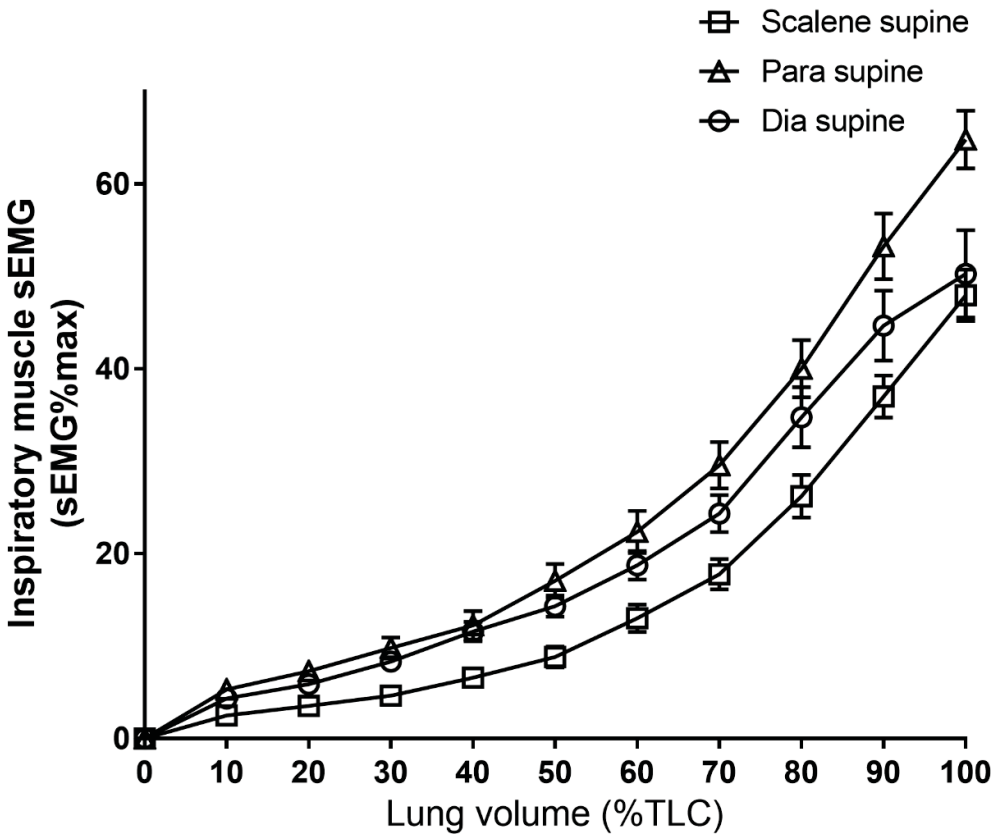
0
10
20
30
40
50
60
70
80
90
100
Lung volume (%TLC)

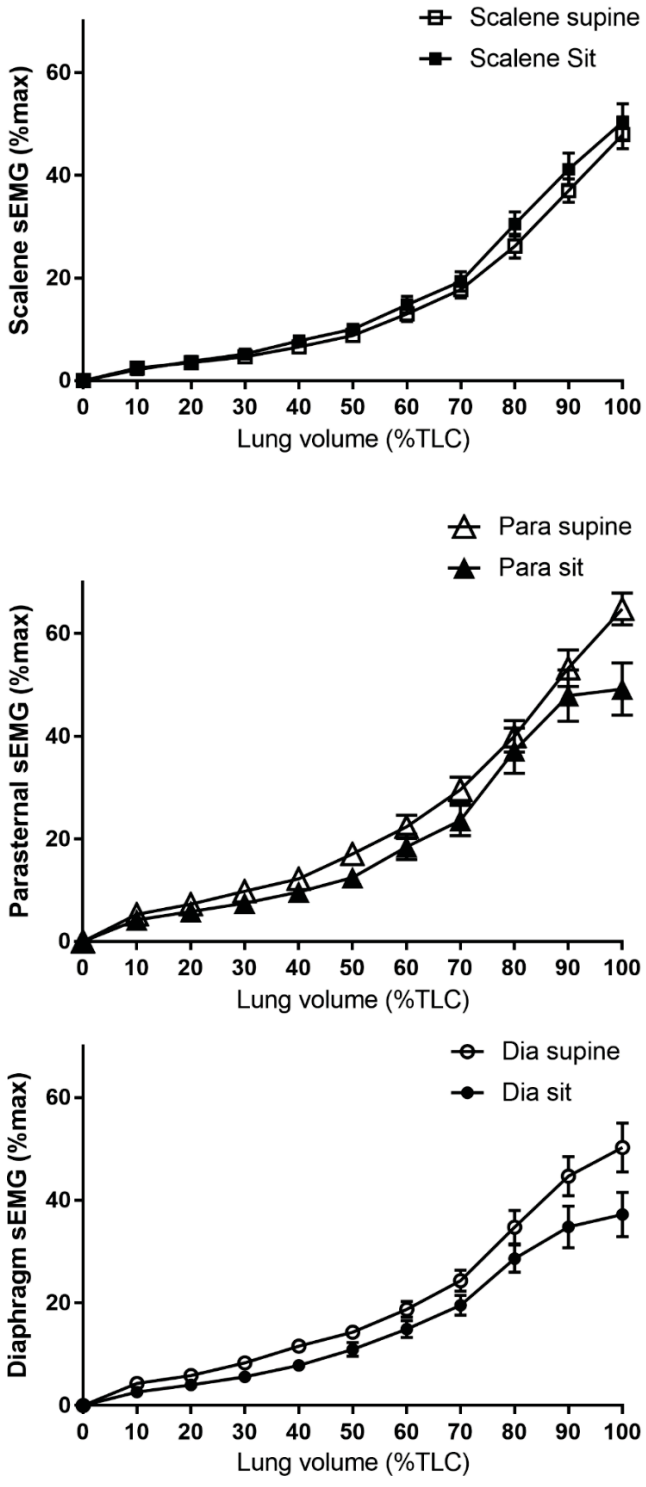

Scalene supine
Para supine
Dia supine
Inspiratory muscle sEMG (sEMG%max)
0
20
40
60
0
10
20
30
40
50
60
70
80
90
100
Lung volume (%TLC)

There was a significant difference between the onset time for inspiratory muscles when performing the TLC ramp manoeuvre when sitting and supine (Table 5.2). When performing the dynamic inspiration to TLC in a sitting position, scalene muscle onset (sitToEMGsc) commenced significantly earlier than parasternal (sitToEMGpara) and diaphragm (sitToEMGdi) muscles, prior to the onset of inspiratory airflow (sitToEMGsc vs sitToEMGpara $p = 0.005$; sitToEMGsc vs sitToEMGdi $p=0.013$). In the supine posture, both scalene (supToEMGsc) and diaphragm (supToEMGdi) were activated prior to the onset of inspiratory airflow when inspiring to TLC. The activity of scalene and diaphragm muscle commenced significantly earlier than parasternal muscle (supToEMGsc vs supToEMGpara $p =0.024$, supToEMGdi vs supToEMGpara $p = 0.031$). There was no significant difference between the sEMG onset time of scalene and diaphragm muscle ($p =0.74$).

In the linear mixed model, posture ($p = 0.002$), respiratory muscle group ($p <0.0001$), and timepoints (0%, 50%, and 100% of TLC) ($p< 0.0001$) were significant predictors of LnEMG%max recorded during TLC ramps. Age ($p = 0.355$), gender ($p =0.482$) and BMI ($p = 0.592$) were not significant predictors of LnEMG%max. sEMG% max increased significantly from 0%, 50%, then 100% of peak volume ($p < 0.0001$) as graphically presented in Figure 5.6. sEMG%max recorded when supine was significantly higher than those obtained when sitting ($p = 0.002$) (Figure 5.7) At 100% TLC, mean difference (SD) in sEMG%max recorded sitting and supine is -4.06% (17.65) for scalene, 2.94% (21.66) for parasternal intercostal muscle, 13.04% (19.33) for diaphragm. Parasternal sEMG%max was significantly greater than diaphragm sEMG%max ($p = 0.001$) and scalene sEMG%max ($p < 0.001$). There was no significant difference between scalene and diaphragm sEMG%max ($p = 0.185$).

Figure 5.7 Scalene, parasternal intercostal, and diaphragm sEMG during dynamic (total lung capacity) ramps in the sitting and supine posture.

Surface EMG presented as normalised value, sEMG%max. Data presented as mean +/- SEM. TLC, total lung capacity. Lung volume expressed as a percentage of TLC represents timepoints on the x-axis.



Discussion

In this chapter, I have demonstrated that similar to adults, where more invasive techniques were used to record inspiratory muscles EMG (Beck et al., 1998b; Ng & Stokes, 1992; Raper et al., 1966; Yokoba et al., 2003), a curvilinear relationship exists in children between obligatory inspiratory muscles' sEMG (including diaphragm, parasternal intercostal and scalene muscle) and increasing lung volume. The relationship between inspiratory muscle sEMG and mouth pressure during a static maximal inspiratory manoeuvre is linear. In this study, recruitment patterns of the scalene, parasternal, and diaphragm muscles varied in timing and amplitude with different volitional maximal inspiratory manoeuvres. When healthy children performed the dynamic TLC ramp, parasternal intercostal muscle sEMG%max was significantly higher than diaphragm and scalene sEMG%max, although the scalene was recruited earliest prior to the onset of airflow. In comparison, the scalene muscle is the main inspiratory muscle activated when performing the static MIP ramp task with no significant difference between the onset time of the three inspiratory muscle studied. Muscle recruitment pattern during maximal inspiratory manoeuvres also varied depending on the posture with significantly higher sEMG%max when supine compared to sitting in children. Not only inspiratory muscles sEMG can reflect respiratory output as measured by lung volume and pressure, but also inspiratory muscle sEMG can be used to explore respiratory muscle activation profiles and further our understanding of respiratory mechanics in children.

Routine clinical measures of respiratory function have focused on the measurement of lung volume and pressure changes from the mouth which reflects the combined actions of synergistic respiratory muscles. The use of pressure or ventilatory

parameters to estimate neural respiratory drive can be influenced by muscle length changes associated with lung volume, chest wall configurations, and the elastance and resistance of the respiratory system (Beck, Sinderby, Lindstrom, & Grassino, 1998a; Beck et al., 1998b). In contrast, respiratory muscle EMG activity mirrors the level of neural respiratory drive delivered to individual muscle to elicit muscle contraction, reflecting both motor unit recruitment and discharge frequency of specific muscles. The findings on this chapter of a linear / curvilinear relationship between inspiratory muscles sEMG and mouth pressure or lung volume changes in healthy children (> 6 years of age) suggest neural respiratory drive is directly related to respiratory output in children, similar to adults (Hudson, Gandevia, & Butler, 2007; Ng & Stokes, 1992; Raper et al., 1966; Yokoba et al., 2003). Replicating the same relationship between inspiratory muscle EMG and respiratory output using the non-invasive and well-tolerated surface electrodes instead of the more invasive intramuscular electrodes or catheter-mounted electrodes used in adult studies further highlights the potential utility of surface electrodes to assess neural respiratory drive especially for children. (Beck et al., 1998b; Hudson et al., 2007; Raper et al., 1966; Yokoba et al., 2003).

In this cohort of children, there was a difference in muscle activation profile between the dynamic TLC ramp and the static MIP ramp. Electrical activity of the parasternal intercostal muscle (sEMGpara%max) was significantly greater than that of the scalene and diaphragm when performing the dynamic TLC ramp task. On the other hand, scalene sEMG%max was significantly higher than parasternal and diaphragm sEMG%max when performing the static MIP ramp task. Differences in muscle recruitment with different voluntary breathing tasks has been shown in adults and that variation in recruitment of inspiratory muscles potentially may be due to individual muscle's relative mechanical advantage for the specific breathing tasks (De Troyer et

al., 1996; Hudson et al., 2007; Hudson, Gandevia, & Butler, 2011). Two small adult studies (n=9 and n=10) reported higher EMGdi compared to EMGpara when healthy adults performed the standard MIP (shMIP) manoeuvre although no direct statistical comparison was made between the magnitude of the two muscles' EMG by the authors (Nava et al., 1993; Segizbaeva et al., 2013). It is known that the maximum force generated by a muscle is a function of its contraction velocity, hence the difference between the speed of contraction between the short MIP and the gradual MIP ramp may have contributed to differences in the muscles' EMG and activation profile. Another potential cause of the difference in muscle activation profile between adults and children is due to the breathing strategies adopted by the children (who were all naïve to lung function testing) and potential developmental differences although age was not a significant predictor of sEMG%max in this study. The slightly higher activation of scalene when the children performed the MIP ramp task may be due to the strain placed on the neck when breathing against an occlusion using a sucking action. Comparatively, when the children breathed in fully to TLC they relied on the expansion of the ribcage and hence the higher drive to parasternal intercostal muscle rather than scalene. Also, parasternal intercostal muscle's optimal length for force generation is preserved even at higher lung volumes near TLC whereas the optimal length for diaphragm is closer to FRC (Farkas & Rochester, 1986; Jiang, Deschepper, Demedts, & Decramer, 1989). Hence the parasternal muscle may be preferentially activated with dynamic inspirations. Future studies with bigger cohorts of children across different age groups and also in children performing different breathing tasks is needed to confirm the muscle recruitment profiles demonstrated in this chapter.

Contrary to my hypothesis, the diaphragm did not have the highest sEMG activity when performing either the TLC or MIP ramp. Although the parasternal and the scalene

muscles in this study had higher activity during the dynamic and static inspiratory ramps respectively, these muscles are not likely to make a large contribution to affect airway pressure or lung volume changes. Contraction of human scalene muscles bilaterally is reported to generate a change in airway opening pressure (ΔPao) of -3cmH_2O over the whole inspiratory capacity range (Legrand, Schneider, Gevenois, & De Troyer, 2003). A similar ΔPao has been estimated for the parasternal intercostals in all interspaces over the inspiratory capacity range (De Troyer et al., 1998). The diaphragm has the greatest potential to change inspiratory pressure and therefore lung volume with a ΔPao of -34.8+/- 2.8 cmH_2O at FRC (De Troyer & Boriek, 2011). Although in my study the magnitude of diaphragm activity during maximal inspiratory manoeuvres was less than those of parasternal and scalene, more detailed studies are required to assess how muscle activities translate to contribution of lung volume and pressure changes in children. Concurrent recordings of inspiratory muscle sEMG and calibrated respiratory inductance plethysmography (RIP) or optoelectronic plethysmography (OEP) in children when performing different breathing tasks may help answer this question by assessing the changes in the volume of chest wall compartments simultaneously with changes in inspiratory muscle sEMG (Laveneziana et al., 2019).

I have also demonstrated in this chapter a postural difference in muscle recruitment pattern when children performed maximal inspiratory manoeuvres, contrary to my hypothesis. The level of inspiratory muscle EMG activity was significantly greater when performing both the dynamic TLC ramp and the static MIP ramp supine compared to sitting. However the magnitude of the differences in sEMG between the two postures is small (as graphically presented in Figure 5.5 and 5.7), with the biggest mean difference in sEMG%max between the postures being 5.84% for parasternal during the MIP ramp, at 13.04% for diaphragm during the TLC ramp. My hypothesis

was made based on adult studies recording respiratory muscle EMG using intramuscular electrodes and surface electrodes (Butler et al., 2001; Segizbaeva et al., 2013). In Segizbaeva et al's study, both sEMGdi and sEMGpara recorded during standard shMIP manoeuvre was not significantly different in sitting and supine positions when compared to standing (Segizbaeva et al., 2013). Direct comparison between sEMG in sitting and supine position was not made by the author. In Butler et al's study using intramuscular electrodes, there was no difference in the single motor unit discharge frequency during tidal breathing when supine or standing for parasternal intercostal muscles in all subjects, and the diaphragm muscle for most subjects (Butler et al., 2001). The difference we found between supine and sitting posture in children's inspiratory muscle activity may be due to the different methods used for recording EMG (intramuscular electrodes measuring single motor unit discharge and frequency compared to surface electrodes measuring amplitude of interference pattern sEMG from asynchronously firing motor units), and the type of breathing tasks (tidal breathing or shMIP compared to maximal volitional ramps). When supine, abdominal contents push up on the diaphragm and displace the diaphragm caudally. Despite the diaphragm being at a more favourable position in the length-tension curve for force generation when supine, the mechanical load of the abdominal contents on the rib cage may have contributed to the higher inspiratory muscle sEMG particularly in the parasternal intercostal muscle when inspiring maximally compared to sitting. The difference associated with posture confirms the importance of consistent positioning when assessing children with lung pathologies.

The onset time of the three inspiratory muscles studied differed between the static and dynamic manoeuvres. The scalene muscle was persistently recruited earlier when performing the dynamic and static ramps, whether sitting or lying supine. When

performing the dynamic TLC ramp in the supine posture, diaphragm sEMG onset time was also earlier than that of parasternal intercostal muscle, with no significant difference between the onset time for diaphragm and scalene. Butler et al's adult study on diaphragm demonstrated that during voluntary inspirations diaphragm motor units were recruited throughout inspiration in a stable order with the majority of the motor units recruited in the first half of the breaths (Butler, McKenzie, & Gandevia, 1999). For the neck inspiratory muscles, scalene consistently activated earlier than sternocleidomastoid muscle in adults performing both dynamic and static maximal inspiratory tasks (Hudson et al., 2007; Raper et al., 1966; Washino et al., 2017; Yokoba et al., 2003). To my knowledge, there are no other studies that compared the inspiratory muscle onset time between the neck, rib cage, and the diaphragm muscle during voluntary maximal inspirations in children and adults. The different inspiratory muscle activation pattern I observed during maximal inspiratory ramps in children with earlier activation of scalene may be related to the differential recruitment of inspiratory muscle depending on the breathing task. Further studies investigating the interactions between different respiratory muscles during tidal spontaneous breathing and volitional inspiratory tasks in children are needed to improve our understanding.

The main strength of this study was the use of the surface electrodes which enabled measurement of sEMG activity from multiple inspiratory muscles simultaneously in children to explore the relationship between inspiratory muscle sEMG and lung volume and pressure changes. Previous studies in adults have focused on the recruitment of neck inspiratory muscles (scalene vs sternomastoid) only (Hudson et al., 2007; Raper et al., 1966; Washino et al., 2017; Yokoba et al., 2003)), or compared the EMG of diaphragm with other inspiratory muscles while performing short MIP (Nava et al., 1993; Segizbaeva et al., 2013) which does not allow characterisation of

muscle activation profiles. Although the use of intramuscular and catheter-mounted electrodes in previous studies would be less susceptible to cross-talk, and less influenced by anatomical differences between individuals, the discomfort and invasiveness of both techniques limit their use in children (American Thoracic Society/European Respiratory Society, 2002). The small sample sizes ranging from n=4 (Butler et al., 1999) to n=20 adults (Ng & Stokes, 1992) in previous studies may have also related to the more invasive techniques used to record respiratory muscle EMG.

As discussed in Chapter 1 Introduction and Literature Review, there are advantages and disadvantages associated with each of the three methods for recording respiratory muscle EMG. Our study had several limitations relating to the use of surface electrodes to evaluate respiratory muscle EMG. There are no consensus guidelines on the optimal location for electrode placement when studying respiratory muscles which can affect the comparison of results between studies (American Thoracic Society/European Respiratory Society, 2002; Cabral et al., 2018; Dos Reis et al., 2019). Surface EMG recording is susceptible to cross talk of signals from ECG and also from other muscles in proximity of the muscle of interest, such as those from abdominal muscles when recording costal diaphragm sEMG at the lower chest wall (American Thoracic Society/European Respiratory Society, 2002). Furthermore, variations between individuals in body habitus such as the amount of subcutaneous fat tissue can produce variable muscle-to-electrode filtering effects especially when sEMG is reported as absolute RMS values (American Thoracic Society/European Respiratory Society, 2002). I have attempted to account for the individual differences in anatomical variations by presenting the respiratory sEMG normalised to a maximal value derived from maximal inspiratory manoeuvres. Both age and BMI were not independent predictors of sEMG%max in the mixed model analysis suggest individual anatomical

differences are minimised. The electrode placement locations used in this study was based on previous studies comparing intramuscular and surface EMG recordings (Hudson et al., 2007; Saboisky et al., 2007; Verin et al., 2002). More studies in children using the same methodology are needed to further validate my results.

Although this study cohort (n=24) is of reasonable size, a bigger sample size with equal distribution from different age groups will be required to further assess potential developmental differences in sEMG and respiratory muscle activation profile between children and adults. My main aim was to investigate the relationship between lung volume and pressure with inspiratory muscle EMG hence I chose to include children older than the age of 6 to ensure they were able to perform routine lung function manoeuvres. To assess maximal inspiratory efforts in infants, one potential is to measure mouth pressure generated during airway occlusion at the end of a crying effort (Shardonofsky, Perez-Chada, & Milic-Emili, 1991). Another area requiring further investigation is the variability in the maximal reference EMG value obtained from the four maximal inhalation manoeuvres. In this study we chose to perform 3 maximal inhalation manoeuvres: inspiring to TLC, maximal sniff inhalation, and MIP (as both the standard short burst of inspiratory effort against occlusion and as a graded ramp). It is known that there is a learning effect associated with lung function manoeuvres, especially with the MIP manoeuvre which has a higher degree of variability than other respiratory manoeuvres (American Thoracic Society/European Respiratory Society, 2002; Quanjer et al., 2012). In practice, it can be difficult to ask children to complete multiple maximal inhalation manoeuvres due to potential fatigue and limited patience. It would be important to investigate whether performing multiple inhalation manoeuvres is necessary and which manoeuvre is more likely to produce the maximal respiratory muscle EMG value.

In summary, in this chapter I have explored the relationship between the inspiratory muscles of the neck (scalene), rib cage (parasternal intercostal), and the diaphragm, and respiratory output by recording sEMG when healthy children performed maximal inspiratory manoeuvres. I have demonstrated that similar to adults, there is a curvilinear relationship between lung volume and inspiratory muscle sEMG and a linear relationship between mouth pressure and inspiratory muscle sEMG. Activation profiles of the scalene, parasternal, and diaphragm muscles varied in time of onset and amplitude depending on the maximal inspiratory manoeuvres and posture adopted. The size and clinical importance of the differences observed between the inspiratory muscles' recruitment profiles need to be confirmed with a bigger cohort of children from different age groups. Although I presented the inspiratory muscle sEMG data as a normalised value (sEMG%max), the accuracy of the data may vary depending on the reliability of the reference peak sEMG value. Hence it is important to explore the variability in the peak sEMG obtained when the children performed the different maximal inspiratory manoeuvre. Having demonstrated a difference in the level of NRD in different inspiratory muscles when children performed maximal volitional tasks, it would be important to explore whether differences in NRD also exists between inspiratory muscles during spontaneous tidal breathing in healthy children.

Key messages

- Inspiratory muscles (scalene, parasternal, and diaphragm) have a curvilinear relationship with increasing lung volume, and a linear relationship with increasing mouth pressure in healthy children.
- Activation profile of the three inspiratory muscles studied varied in onset time and amplitude depending on the maximal inspiratory manoeuvres and posture adopted.

Chapter 6. Inspiratory muscle sEMG during tidal breathing in healthy children and factors affecting sEMG

Introduction

In Chapter 5, I used surface EMG recorded when children performed maximal volitional inspiratory ramps to explore the relationship between inspiratory muscle sEMG and respiratory output of volume and pressure. Previous studies in both children and adults have highlighted the potential use of sEMG recorded from inspiratory muscles during tidal breathing as a non-volitional method of assessing respiratory function (Maarsingh, Oud, van Eykern, Hoekstra, & van Aalderen, 2006; Maarsingh, van Eykern, Sprikkelman, Hoekstra, & van Aalderen, 2000; MacBean, Hughes, Nicol, Reilly, & Rafferty, 2016; MacBean, Jolley, et al., 2016; Steier, Jolley, Polkey, & Moxham, 2011). Compared to performing maximal inspiratory manoeuvres which requires cooperation and motivation from the children, recording sEMG during tidal breathing does not require specific collaboration from the child and potentially can be performed in a wider age range of children including babies, infants and pre-schoolers (Kraaijenga, de Waal, Hutten, de Jongh, & van Kaam, 2017; Kraaijenga, Hutten, de Jongh, & van Kaam, 2015; Maarsingh et al., 2006; Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). The respiratory muscle activation profile during tidal breathing is likely different when performing volitional inspiratory manoeuvres where lung volume and flow rates can differ (Butler, McKenzie, & Gandevia, 1999). Having explored the NRD in different inspiratory muscles when healthy children performed maximal inspiratory manoeuvres in Chapter 5, this chapter explores the NRD of the same inspiratory muscles (scalene, parasternal, and diaphragm muscles) in healthy children

during tidal breathing and investigates whether NRD varies in different inspiratory muscles during tidal spontaneous breathing.

Surface recordings of inspiratory muscle EMG can be influenced by anthropometric factors. In Chapters 3 and 4, sEMGdi recorded during tidal breathing in sleeping children had an inverse relationship with age and BMI. Other studies in children and adults have also suggested that inspiratory muscle EMG can be affected by age, height, and weight, potentially due to the anatomical variations between individuals causing a filtering effect on EMG (American Thoracic Society/European Respiratory Society, 2002; Beck, Sinderby, Weinberg, & Grassino, 1995; Jolley et al., 2009; MacBean, Jolley, et al., 2016; Steier et al., 2009). Tidal inspiratory muscle sEMG during spontaneous breathing can also be affected by postures (Druz & Sharp, 1981; Hudson et al., 2016; Steier et al., 2009; Williams, Porter, Westbrook, Rafferty, & MacBean, 2019). To further our understanding of using sEMG to estimate inspiratory muscle neural drive in children, it is important to develop normal reference values of inspiratory muscle sEMG and explore potential factors which can affect interpretation of sEMG during tidal breathing including age, BMI, and different postures in healthy children.

In recent research, inspiratory muscle sEMG magnitude has been presented as a value normalised to a reference value to allow comparison between individuals (Jolley et al., 2009; MacBean, Hughes, et al., 2016; Reilly et al., 2011; Sinderby, Beck, Spahija, Weinberg, & Grassino, 1998; Steier et al., 2011; Steier et al., 2009; Williams et al., 2019). The most reproducible and consistent reference value is an involuntary maximal EMG signal to represent maximal motor unit recruitment and firing rate, such as diaphragm compound muscle action potential elicited by phrenic nerve stimulation (Kassim, Jolley, Moxham, Greenough, & Rafferty, 2011). However this is difficult to

perform, uncomfortable for the participant and likely not tolerated by children. Previous research in children has used tidal breathing sEMG at baseline as the reference value for normalisation of EMG which is not ideal as some muscles are not activated during tidal breathing (Maarsingh et al., 2006; Maarsingh et al., 2000; Maarsingh, van Eykern, Sprikkelman, & van Aalderen, 2004). The alternative would be to normalise to a maximal voluntary EMG signal obtained during a maximal inspiratory manoeuvre as I have done in Chapter 5 (Laveneziana et al., 2019; MacBean, Jolley, et al., 2016; Reilly et al., 2011; Sinderby et al., 1998; Steier et al., 2009). In adults peak EMG is achieved through different maximal volitional manoeuvres in different individuals (Jolley et al., 2009; Reilly et al., 2011; Sinderby et al., 1998; Steier et al., 2009). In children it is not known which maximal volitional manoeuvre is more likely to produce a peak inspiratory muscle sEMG which is important to explore if sEMG is to be interpreted as a normalised value.

To explore the potential of using sEMG measured during tidal breathing as a clinical tool in assessing respiratory function in children, I needed to address the following aims (Aims 5a and 5b): 1) to determine the reference values of inspiratory muscles' (i.e. scalene, parasternal, and diaphragm muscles) sEMG during tidal breathing in awake healthy children both as sEMG and sEMG%max; 2) evaluate factors which may affect inspiratory muscle sEMG including age, BMI, and posture; and 3) to explore which maximal inspiratory manoeuvre is more likely to provide the peak sEMG value for children and assess the degree of variability in peak sEMG between attempts of maximal inspiratory manoeuvres. Based on the data from adult studies, I hypothesised that inspiratory muscle sEMG during tidal breathing is largely unchanged when supine (Druz & Sharp, 1981; Hudson et al., 2016; Steier et al., 2009; Williams et al., 2019). I also hypothesised that inspiratory muscle sEMG in healthy children has a

negative relationship with age and BMI similar to the findings in sEMGdi of snoring children in Chapter 3. Finally, I hypothesised that in children, sniff inhalation and/or inspiration to TLC are the two maximal inspiratory manoeuvres that children are more likely to achieve peak sEMG values from as these two manoeuvres are more similar to natural breathing.

Method

The study protocol was approved by the Sydney Children's Hospitals Network's Human Research Ethics Committee (LNR/14/SCHN/424). Written informed consent was obtained from the parents or carer of all enrolled children prior to commencing the study.

The methodology including the experimental protocol has already been described in detail in Chapter 5. Briefly, healthy children > 6 years of age were recruited from friends and family of hospital staff and the local community. All enrolled children's height, weight, and BMI were measured and recorded on the day of testing. Standard spirometry with forced expiratory volume manoeuvres was performed in all children and the results were expressed in reference to published values as z scores (Quanjer et al., 2012).

Experimental set-up

Respiratory muscle activity from the right scalene (sEMGsc), right parasternal intercostal (sEMGpara), and the right costal diaphragm muscles (sEMGdi) were recorded simultaneously with breathing variables. The sEMG signals were amplified

and bandpass filtered between 10 Hz and 1000 Hz with a sampling rate of 2000 Hz (Cambridge Electronic Design (CED) data acquisition and analysis interface, Cambridge, UK). Respiratory flow and airway pressure were measured simultaneously from the children by breathing through a mouthpiece connected in series to a calibrated pneumotachograph (4,500 series, Hans Rudolph, Kansas City, MO). The children wore a nose clip for all measurements except for sniff nasal inhalation. The flow and pressure signals were sampled at 100 Hz (CED data acquisition and analysis interface, Cambridge, UK).

Study protocol

The study protocol for this chapter is an extension of the protocol described in Chapter 5 with the addition of a period of relaxed tidal breathing in both sitting and supine postures. In brief, children were asked to sit in a chair with handrests in an upright posture. The children were asked to sit quietly looking forward with their head in a neutral position and both arms on the chair's arm rests or in their lap (whichever was more natural for the child) while breathing through the mouthpiece for at least 5 minutes to ensure stable consistent inspiratory muscle sEMG had been recorded. Children were then asked to perform 4 different maximal volitional manoeuvres as followed: 1) TLC ramp, 2) standard short MIP (shMIP), 3) MIP ramp, 4) maximal sniff inhalation (SNIP). Each manoeuvre was repeated at least 3 times until at least two reproducible maximum efforts had been obtained with less than 20% variance in the maximum pressure recorded or less than 5% variance in the maximum volume recorded (American Thoracic Society/European Respiratory Society, 2002). The testing

procedure was then repeated in the supine posture with the child lying on a firm mattress with hands by the side of their body.

Data analysis

All signals were acquired and stored on a computer via a Cambridge Electronic Design 1401 interface (Cambridge, UK) for analysis. sEMG signals were converted to root mean square values (RMS) using a time constant of 50 ms and a moving window. The recordings were annotated to ensure any periods incorporating coughing or movement artefact were excluded from analysis. Analysis was performed with custom-written software that measured the peak and trough of all signals during the inspiratory phase of the respiratory cycle (based on lung volume traces) in a manual breath-by-breath fashion based on visual inspection. The maximum RMS-sEMG for each of the three muscles (scalene, parasternal intercostal, and diaphragm muscles) were manually determined by selecting the RMS-sEMG signal falling between QRS complexes of the ECG. The difference between the maximum and minimum RMS-sEMG signals were used to determine the absolute amplitude of the RMS-sEMG signal per breath. The mean amplitude of RMS-sEMG per breath over 10 stable consecutive breaths (based on stable tracings on the respiratory flow and lung volume channel) was then calculated for each muscle. Volume, pressure, and sEMG data recorded from the best 3 attempts (i.e. with the highest volume or pressure) of the maximal inspiratory manoeuvres were included for analysis. RMS-sEMG signals recorded from the three muscles during the best 3 attempts of each of the maximal inspiratory manoeuvres were also measured and averaged (sEMGsc,peak, sEMGpara,peak, sEMGdi,peak). Irrespective of the maximal inspiratory manoeuvre, the numerically largest sEMG,peak for each individual muscle

was used as the reference value for normalisation of sEMG (sEMGsc%max, sEMGpara%max, sEMGdi%max).

Statistical analysis

All data normally distributed were expressed as mean and SD. Non-normal data were expressed as median and interquartile range (IQR). Paired data were compared using paired t-tests for normal data, and Wilcoxon matched-pairs signed rank test for non-normal data. Comparisons between 3 or more variables recorded from the same child were conducted using the Friedman test for non-normal data, and repeated measure one-way ANOVA for normal data. Coefficient of variation (CV) was calculated to assess within-individual variability between the peak sEMG from the 3 highest attempts for each of the maximal inspiratory manoeuvres. Repeated measure one way ANOVA was used to compare the CV for peak EMG of the four maximal inspiratory manoeuvres. Within-subject CV was also calculated over 10 breaths for tidal sEMGsc, sEMGpara, and sEMGdi recorded during sitting and supine postures for each participant. Logarithmic transformation of sEMG%max (LnEMG%max) was performed to normalise the data. Relationship between LnEMG%max and anthropometric variables (BMI and age) were investigated by calculating the Spearman' correlation coefficient and performing linear regression analysis. The strength of the correlations was categorized as very strong (0.90-1.00), strong (0.70-0.89), moderate (0.4-0.69), weak (0.1-0.39), or insubstantial (< 0.1)(Hinkle, Wiersma, & Jurs, 2003). Linear mixed model analysis with a diagonal covariance structure and separate random effects intercept for each child were used for assessing the effects of age, BMI z score, gender, posture (sitting vs supine), muscle groups (scalene vs parasternal intercostal vs diaphragm) on LnEMG%max. Statistical analysis of the data was performed using

SPSS Version 25 (SPSS, Chicago, Illinois, USA). A p value < 0.05 was considered statistically significant.

Sample size calculation

Due to the exploratory nature of the study, no a priori sample size calculation was undertaken. I used a convenience sample of 24 children.

Results

As reported in Chapter 5, 24 healthy children with a mean (SD) age of 11.15 (3.14) years were recruited with equal distribution from each gender. All included children were naïve to lung function testing. Two children were obese with a BMI z score > 1.64 (Cole, Faith, Pietrobelli, & Heo, 2005). All children had essentially normal lung function on spirometry, with a z score ≥ -1.64 (Quanjer et al., 2012). The anthropometric characteristics and baseline pulmonary function variables are summarised in table 6.1. For detailed description of the baseline demographic data for each participant please refer to table 5.1b in Chapter 5.

Table 6.1: Demographic and anthropometric data for all healthy children

	N=24
Age, year	11.15 (3.14)(Range 6.8-17.2)
Male (%)	12 (50%)
Height z score	0.50 (1.04)
BMI z score	0.10 (0.84)
FVC, z score	0.15 (0.68)
FEV1, z score	0.11 (0.75)
FEV1/FVC, z score	-0.08 (0.89)

Data are presented as mean (SD), unless otherwise stated. BMI: body mass index: FEV1: forced expiratory volume in one second; FVC: forced vital capacity.

Manoeuvres for determining peak inspiratory muscle sEMG in healthy children

When sitting, the sniff inhalation manoeuvre for all three muscle groups (37.5% of cohort for parasternal, and 45.8% of cohort for scalene and diaphragm muscles) yielded the peak sEMG value most frequently (Table 6.2a). The highest sEMGsc,peak (median(IQR) 310.9 μV (291.8-427.9)), sEMGpara,peak (53.47 μV (33.36-79.28)), and sEMGdi,peak (48.77 μV (34.88-62.86)) were also achieved during the SNIP manoeuvre when sitting (Table 6.2b). When supine, the peak RMS-sEMG was most frequently obtained from the MIP ramp for scalene (37.5%), TLC ramp for parasternal (45.8%), and the SNIP manoeuvre for diaphragm (33.3%) (Table 6.2a). However, the highest sEMGdi,peak when supine was achieved through the TLC instead of the SNIP manoeuvre although the difference between the sEMGdi,peak recorded from the two manoeuvres was small and not statistically significant (median difference 3.74μV, p =0.855) (Table 6.2b).

Table 6.2a: Number and percentage of children who produced peak RMS-EMG values for scalene, parasternal and diaphragm muscles in each of the four manoeuvres performed while sitting and supine

Sitting	**TLC ramp**	**Short MIP**	**MIP ramp**	**SNIP**
Scalene	4 (16.7%)	3 (12.5%)	6 (25%)	**11 (45.8%)**
Parasternal	7 (29.2%)	1 (4.1%)	7 (29.2%)	**9 (37.5%)**
Diaphragm	3 (12.5%)	3 (12.5%)	7 (29.2%)	**11 (45.8%)**
Supine				
Scalene	3 (12.5%)	6 (25%)	**9 (37.5%)**	6 (25%)
Parasternal	**11 (45.8%)**	5 (20.8%)	6 (25%)	2 (8.4%)
Diaphragm	6 (25%)	4 (16.6%)	6 (25%)	**8 (33.3%)**

Values represent number of children (%). Highlighted in bold are the manoeuvres that most frequently produced the maximum peak RMS-sEMG value for each of the three muscles. MIP, maximal inspiratory pressure; SNIP, sniff inspiratory pressure; TLC, total lung capacity.

Table 6.2b Peak sEMG of the scalene, parasternal intercostal, and diaphragm muscles during four different maximal respiratory manoeuvres while sitting and supine.

	TLC ramp	Short MIP	MIP ramp	SNIP
Sitting				
sEMGsc,peak (μV)	257.3 (205.9-330.9)	287.7 (236.2-392.3)	293.7 (239.7-401)	**310.9 (291.8-427.9)**
sEMGpara,peak (μV)	42.12 (28.89 – 48.59)	37.53 (18.58-67.28)	35.41 (22.16 – 64.61)	**53.47 (33.36-79.28)**
sEMGdi,peak (μV)	38.92 (25.02-68.64)	46.01 (26.84-65.75)	38.81 (25.45-86.15)	**48.77 (34.88-62.86)**
Supine				
sEMGsc,peak (μV)	229.6 (196.4-315.6)	277.3 (201.3-330.6)	**304.8 (216.4 – 353.2)**	294.9 (229.6-372.6)
sEMGpara,peak (μV)	**42.31 (34.6-59.73)**	36.32 (24.92-63.7)	37.44 (25.21-59.6)	36.32 (25.06-48.71)
sEMGdi,peak (μV)	**52.4 (32.62-90.17)**	41.63 (26.79-72.41)	43.64 (28.17-69.2)	51.61 (34.01-80.07)

All EMG data are presented as median (IQR) in μV. Highlighted in bold are the manoeuvres that produced the highest sEMG value for each of the three muscles. MIP, maximal inspiratory pressure; sEMGdi,peak, peak surface electromyography of diaphragm; sEMGpara,peak, peak surface electromyography of parasternal intercostal muscle; sEMGsc,peak, peak surface electromyography of scalene; SNIP, sniff inspiratory pressure; TLC, total lung capacity.

When performing the maximal inspiratory manoeuvres when sitting, there were no significant differences between the within-subject CV of the peak RMS-sEMG obtained from the three attempts of each manoeuvres (Table 6.3). The mean within-subject CV for the four maximal inspiratory manoeuvres ranged from 18.8% to 38.28%. When supine, there were no significant differences between the within-subject CV of the peak RMS-sEMG between attempts of the inspiratory manoeuvres except for diaphragm (p =0.002). For diaphragm, the variability of the sEMGdi,peak obtained during attempts of the MIP ramp manoeuvre (mean (SD) 37.63% (18.94)) and SNIP manoeuvre (34.29% (22.48)) were significantly greater than during the standard short MIP manoeuvre (20.87% (17.09)) and TLC ramps (18.78% (15.24)).

Table 6.3 Coefficient of variation within individuals for the peak scalene, parasternal, and diaphragm sEMG obtained during attempts of the different maximal manoeuvres when sitting and supine.

	TLC ramp	Short MIP	MIP ramp	SNIP	*P* Value
Sitting					
Scalene	20.97% (14.05)	20% (12.84)	18.82% (9.32)	18.8 % (9.82)	0.81
Parasternal	19.68% (14.93)	19.68% (14.93)	19.68% (14.93)	19.68% (14.93)	0.07
Diaphragm	25.78% (19.67)	38.28% (22.21)	34.7% (19.55)	33.25% (18.14)	0.16
Supine					
Scalene	18.69% (15.25)	13.42% (5.83)	20.47% (13.75)	19.24% (9.85)	0.16
Parasternal	19.22% (12.93)	25.79% (13.92)	28.31% (21.62)	24.79% (15.86)	0.17
Diaphragm	18.78% (15.24)	20.87% (17.09)	37.63% (18.94)	34.29% (22.48)	0.002[#]

Data are presented as mean (SD); TLC, total lung capacity; MIP, maximal inspiratory pressure; SNIP, sniff nasal inspiratory pressure.
[#] Comparison of CV of sEMGdi,peak in supine position – MIP vs short MIP $p = 0.003$; MIP vs TLC $p = 0.003$; MIP vs SNIP $p = 0.55$. SNIP vs TLC $p = 0.014$; SNIP vs short MIP $p = 0.04$; TLC vs short MIP $p = 0.68$

Factors affecting peak sEMG of inspiratory muscles recorded during maximal inspiratory manoeuvres

For the cohort, the largest peak RMS-sEMG out of any of the four inspiratory manoeuvres was used as the reference peak RMS-sEMG for normalisation of the tidal sEMG. When sitting, the median (IQR) peak RMS-sEMG for the scalene muscle was 379.6 μV (IQR 306.3-478.8), 61.01 μV (41.36-82.51) for the parasternal intercostal muscle, and 78.81 μV (50.58-91.96) for the diaphragm (Table 6.4). Peak RMS-sEMG was significantly higher when sitting compared to supine for the scalene ($p = 0.001$) and parasternal intercostal muscles ($p = 0.040$). Univariate analyses did not demonstrate any significant relationship between peak RMS-sEMG and age for the three inspiratory muscles (*when sitting,* sEMGsc,peak vs age $p = 0.522$, sEMGpara,peak vs age $p = 0.032$,

sEMGdi,peak vs age p = 0.426; *when supine,* sEMGsc,peak vs age p =0.817, sEMGpara,peak vs age p =0.760, sEMGdi,peak vs age p =0.831). There was no relationship between peak RMS-sEMG and BMI for the scalene and parasternal muscles in both postures (*when sitting,* sEMGsc,peak vs BMI p =0.51, sEMGpara,peak vs BMI p = 0.79; *when supine,* sEMGsc,peak vs BMI p =0.82, sEMGpara,peak vs BMI p =0.76). For sEMGdi,peak, there was an inverse relationship with BMI when sitting and supine (sitting*:* β = -0.42, p =0.001; *supine:* β = -0.38, p =0.018*)*. A low to moderately negative correlation existed between sEMGdi,peak and BMI for both sitting (r= -0.61, p=0.002) and supine (r= -0.48, p = 0.02) postures.

Table 6.4 Lung function and peak EMG data recorded when healthy children perform maximal inhalation manoeuvres in sitting and supine postures

	Sitting	**Supine**	***p* value**
Short MIP, cmH_2O	108.2 (94.93-123.3)	114.7 (97.74-124.3)	0.22
MIP ramp, cmH_2O	97.87 (24.36)	96.73 (20.28)	0.76
SNIP, cmH_2O	84.21 (26.34)	88.59 (22.13)	0.16
IC, L	1.71 (0.52)	1.89 (0.59)	<0.0001
sEMGsc,peak µV	379.6 (306-3-478.8)	330.3 (279.8-409.1)	0.001
sEMGpara,peak µV	61.01 (41.36-82.51)	49.46 (36.32 – 73.7)	0.040
sEMGdi,peak µV	78.81 (50.58-91.96)	68.28 (50.29-110.3)	0.75

Data are presented as mean +/- SD, or median (IQR) unless otherwise stated. MIP: maximal inspiratory pressure; SNIP: sniff nasal inspiratory pressure; IC: inspiratory capacity, obtained from the inhalation to total lung capacity manoeuvre; sEMGsc,peak, maximal surface EMG of the scalene across the four inspiratory manoeuvres; sEMGpara,peak, maximal surface EMG of the parasternal intercostal muscle across the four inspiratory manoeuvres; sEMGdi,peak, maximal surface EMG of the diaphragm across the four inspiratory manoeuvres.

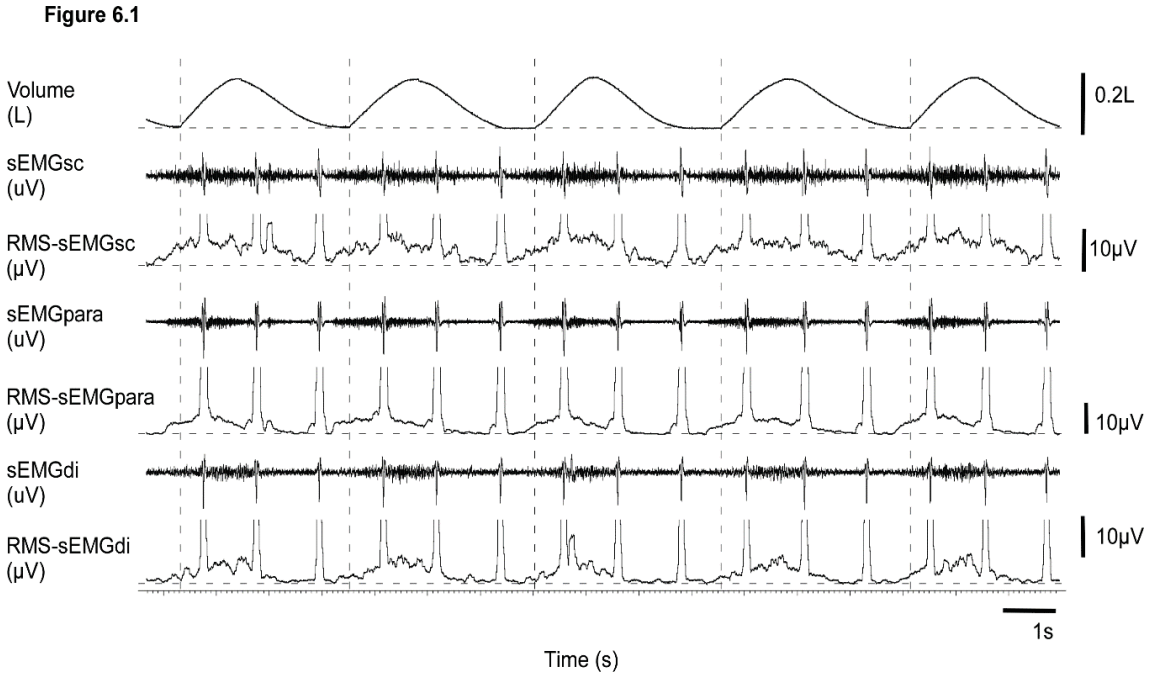
Respiratory muscle activity in healthy children during quiet breathing

Representative traces of tidal breathing recordings from a healthy child are shown in Figure 6.1. The levels of scalene, parasternal intercostal, and diaphragm sEMG

during tidal breathing in sitting and supine postures are presented in Table 6.5, and Figure 6.2.

Figure 6.1: Representative traces of surface scalene electromyogram (sEMGsc), parasternal intercostal EMG (sEMGpara), and diaphragm EMG (sEMGdi) and respiratory volume recorded during resting tidal breathing in a healthy child.

From top to bottom, panels show lung volume, raw and RMS-EMG from scalene (sEMGsc), parasternal intercostal (sEMGpara), and diaphragm (sEMGdi) muscles respectively. Vertical dashed line indicates the onset of inspiration based on deflection from baseline in the lung volume channel.



When sitting, median (IQR) scalene muscle sEMG was 15.02µV (7.36- 27.8), 2.67µV (1.30-4.75) for the parasternal intercostal muscle, and 4.03µV (1.75-7.49) for the diaphragm muscle (Table 6.5). The corresponding median (IQR) sEMG%max of the inspiratory muscles were 3.44% (1.74-7.20) for scalene, 4.83% (1.27-7.43) for parasternal intercostal muscles, and 5.52% (2.86-8.69) for diaphragm (Figure 6.2). Postural differences in inspiratory muscle sEMG will be discussed later.

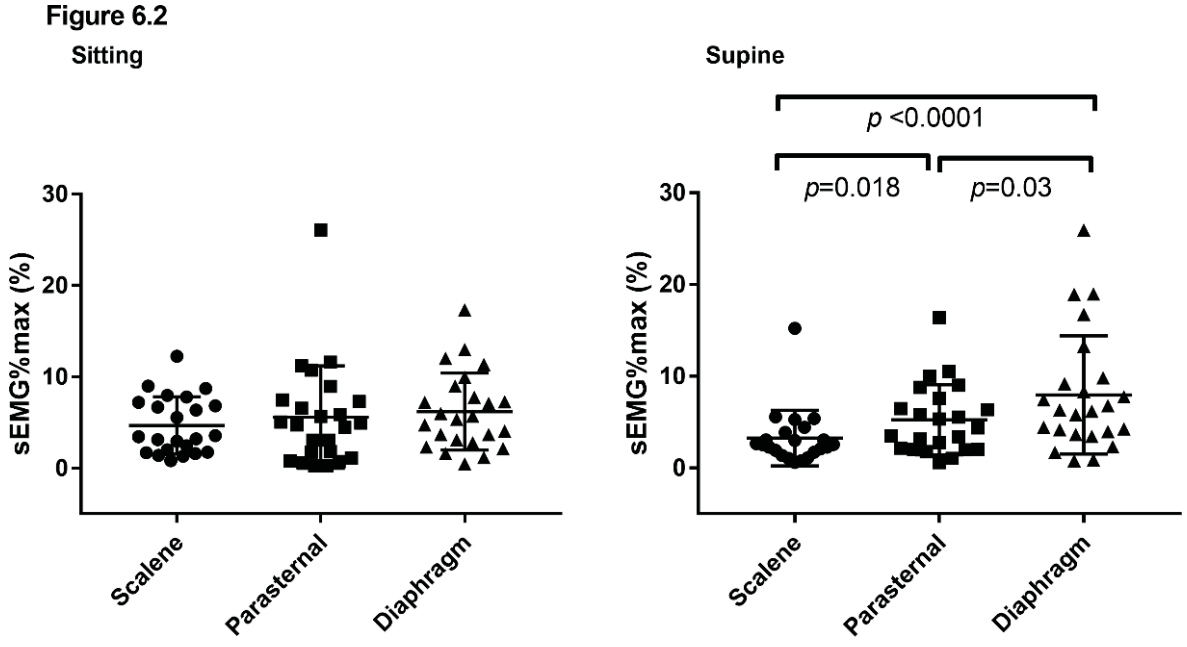
Table 6.5 Respiratory parameters including respiratory muscle EMG activity measured in healthy children in sitting and supine posture

	Sitting	Supine	*P* value
Tidal volume, L	0.36 (0.32-0.49)	0.34 (0.28-0.46)	0.06
RR at rest, bpm	18.04 (14.03-23.39)	19.53 (13.78-22.88)	0.46
sEMGsc, µV	15.02 (7.36-27.8)	8.27 (5.11-12.24)	0.001
sEMGpara, µV	2.67 (1.30-4.75)	2.25 (2.55-7.53)	0.13
sEMGdi, µV	4.03 (1.75-7.49)	3.86 (2.55-7.53)	0.49
sEMGsc%max, %	3.44 (1.74-7.20)	2.56 (1.54 - 4.02)	0.02
sEMGpara%max, %	4.83 (1.27-7.43)	4.38 (2.05 – 7.58)	0.88
sEMGdi%max, %	5.52 (2.86-8.69)	6.26 (3.72-9.67)	0.22

Data are presented as median (IQR). RR, respiratory rate as breaths per minute; sEMGdi, surface electromyography of the diaphragm; sEMGdi%max, sEMGdi % maximal value; sEMGpara, surface electromyogram of the parasternal intercostal muscle; sEMGpara%max, sEMGpara % maximal value; sEMGsc, surface electromyography of the scalene; sEMGsc%max, sEMGsc % of maximal value.

Figure 6.2 Resting tidal sEMG of scalene, parasternal and diaphragm muscles in sitting and supine posture.

EMG magnitude presented as sEMG%max. The horizontal bars indicate the median and the 25th and 75th percentiles.



Intrasubject variability in inspiratory muscle EMG activity during quiet breathing

Coefficient of variation for tidal sEMGsc, sEMGpara, and sEMGdi was calculated for each child over 10 breaths and the averaged result from the cohort is presented in Table 6.6. There was a significant difference between the CV of the tidal sEMG for the three muscle groups for both sitting (p=0.003) and supine (p=0.040) posture. While sitting, the CV of sEMGpara, and sEMGdi was significantly greater than sEMGsc (p= 0.004 for both comparisons to CV of sEMGsc). In the supine position, the CV of sEMGpara was significantly greater than sEMGsc (p=0.040), and sEMGdi (p = 0.045).

Table 6.6 Breath-to-breath coefficient of variation for the RMS-sEMG of scalene, parasternal intercostal and diaphragm within individual children during tidal breathing when sitting and supine.

	CV sEMGsc	CV sEMGpara	CV sEMGdi	*P* value
Sitting	23.09 (9.46)	36.76 (21.38) ^	30.42 (14.33)^	0.003
Supine	22.19 (7.92)*	30.68 (15.87)	23.02 (6.6)*	0.040

Values presented as mean (SD) expressed as percentage. CV, coefficient of variation; RMS, root mean square; sEMGdi, surface electromyography of diaphragm; sEMGpara, surface electromyography of parasternal intercostal muscles; sEMGsc, surface electromyography of scalene.

^ CV sEMGpara vs CV sEMGsc p = 0.004; CV sEMGdi vs CV sEMGsc p = 0.004; CV sEMGdi vs CV sEMGpara p =0.13.

* CV sEMGdi vs CV sEMGpara p=0.040; CV sEMGsc vs CV sEMGpara p= 0.045; CV sEMGdi vs CV sEMGsc p=0.62;

Determinants of inspiratory muscle EMG activity during tidal breathing

The effects of factors such as posture, age, and BMI on inspiratory muscle sEMG were explored. There was no significant difference between sEMG%max of the three muscles in the sitting posture (p = 0.293) (Table 6.5, Figure 6.2). There was a

significant difference between sEMG%max of the three muscles in the supine posture (p <0.0001), with scalene having the lowest sEMG%max (median (IQR) 2.56% (1.54-4.02)), followed by parasternal (4.38% (2.05-7.58)), then the diaphragm (6.26% (3.72-9.67)) (sEMGsc%max vs sEMGpara%max p = 0.018; sEMGsc%max vs sEMGdi%max p<0.0001; sEMGpara%max vs sEMGdi%max p =0.03) (Figure 6.2). Scalene sEMG during tidal breathing was significant lower when supine compared to sitting (median difference -0.91%, p =0.02) (Table 6.5). There was no significant difference between parasternal and diaphragm muscles' sEMG in the two postures.

A significantly negative linear relationship (p =0.024) and a low negative correlation between BMI and sEMGsc when sitting was observed (r=-0.46, p=0.03), where increasing BMI z score was associated with decreasing sEMGsc (Figure 6.3a, Table 6.7). There was no significant relationship between BMI and sEMGpara or sEMGdi in both postures (Table 6.7, Figure 6.3b). When inspiratory muscle sEMG was presented as a normalised value, (sEMGsc%max, sEMGpara%max, sEMGdi%max), there was no longer a significant relationship between tidal inspiratory muscle sEMG (including scalene) and BMI in both postures (Figure 6.3b, Table 6.7).

Table 6.7 Univariate linear regression between EMG of inspiratory muscles and BMI z score or age

	BMI (z score)		**Age (years)**	
Sitting	β value	*p* value	β value	*p* value
Ln sEMGsc	-0.448	**0.024**	-0.105	**0.034**
Ln sEMGsc%max	-0.386	0.055	-0.113	**0.020**
Ln sEMGpara	-0.392	0.146	-0.048	0.516
Ln sEMGpara%max	-0.347	0.264	-0.016	0.846
Ln sEMGdi	-0.435	0.050	-0.136	**0.020**
Ln sEMGdi%max	-0.015	0.946	-0.166	**0.001**
Supine				
Ln sEMGsc	-0.192	0.315	-0.116	**0.007**
Ln sEMGsc%max	-0.133	0.521	-0.120	**0.010**
Ln sEMGpara	-0.026	0.875	-0.098	**0.018**
Ln sEMGpara%max	-0.041	0.846	-0.083	0.128
Ln sEMGdi	-0.234	0.270	-0.110	**0.045**
Ln sEMGdi%max	0.145	0.528	-0.101	0.091

All the EMG variables was logarithmically transformed (Ln) to normalise the data. Significant *p* values (< 0.05) were highlighted in bold. sEMGdi, surface electromyography of diaphragm; sEMGdi%max, sEMGdi % maximal value; sEMGpara, surface electromyography of parasternal intercostal muscles; sEMGpara%max, sEMGpara % maximal value; sEMGsc, surface electromyography of scalene; sEMGsc%max, sEMGsc % maximal value.

Figure 6.3a Scatterplot showing the relationship between scalene muscle sEMG and body mass index in healthy children when sitting.

Top panels – scatterplot of BMI z score vs scalene sEMG. Bottom panels - scatterplot of BMI z score vs scalene sEMG%max. BMI, body mass index; sEMG, surface electromyography; % max, % of maximal value.

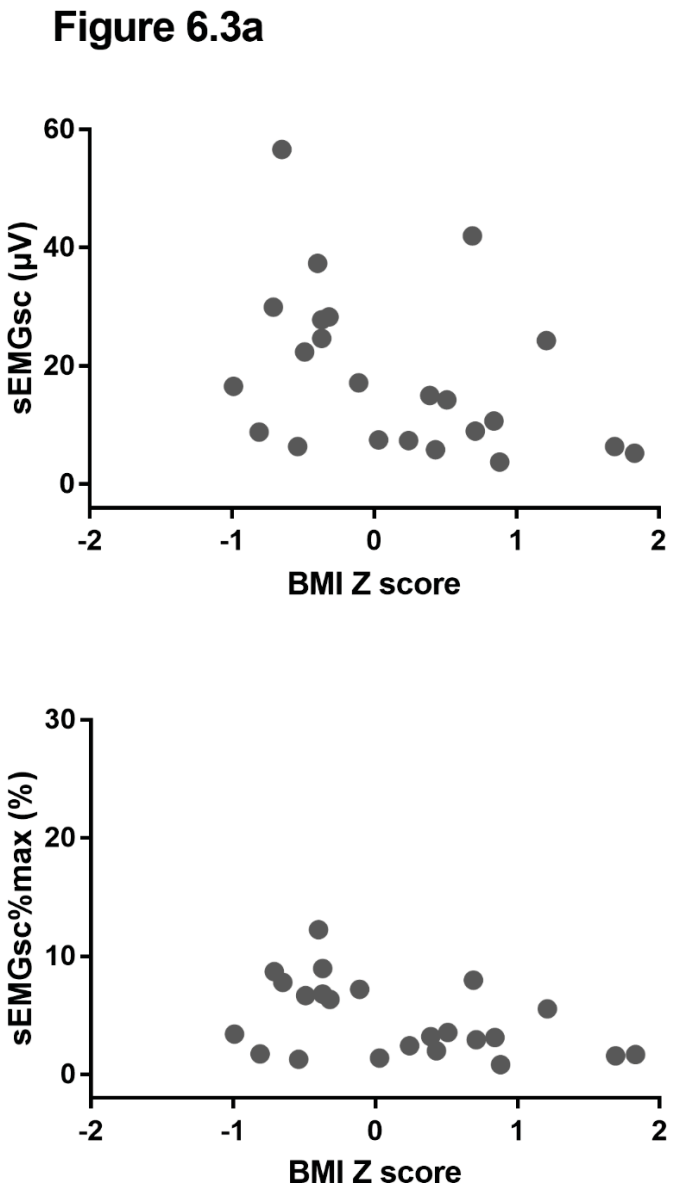
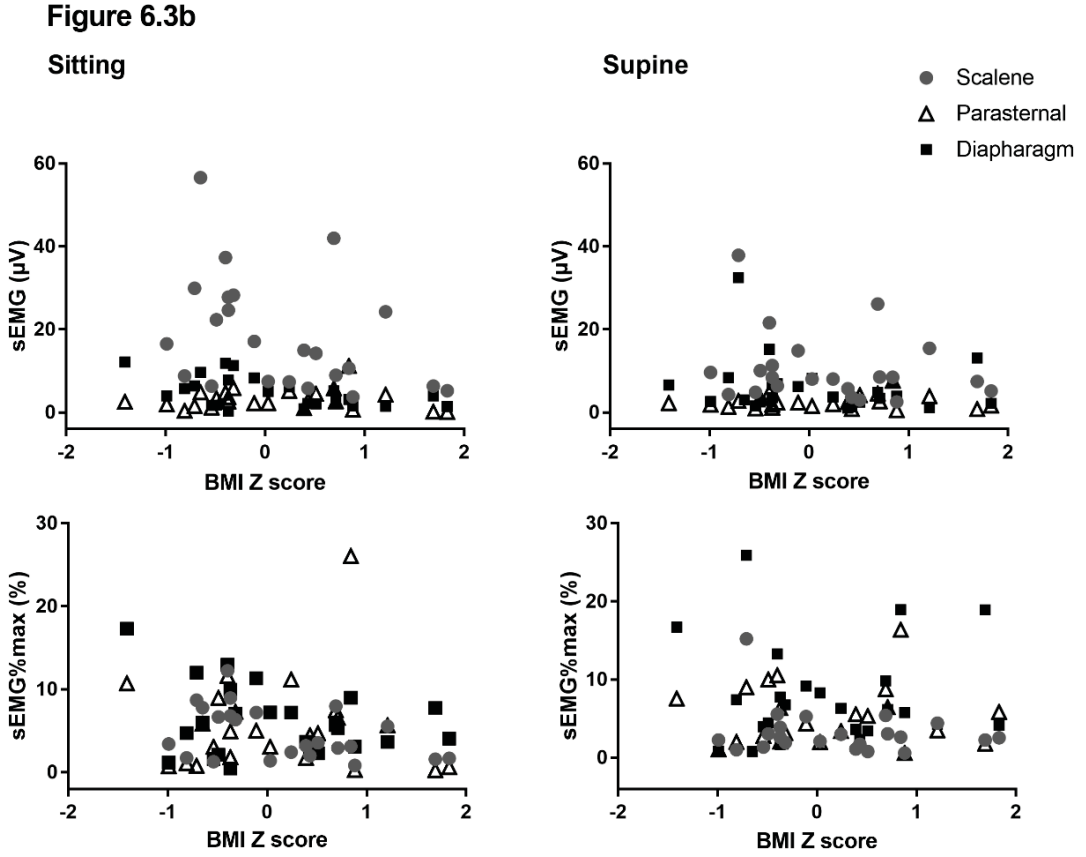


Figure 6.3b Scatterplot showing the relationship between inspiratory muscle sEMG and body mass index in healthy children.

Top panels – scatterplot of BMI z score vs sEMG of scalene, parasternal intercostal, and diaphragm muscle. Bottom panels - scatterplot of BMI z score vs sEMG%max from scalene, parasternal intercostal, and diaphragm muscles. BMI, body mass index; sEMG, surface electromyography; % max, % of maximal value.



Age was another factor affecting tidal inspiratory muscle sEMG. When sitting, there was a significant inverse relationship between tidal sEMGsc and sEMGdi and age (p = 0.034 and 0.02 respectively), with decreasing scalene and diaphragm sEMG associated with increasing age (Figure 6.4a, Table 6.7). This inverse relationship with age remained even when sEMGsc and sEMGdi were normalised (sEMGsc%max, p =0.02; sEMGdi%max, p =0.001) (Figure 6.4a). There were moderately negative correlations between age and sEMGsc%max (r=-0.54, p=0.008), age and sEMGdi%max (r=-0.60, p =0.002). When the posture changed to supine, there was an inverse relationship between age and all three inspiratory muscles' sEMG (sEMGsc p = 0.007;

sEMGpara p = 0.018; sEMGdi p=0.045) (Table 6.7, Figure 6.4b). The inverse relationship with age persisted after normalisation only for scalene (sEMGsc%max, p= 0.01). There was also a moderately negative correlation between sEMGsc (r=-0.56, p= 0.007) and sEMGsc%max (r=-0.54, p=0.009) and age.

Figure 6.4a Scatterplot showing the relationship between inspiratory muscle sEMG and age in healthy children when sitting.

Top panels – scatterplot of age vs sEMG of scalene, parasternal intercostal, and diaphragm muscle. Bottom panels - scatterplot of age vs sEMG%max from scalene, parasternal intercostal, and diaphragm muscles. sEMG, surface electromyography; % max, % of maximal value.

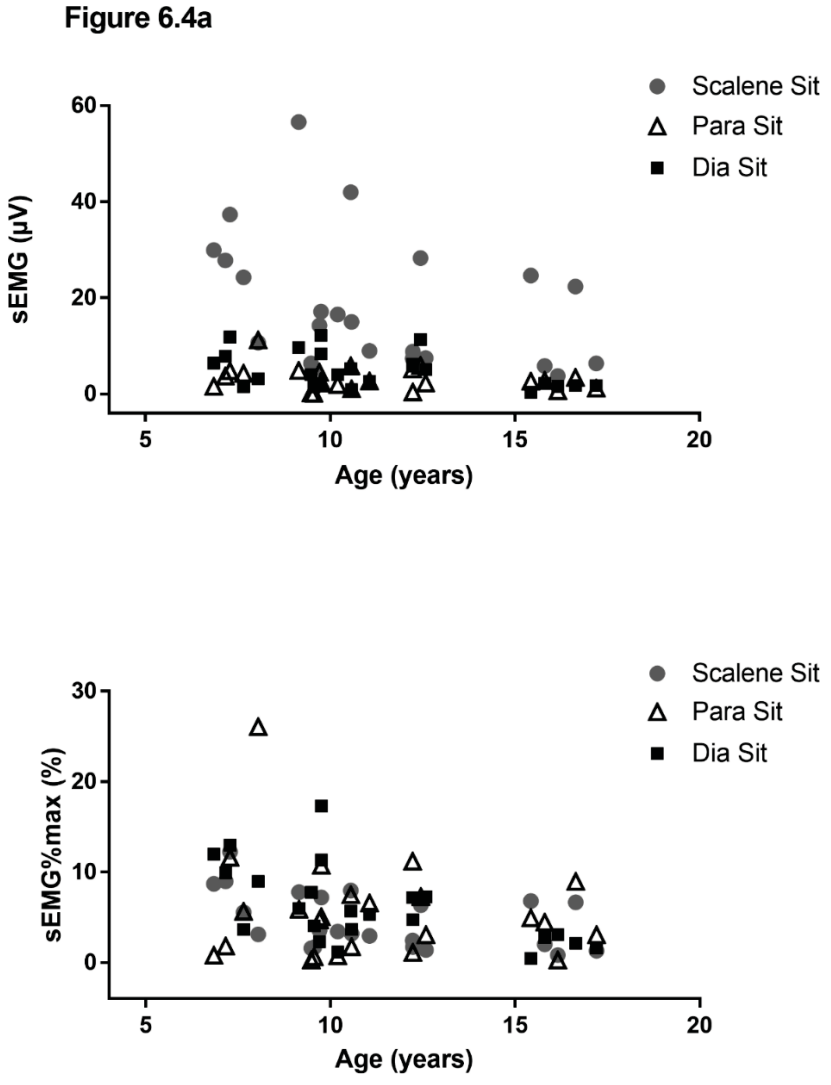
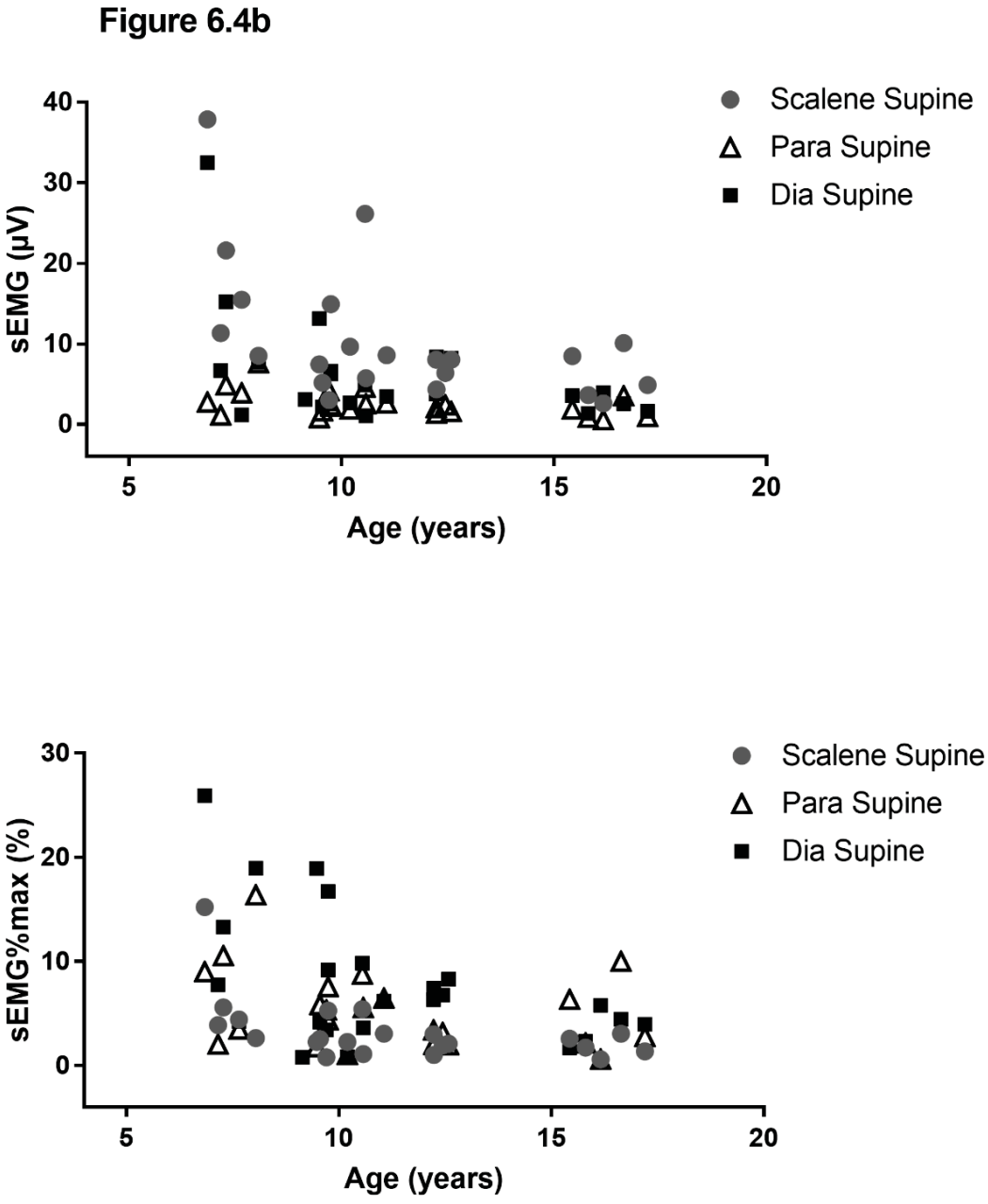


Figure 6.4b Scatterplot showing the relationship between inspiratory muscle sEMG and age in healthy children when supine.

Top panels – scatterplot of age vs sEMG of scalene, parasternal intercostal, and diaphragm muscle. Bottom panels - scatterplot of age vs sEMG%max from scalene, parasternal intercostal, and diaphragm muscles. sEMG, surface electromyography; % max, % of maximal value.



Linear mixed model analysis was performed. Age and muscle groups (scalene vs parasternal intercostal vs diaphragm muscle) were independent predictors of tidal LnEMG%max (Table 6.8). Posture (p=0.23), gender (p= 0.55) and BMI (p= 0.08) were

not significant predictors of tidal sEMG%max. Age was a negative predictor of sEMG%max where a 1.13% decrease in sEMG%max (95% CI 1.05-1.20) was associated with each year of increase in age ($p = 0.001$). Between the three inspiratory muscles studied, scalene had significantly lower activity during tidal breathing than the diaphragm ($p < 0.0001$). There was no significant difference between sEMGpara vs sEMGsc ($p = 0.075$), sEMGpara vs sEMGdi ($p = 0.146$). In the predicted model where BMI z score was fixed at 0.13, and a fixed age of 11.19 years, tidal sEMGsc%max would be 3.06%, sEMGpara%max 3.91%, and sEMGdi%max 4.96%.

Table 6.8 Linear mixed model results for factors affecting interpretation of inspiratory muscle sEMG

Covariates	Coefficient of beta (95% CI)	*P* value
Intercept	2.91 (2.11-3.70)	<0.0001
BMI z score	-0.22 (-0.47- 0.03)	0.08
Gender Male	-0.12 (-0.54- 0.30)	0.554
Female	Reference	
Age (years)	-0.12 (-0.19 - -0.05)	0.001
Muscle groups Scalene	-0.48 (-0.74- -0.22)	<0.0001
Parasternal	-0.24 (-0.56-0.81)	0.146
Diaphragm	Reference	
Posture Sitting	0.13 (-0.09 – 0.35)	0.223
Supine	Reference	

Dependent variable: LnEMG%max

Discussion

The work undertaken in this chapter is the first to investigate the factors influencing interpretation of multiple inspiratory muscle (scalene, parasternal intercostal and diaphragm) sEMG during tidal breathing in a cohort of mostly non-obese (92%) healthy children (> 6 years of age). Scalene sEMG during tidal breathing was

significantly lower than diaphragm sEMG in the linear mixed model, with no significant difference between parasternal and diaphragm sEMG. Inspiratory muscle sEMG decreased linearly with age even when the sEMG is normalised to a maximal value. BMI, and postures were not independent predictors of tidal sEMG%max after other factors including age and muscle groups were included in the mixed model analysis. On the other hand, when inspiratory muscle sEMG recorded during sitting or supine were analysed separately, supine posture is associated with significantly lower tidal sEMGsc and also lower sEMG,peak for parasternal and scalene muscle compared to sitting. The majority of the children (37.5-45.8% of the cohort) achieved peak inspiratory muscle sEMG by performing the SNIP manoeuvre while sitting. In the supine posture, peak sEMG was achieved in different inspiratory muscle by different maximal inspiratory manoeuvres (MIP ramp manoeuvre for the scalene muscle, TLC manoeuvre for the parasternal intercostal muscle, and the SNIP manoeuvre for the diaphragm). There was no significant difference in the variability of the peak sEMG recorded between attempts of different maximal inspiratory manoeuvres within an individual when sitting. However, when supine, there was greater variability in the peak sEMG recorded in the MIP ramp manoeuvre and the SNIP manoeuvre compared to the standard MIP and TLC ramp manoeuvre.

Inspiratory sEMG in healthy children

In this cohort of healthy children, tidal inspiratory muscle sEMG in a sitting position was 3.44% (1.74-7.20) for the scalene (sEMGsc%max), 4.83% (1.27-7.43) for the parasternal intercostal muscle (sEMGpara%max), and 5.52% (2.86-8.90) for the diaphragm (sEMGdi%max). The results are comparable to previously reported level of

sEMG in children although the locations of electrode placement in this study was different from that previously reported (Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). In MacBean et al's study of 92 healthy children of similar age to our cohort, median sEMGpara%max was 6.8% (5.06-10.16)(MacBean, Jolley, et al., 2016). The active electrodes were placed bilaterally at the second intercostal space with a reference electrode at the right scapula or the right olecranon, which was also the same location used in Maarsingh et al's study (Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). In my study, the active electrode for the parasternal intercostal muscle was placed on the right second intercostal space with the reference electrode on the sternum to assess the sEMG of the right parasternal intercostal muscle. The decision to place the electrodes on the right side of the body was to reduce ECG artefact, in keeping with other studies investigating inspiratory muscle sEMG and routine sleep study set-up (Berry et al., 2010; Dos Reis et al., 2019). Potentially in clinical situations where the inspiratory muscle sEMG is anticipated to be different on the left compared to the right side, such as hemidiaphragm paralysis, active and reference electrodes placed on the same side, similar to the electrode locations in this study would be more appropriate than bilateral electrode placement where the recorded EMG would presumably reflects combined output from both the left and right muscles of interest.

In terms of sEMG magnitude, our cohort's median (IQR) diaphragm sEMG was 4.93μV (1.75-7.49), which is less than the pooled diaphragm sEMG (5-15 μV) recorded from 39 school-aged (6-13 years) children with and without asthma reported by Maarsingh et al (2000). It is likely that the higher level of diaphragm sEMG from Maarsingh et al's cohort is due to the higher level of tidal sEMGdi in children with obstructive airway. Also, in Maarsingh et al's study diaphragm sEMG were recorded with bilateral placement of the active electrodes at the costal margin whereas I placed

the electrode only on the right side at the same intercostal space (Maarsingh et al., 2000). In Chapter 3, tidal sEMGdi in healthy snoring children during sleep was 4.63 μV (3.04-6.65)(Chuang et al., 2017), which is similar to the sEMGdi recorded from healthy children in supine position awake in this study (3.86 μV (2.55-7.53)). This was surprising as increased upper airway resistance and hence higher sEMGdi is expected in sleeping snoring children compared to non-snorers although there has been no previous study on inspiratory muscle sEMG recorded from non-snoring healthy children during sleep to confirm this (Schwartz, Smith, Wise, Gold, & Permutt, 1988). Furthermore, inspiratory muscle sEMG is reported to be higher during sleep compared to awake in children (Tabachnik, Muller, Bryan, & Levison, 1981). Future larger studies recording inspiratory muscle sEMG using right sided electrode placement as I did in this study is needed to confirm the reference values of inspiratory muscle sEMG in healthy children, awake and asleep.

Inspiratory muscle activation profile during tidal spontaneous breathing in children

In this cohort of healthy children, neural respiratory drive (as measured by sEMG%max) to the diaphragm muscle during tidal breathing is significantly higher than scalene muscle even after factors such as age and postures has been accounted for, although the magnitude of the difference is small. It is known that different inspiratory muscles would have different patterns of motoneurone activity during tidal breathing (Saboisky, Gorman, De Troyer, Gandevia, & Butler, 2007). Single motor unit recordings made using intramuscular electrodes demonstrated earlier and highest motor unit discharge in the diaphragm during tidal breathing compared to other inspiratory muscle such as scalene and parasternal intercostal muscle (Saboisky et al., 2007). The

non-uniform distribution of neural respiratory drive across different inspiratory muscles during tidal breathing may be related to the principle of neuromechanical matching for optimal ventilatory efficiency (Butler, 2007; De Troyer & Boriek, 2011). The diaphragm muscle's optimal length for force generation is close to FRC and has the greatest capacity to change inspiratory pressure at FRC. Hence potentially diaphragm was the main inspiratory muscle activated due to its efficiency at generating pressure and hence volume change during tidal breathing (De Troyer & Boriek, 2011; Farkas & Rochester, 1986). My study highlights the potential of using surface electrodes to investigate respiratory muscle activation profiles under different breathing conditions and interventions especially for children since it is non-invasive and requires less expertise than recording EMG using intramuscular electrodes.

Factors affecting interpretation of inspiratory muscle sEMG

The negative relationship between inspiratory muscle EMG activity with age that I observed in this cohort was similar to those reported in another paediatric study measuring tidal sEMGpara and in Chapter 3 (Chuang et al., 2017; MacBean, Jolley, et al., 2016). However, in both my cohort and also in MacBean's cohort (2016), there was no relationship between age and the magnitude of sEMG,peak, suggesting different muscle-to-electrode distance between individuals is not the only reason underlying the inverse relationship between tidal sEMG and age. In addition, presenting sEMG as a normalised value (sEMG%max) did not correct the inverse relationship between age and sEMG% max (both in the univariate analyses and the linear mixed model), suggesting there are causes other than anatomical factors for the decreasing inspiratory muscle sEMG with age. A possible explanation is maturational changes in the

respiratory system with age for children (Bryan & Wohl, 2011). Decreases in airways resistance as a result of lung and airway growth, together with increased ribcage ossification may all contribute to the overall decrease in the load of the respiratory system and hence reductions in the neural respiratory drive with age. Since expressing sEMG as a percentage of a maximal value may not correct the age-related differences in sEMG in children, a large database of age-matched reference values of inspiratory muscle sEMG is needed similar to those used for spirometry testing in children to assess differences in inspiratory muscle sEMG in children with and without respiratory conditions (MacBean, Jolley, et al., 2016; Quanjer, et al., 2012).

Other known factors negatively affecting the amplitude of surface sEMG are physical factors such as weight and BMI due to the filtering effect of sEMG with increasing subcutaneous fat (Chuang et al., 2017; MacBean, Jolley, et al., 2016). In our cohort, BMI z score had a significantly negative relationship with sEMGsc. The relationship dissipated when sEMGsc was expressed as sEMGsc%max. In the linear mixed model BMI was not a significant predictor of sEMG%max. This highlights the importance of expressing sEMG as a normalised value in future studies in children especially for conditions such as OSA where a higher proportion of children with increased BMI is anticipated, if an age-matched and BMI matched- reference control group is not available.

The effect of different postures on sEMG was previously described in adults but not in children (Druz & Sharp, 1981; Hudson et al., 2016; Steier et al., 2009; Williams et al., 2019). Inspiratory muscle EMG can alter with changes in posture due to change in muscle fibre lengths, effect of gravity on abdominal contents and hence altered lung volume, and length-tension relationship in different postures. In my cohort of healthy children, postural changes affected the activation of different inspiratory muscles

differently, with a decrease in tidal scalene sEMG in supine position compared to sitting but no significant difference in diaphragm and parasternal sEMG. However, posture was not an independent predictor of tidal sEMG%max when the effects of age, BMI, gender, and muscle groups were included in the linear mixed model. The lack of differences in diaphragm and parasternal tidal sEMG in healthy children are consistent with studies in healthy adults where diaphragm and parasternal EMG was recorded using more invasive techniques (Hudson et al., 2016; Steier et al., 2009; Williams et al., 2019). A potential explanation for the unchanged EMGdi is the increase in diaphragm efficiency when supine compared to sitting as shown in adults, where higher transdiaphragmatic pressure was generated for a given level of neural respiratory drive (ΔPdi / EMGdi) when supine (Hudson et al., 2016; Steier et al., 2009). In comparison, the decrease in scalene sEMG when changing from sitting to supine position may be due to the decreased requirement of the scalene to elevate the ribcage in the supine position, an observation also reported in adults (Hudson et al., 2016). My data suggest depending on the inspiratory muscle of interest, postural differences may need to be accounted for when assessing tidal sEMG recorded in children.

Maximal inspiratory manoeuvres to determine peak sEMG value for normalisation

As aforementioned, normalising sEMG to a maximal value can be helpful in minimising the influence of anatomical factors such as BMI on sEMG between individuals, and also allow comparison of neural respiratory drive between different inspiratory muscles. However, it is not known which manoeuvre is more likely to obtain a peak sEMG value in children. In my cohort of healthy children, the maximal inspiratory manoeuvre which most commonly generated the peak sEMG for the

inspiratory muscles evaluated (scalene, parasternal intercostal, and diaphragm muscle) when sitting was the maximal sniff inhalation (SNIP) manoeuvre. This was not surprising as the SNIP manoeuvre has been shown to be a relatively easy and reproducible volitional test to assess inspiratory muscle strength in children including children with neuromuscular weakness (Nicot et al., 2006; Rafferty, Leech, Knight, Moxham, & Greenough, 2000). However, when supine, different maximal inspiratory manoeuvres achieved peak sEMG for different inspiratory muscles. Studies in adults have also shown that peak diaphragm EMG was achieved through different manoeuvres in different healthy individuals (Jolley et al., 2009; Nava, Ambrosino, Crotti, Fracchia, & Rampulla, 1993; Reilly et al., 2011; Sinderby et al., 1998; Steier et al., 2011). Possible reasons why peak inspiratory muscle sEMGs are not consistently achieved from the same maximal manoeuvres between individuals and for different muscles may be due to: the degree of muscle shortening for individual muscles in each manoeuvre (e.g. isometric MIP manoeuvre is expected to generate the highest Pdi (and hence EMGdi) at FRC with the diaphragm muscle being at optimal length)(Gandevia & McKenzie, 1986), ease of performing the manoeuvre (e.g. Static MIP manoeuvre has greater learning effect and hence may be more difficult to achieve peak EMG in inexperienced subjects than the TLC manoeuvre)(Sinderby et al., 1998), or anatomical factors preventing best performance of certain manoeuvres (e.g. difficulty in performing maximal sniff inhalation in subjects with nasal obstruction)(American Thoracic Society/European Respiratory Society, 2002). The differing proportion of subjects achieving peak EMG with different maximal inspiratory manoeuvres suggest it is best for each individual to perform all the different manoeuvres to obtain the highest peak sEMG when possible. However, if only limited maximal manoeuvres are to be chosen,

the sniff inhalation manoeuvre would be the one most likely to record peak sEMG from especially for children.

Another factor to consider is the repeatability and reproducibility of the peak EMG value between manoeuvres in children. In my cohort, the coefficient of variation for the peak sEMG value obtained from the best three attempts of the four inspiratory manoeuvres when sitting varied from 18.8-20.97% for scalene, 19.68% for parasternal, and 25.78-38.28% for diaphragm. There was no significant difference in the within-individual CV for the peak RMS between the four inspiratory manoeuvres (except when recording peak sEMGdi when supine where TLC ramp and standard MIP manoeuvre had less variability between attempts than MIP ramp and SNIP manoeuvre). The larger CV of peak sEMG in the healthy children compared to those reported from healthy adults (CV 10-15%) may be related to the children being naive to lung function testing procedures, potential difficulties in respiratory muscle co-ordination leading to submaximal efforts, differing level of understanding of the inspiratory manoeuvres, and insufficient time to practice the manoeuvres (Reilly et al., 2011; Sinderby et al., 1998; Williams et al., 2019). Further, maximal inhalation manoeuvres may be more challenging to perform in the supine posture leading to higher variability in the peak sEMG (Williams et al., 2019). However, the MIP recorded from the children in my cohort is comparable to age-related reference of MIP reported suggesting the children's best efforts were likely maximal although variable between attempts (Domenech-Clar et al., 2003; Gaultier & Zinman, 1983). The current study was not designed to assess repeatability and reproducibility of the peak sEMG value obtained from different manoeuvres within and between occasions in children. Future studies involving children repeating the maximal manoeuvres on the same testing day, and also on two separate occasions is needed to address this question.

Limitations

As discussed in previous chapters, one of the main criticisms of surface EMG recordings of respiratory muscle is the lack of consensus for optimum surface electrode locations, limiting the ability to compare results between studies even when the same muscles are being studied (American Thoracic Society/European Respiratory Society, 2002). Both the 2002 ATS/ERS statement on respiratory muscle testing and also the 1998 European surface EMG for non-invasive assessment of muscle (SENIAM) project have discussed recommended methodologies for recording EMG at length without suggesting a consensus for electrode placement location when recording respiratory muscle sEMG (American Thoracic Society/European Respiratory Society, 2002; Stegeman & Hermens, 1998). As I have discussed in Chapter 5, the location of the electrode placement used in my study was based on the locations used in other studies comparing intramuscular and surface EMG recordings of the scalene, parasternal, and diaphragm muscle (Hudson, Gandevia, & Butler, 2007; Saboisky et al., 2007; Verin et al., 2002). Although the tidal inspiratory muscle sEMG I have recorded are comparable to those reported from other studies in children using bilateral electrode placement locations, there are currently no studies assessing the effect of surface electrode placement (especially for unilateral to bilateral placement) to ensure comparisons between studies are valid. Future works into assessing respiratory muscle sEMG needs to ensure consistency in the methodology including electrode placement location to allow reliable data interpretation and comparisons between studies.

Another limitation of the current study is I did not assess the repeatability and reproducibility of the tidal sEMG recorded from healthy children. The coefficient of

variation of the tidal inspiratory muscle sEMGdi within individual healthy children (23.02-30.42%) was similar to the variability of tidal crural diaphragm EMG reported in healthy adults (27.6%), sEMGdi recorded from neonates (33-47%), and also what I observed in snoring children during sleep (32.55-34.22%) in Chapter 3 (Chuang et al., 2019; de Waal, Hutten, Kraaijenga, de Jongh, & van Kaam, 2017; Sinderby et al., 1998). The interminute coefficient of variation in sEMGpara reported in another study involving healthy children was smaller than my data (11.2%) which may be due to the difference in the method of calculating coefficient of variation (MacBean, Jolley, et al., 2016). In my study, the CV was derived from breath-to-breath variability in the sEMG measured within each participant, whereas MacBean et al calculated the interminute coefficient of variation within-subjects (MacBean, Jolley, et al., 2016). Recent studies have suggested that tidal inspiratory muscle sEMG may be more reliable than sEMG%max as an index of NRD due to submaximal and/or inconsistent efforts when performing maximal inspiratory manoeuvres especially in subjects who are naïve to lung function measurements (MacBean, Hughes, et al., 2016; Williams et al., 2019). Also, reporting NRD as non-normalised sEMG would allow sEMG being used as a non-volitional method of assessing respiratory function across a wider range of children, not limiting to only children who are at least 6-18 years of age who can perform maximal inspiratory manoeuvres. Hence more studies are needed to establish the within- and between- occasion variability of scalene, parasternal, and diaphragm sEMG in children using the same electrode placement locations I have utilised to ensure reliability of the sEMG measured before introduction of sEMG into clinical settings.

Summary

In this chapter, I have reported the level of tidal sEMG for inspiratory muscles including scalene, parasternal intercostal, and diaphragm muscle in a small healthy cohort of children in different postures, adding to the limited reference values of inspiratory muscle sEMG in children which were recorded using mainly bilateral placement of surface electrodes in sitting children (Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). Variability exists between children as to which maximal volitional manoeuvre produces the peak sEMG for individual muscles, although sniff inhalation manoeuvre was most likely to record peak sEMG when sitting and it is a relatively easy manoeuvre for children to perform. Although normalisation of respiratory sEMG to a maximal value obtained from a peak inspiratory manoeuvre can help correct the effect of anatomical factors such as BMI between individuals, age related effects on sEMG cannot be fully corrected. Hence for future studies on tidal inspiratory muscle sEMG in children, a much larger age-matched and BMI-matched healthy reference cohort is needed. Development of such reference values would also allow the application of tidal inspiratory sEMG as a non-volitional, non-invasive, and objective method of assessing lung function in children, including those who are too young or not able perform spirometry testing.

Key messages

- Tidal inspiratory muscle sEMG in children can be influenced by anthropometric factors including age and BMI, but not gender.
- Postural changes may affect tidal sEMG of certain inspiratory muscles likely due to the different mechanical effects on different inspiratory muscles in different postures.
- Peak sEMG is achieved from different maximal inspiratory manoeuvres in different individuals. Sniff inhalation manoeuvre is more likely to achieve peak inspiratory muscle sEMG in children than other maximal inspiratory manoeuvres.
- Establishing a database of age- and BMI-matched reference values of inspiratory muscle sEMG recorded using consistent methodology will allow tidal inspiratory muscle sEMG to be used as a non-volitional measure of lung function assessment in children across the ages.

Chapter 7. Conclusions and future directions

This chapter begins with a discussion on how the findings in the previous chapters address the aims and hypotheses of the thesis. This is followed by a critical appraisal of the weaknesses, strengths, and potential significance of the research undertaken. Finally, the thesis concludes with the potential translational impact of the work undertaken, and future research directions required to further explore the utility of sEMG of inspiratory muscles as a non-invasive measure of NRD in children with and without health conditions. In an era where lung disease is being viewed as a continuum from infancy to adulthood, a non-volitional and non-invasive method of assessing lung function across the age ranges is becoming even more important. The studies undertaken in this thesis establish a foundation on which to incorporate inspiratory muscle sEMG as an objective and quantitative assessment of respiratory function in both clinical and research settings in children.

7.1 Addressing the aims and hypotheses

The findings of this body of work are discussed in relation to the key aims and hypotheses.

Aim 1 – To develop a reproducible method of quantitatively assessing diaphragm surface EMG recorded using standard clinical polysomnography equipment and setting.

I have established a reproducible and reliable method for quantitatively assessing sEMGdi recorded using standard clinical polysomnography equipment and settings in Chapter 2. The measurement method was robust between occasions and also between assessors of different level of expertise. I also compared sEMGdi magnitude analysed using the standard EMG filter setting recommended by AASM (22-100 Hz) to those recommended for respiratory muscle EMG recordings (10-1000Hz) and found a high correlation between the sEMGdi measured using the two different filters. The development of a feasible method to quantitively assess sEMGdi recorded during clinical PSG allows the evaluation of sEMGdi (and hence NRD) in children with and without sleep-disordered breathing and highlights the possibility of introducing sEMGdi as an objective quantitative respiratory variable evaluated in clinical paediatric PSG.

Aim 2 – To determine whether tidal diaphragm surface EMG (as a marker of neural respiratory drive) during sleep is different between children with and without OSA.

The hypothesis 'tidal diaphragm surface EMG is higher in children with OSA than children without' was partially supported by Chapter 3. Not only is tidal sEMGdi significantly higher in children with OSA compared to healthy snoring children, tidal sEMGdi in children without OSA (based on the AASM's diagnostic criteria of OAHI ≤1/h) with subjective increased work of breathing (based on criteria described in Chapter 3, including video observation of the child's respiratory effort, evidence of out-of-phase chest and abdominal RIP traces, and visual evaluation of sEMGdi magnitude) was also elevated compared to healthy snorers. The wide range of sEMGdi in children without OSA and the weak correlation between sEMGdi magnitude and routine

PSG respiratory variables (including OAHI, oxygen saturation nadir, and peak transcutaneous carbon dioxide level) highlights that sEMGdi (and hence NRD) evaluates a different aspect of breathing and may aid in the diagnosis of sleep-disordered breathing by providing a non-invasive and quantitative technique for assessing respiratory effort. My study is the first study which provided a quantitative assessment of NRD during tidal breathing in sleeping snoring children.

Aim 3 – To determine whether neural respiratory drive (as measured by tidal diaphragm surface EMG) decreases in children treated with positive airway pressure support for sleep-disordered breathing.

NRD as determined by tidal sEMGdi in children with sleep-disordered breathing was confirmed to decrease in the majority (90%) of the children after treatment with positive airway pressure support during overnight split diagnostic-titration studies as I hypothesised. Further there were no correlation between the degree of change in OAHI or gas exchange indices and sEMGdi before and after PAP support, suggesting sEMGdi is an independent respiratory variable compared to OAHI when assessing breathing in children. The reduction in sEMGdi after commencement of PAP reflects the unloading of the diaphragm once airway patency was able to be maintained using PAP.

Aim 4 – To explore the relationship between inspiratory muscle (scalene, parasternal intercostal, and diaphragm) sEMG and conventional lung function measures of lung volume and pressure in healthy children.

The hypothesis that "a positive linear or curvilinear relationship exists between inspiratory muscle sEMG and lung volume and mouth pressure" was proven. The finding was consistent with adult studies where EMG was recorded using more invasive techniques (Hudson, Gandevia, & Butler, 2007; Ng & Stokes, 1992; Raper, Thompson, Shapiro, & Patterson, 1966; Yokoba, Abe, Katagiri, Tomita, & Easton, 2003). Further, I observed that the recruitment pattern of the inspiratory muscles varied in timing and amplitude with different volitional maximal inspiratory manoeuvres which is also in accordance with adult studies (Butler, McKenzie, & Gandevia, 1999; Hudson et al., 2007; Hudson, Gandevia, & Butler, 2011). This is the first study undertaken to assess the relationship between inspiratory muscle sEMG and lung volume and pressure in children. It is also the first study to explore inspiratory muscle activation profiles in children performing volitional tasks.

Aim 5a – To measure tidal inspiratory muscle sEMG in healthy children, and to explore the relationship between inspiratory muscle sEMG and physiological factors such as age, gender, body mass index, and posture.

The hypothesis that "surface EMG can be recorded from multiple inspiratory muscles (scalene, parasternal intercostal, and diaphragm muscles) simultaneously during tidal breathing from healthy children" was proven. I explored non-normalised sEMG and also normalised sEMG (sEMG%max) activity of scalene, parasternal intercostal, and diaphragm muscle in a small cohort of healthy school-aged children (n=24). My study is the first study to investigate reference values of the scalene muscle in children. The magnitude of the sEMG of diaphragm and parasternal intercostal muscle are in keeping

with those previously reported although the locations of electrode placement in my study is on the right side only, whereas the two studies in children placed electrodes bilaterally (Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). It is not possible to pool existing results together to establish a bigger database of reference inspiratory muscle sEMG values without first investigating the impact of different electrode placement location on sEMG magnitude.

I also confirmed my finding from chapter 3, that age and BMI are negatively associated with non-normalised inspiratory muscle sEMG during tidal breathing. Gender is not a predictor of inspiratory muscle sEMG. Expressing sEMG of inspiratory muscles as a value normalised to the peak sEMG obtained during a maximal inspiratory manoeuvre only minimised the effect of BMI on sEMG magnitude between individuals but not age. Age remained an independent predictor of sEMG%max of the inspiratory muscles in the mixed model analysis. Ideally, an age-matched and BMI-matched healthy cohort is needed to develop inspiratory muscle sEMG reference values similar to those developed for spirometry.

I demonstrated that posture is another factor that can affect sEMG. Although there was no difference in tidal sEMG and sEMG%max for diaphragm and parasternal muscles between postures, there was a decrease in scalene sEMG and sEMG%max recorded in the supine position compared to sitting (Chapter 6). When performing maximal inspiratory ramps, sEMG%max recorded when supine was significantly higher than those obtained when sitting in the linear mixed model analysis, although the magnitude of difference was small (Chapter 5).

Aim 5b – To assess and compare the peak sEMG recorded from different inspiratory muscles (scalene, parasternal intercostal, and diaphragm muscle) when children perform various maximal inspiratory manoeuvres.

The hypothesis "not all children will achieve the peak sEMG for normalisation by performing the same maximal inspiratory manoeuvre" was confirmed, and the variability in the peak sEMG achieved during the four different maximal inspiratory manoeuvres within individual children studied. When sitting, the sniff inhalation manoeuvre was the manoeuvre which most commonly generated the peak sEMG for the inspiratory muscles evaluated, confirming my hypothesis that "maximal sniff inhalation will produce the peak sEMG for most children as it is a natural and relatively easy manoeuvre for children to perform". However, when supine, different maximal inspiratory manoeuvres achieved peak sEMG for different inspiratory muscles (MIP ramp for scalene, TLC ramp for parasternal, and SNIP for diaphragm). This suggests that more than one maximal inspiratory manoeuvres should be performed to select the peak sEMG from, but if only one manoeuvre is selected SNIP would be a good choice for children. However, further studies to explore repeatability and reproducibility of peak sEMG achieved during maximal inspiratory manoeuvre are needed to confirm this finding.

7.2 Weaknesses of the thesis

This body of work illustrates the potential challenges that are associated with recording inspiratory muscle EMG using surface electrodes in children, including the method used to record, process, and evaluate sEMG; different methods of presenting

magnitude of sEMG (normalised vs not normalised); and factors affecting interpretation of sEMG between children with and without lung conditions.

The limitations and disadvantages associated with using surface electrodes to assess respiratory muscle EMG has been described in detail in Chapter 1, and also discussed in each chapter. These limitations include the potential effect of cross-talk of signals (from both ECG and also EMG from adjacent muscles in the vicinity), filtering effects of varying muscle-to-electrode distance between individual subject, and the lack of standardisation for electrode positioning. I have explored the potential physiological factors influencing sEMG interpretations in this thesis, including age, gender, and body mass index (Chapter 2 and Chapter 6 in particular). I have also explored potential differences when presenting sEMG as non-normalised and normalised values (Chapter 6). The potential effects of different placement location on the sEMG measured was not within the scope of this thesis, but is a major challenge that needs to be addressed for ongoing explorations of clinical uses of sEMG as highlighted by two recent systematic review of sEMG research in adults (Cabral et al., 2018; Dos Reis et al., 2019). Validation of different recording sites for surface electrodes to assess inspiratory muscle EMG against intramuscular electrode recordings of the same muscle would be ideal to address this question although the acceptability of intramuscular electrodes in children is likely to be low (Verin et al., 2002). Another possibility is to assess crural diaphragm EMG measured using oesophageal-catheter mounted electrodes against different sites for measuring sEMGdi in children, including bilateral versus ipsilateral placement of recording electrodes. If no significant differences between inspiratory muscle sEMG measured using bilateral or ipsilateral placement of the surface electrodes was found, then potentially pooling of research data to establish healthy reference ranges of inspiratory muscle sEMG would be possible.

One weakness that needs to be addressed with the technique I used to assess inspiratory muscle sEMG is the repeatability and reproducibility of the sEMG measured. Other studies in children and adults where bilateral electrodes were used to assess diaphragm and parasternal sEMG have demonstrated good repeatability and reproducibility of tidal sEMG measured within and between occasions (Maarsingh et al., 2000; MacBean, Hughes, Nicol, Reilly, & Rafferty, 2016; Reilly et al., 2011). Further, if inspiratory muscle sEMG needs to be presented as a value normalised to a peak sEMG value, then it is imperative to assess the repeatability and reproducibility of the peak sEMG recorded from different inspiratory muscles when children perform different maximal inspiratory manoeuvres as discussed in Chapter 6 (MacBean, Hughes, et al., 2016; Williams, Porter, Westbrook, Rafferty, & MacBean, 2019).

Another weakness that I was not able to address in this thesis is whether the average value of inspiratory muscle sEMG over 10 consecutive tidal breaths at a period of stable breathing (as assessed by fluctuations in respiratory flow or RIP bands tracings) is adequate to represent individual children's NRD. As discussed in chapter 3, the choice of 10 consecutive to evaluate inspiratory muscle sEMG during tidal breathing was made based on previous studies of inspiratory muscle sEMG evaluation in children (Maarsingh et al., 2000; Maarsingh, van Eykern, Sprikkelman, & van Aalderen, 2004). Other studies in neonates and children chose to calculate the average sEMG measured from all the breaths recorded over 30 seconds to 1 minute (which would be equivalent to 15-25 breaths for a healthy 6 year old child) (Kraaijenga, de Waal, Hutten, de Jongh, & van Kaam, 2017; Kraaijenga, Hutten, de Jongh, & van Kaam, 2015; MacBean, Jolley, et al., 2016). Relatively large breath-to-breath variations in the tidal inspiratory sEMG magnitude within individuals were observed in both my study (Chapter 6, CV of inspiratory muscle sEMG ranged from

23% to 30% for healthy children over 10 breaths) and other studies in children and adults (de Waal, Hutten, Kraaijenga, de Jongh, & van Kaam, 2017; Kassim, Jolley, Moxham, Greenough, & Rafferty, 2011; Sinderby, Beck, Spahija, Weinberg, & Grassino, 1998). Interestingly in Chapter 3, the breath-to-breath CV of tidal sEMGdi remained 32-34% even when it was calculated over 60 breaths (2 x 10 breaths selected from three different sleep stages) in healthy snoring children although the difference in sleep stages may have contributed to the variability in sEMGdi between breaths. Future studies to explore how many breaths are adequate to represent stable tidal breathing sEMG in children are needed, and whether the number of breaths assessed would vary across different age group due to the variations in breathing pattern with age.

The lack of reference inspiratory muscle sEMG values from healthy children across different age groups awake and asleep in specific postures is a challenge that all future sEMG research in children will need to address. Just as clinical spirometry can only be interpreted using robust, relevant, and reliable reference values, reference values for inspiratory muscle sEMG across the age groups are needed for children as my studies have indicated that age is an independent predictor of inspiratory muscle sEMG which cannot be corrected even if the EMG value is normalised (Chapter 6). One study in children has attempted to develop a reference equation to predict sEMGpara based on a linear regression equation derived from the relationship between age and sEMGpara recorded from 92 healthy children (median age 9.51 years (IQR 5.14-13.99)) (MacBean, Jolley, et al., 2016). This equation was then extrapolated to determine the "normal" predicted sEMGpara value to allow comparison with sEMGpara recorded from preschool children with wheeze (median age 3.70 years IQR (2.23-y.43)) (MacBean, Jolley, et al., 2016). However, anthropometric variables including weight, height, BMI are known to be negatively correlated with inspiratory muscle sEMG in children as well

(Chapter 6) (MacBean, Jolley, et al., 2016). It is not known whether there are ethnic differences in inspiratory muscle sEMG which can potentially occur with differences in body habitus and hence chest wall configuration in subjects from different ethnic groups. Recent experiences in the development of an international spirometry reference equation applicable to children and adults (age range 3-95 years) that adjust for factors including gender, ethnic group, age, and height may be adopted to develop a reference equation for inspiratory muscle sEMG with pooling of inspiratory muscle sEMG data recorded from healthy children from different research groups once the methodology for recording inspiratory sEMG is standardised (Quanjer et al., 2012). In the meantime, it is my opinion, supported from the work undertaken in this thesis, that all future inspiratory muscle sEMG research in children with respiratory conditions requires a contemporary age- and BMI- matched healthy reference cohort.

7.3 Strengths of the thesis

One of the main strengths of the thesis is the choice of using surface electrodes to assess NRD in children. Surface electrodes were well tolerated by all the children, including when used for prolonged periods during sleep (Chapters 2, 3, and 4) and were able to assess multiple inspiratory muscles simultaneously whilst children performed various breathing tasks such as tidal breathing and maximal inspiratory ramps (Chapters 5 and 6). The electrodes are non-invasive with no safety concerns (other than minor irritation to the skin) in comparison to invasive techniques such as intramuscular and catheter-mounted electrodes.

The findings from this thesis have provided further insights into the methodology of evaluating sEMG in children, interpretation of sEMG results, and

highlighted other potential clinical applications of inspiratory muscle sEMG. As the published systematic reviews on research conducted using sEMG of inspiratory muscles in adults highlighted, inadequate reporting of the methodologies used in these studies is a common flaw (Cabral et al., 2018; Dos Reis et al., 2019; Hutten, van Thuijl, van Bellegem, van Eykern, & van Aalderen, 2010). In all the chapters, I have provided clear descriptions of EMG electrode placement locations, EMG signal filtering and processing techniques. I have adopted a right-sided approach to recording inspiratory muscle sEMG to minimise ECG artefacts and also in accordance with sleep study set ups (Cabral et al., 2018; Dos Reis et al., 2019; Berry et al., 2010). The ipsilateral approach also allows comparison of left- and right-sided differences in respiratory muscle activation, such as those experienced by children with unilateral diaphragm pathologies, or chest wall surgery for conditions such as congenital diaphragmatic hernia (Kassim et al., 2011; Steier et al., 2008). A novel contribution I have made to the methodology of evaluating inspiratory muscle sEMG is the validation of a reliable and reproducible method for quantitatively assessing sEMGdi recorded using clinical polysomnography equipment and settings (Chapter 2). The use of commercially available equipment to record sEMGdi allows for the potential introduction of sEMGdi measurements into clinical sleep medicine as I have demonstrated in children with and without OSA (Chapter 3), and children requiring PAP support for treatment of sleep-disordered breathing (Chapter 4).

Another strength of the thesis is the additional knowledge gained on factors affecting sEMG interpretations in children. Although other studies in adults have suggested that presenting inspiratory muscle EMG as a normalised value would minimise anatomical differences (such as variations in height, weight, and BMI) between individuals (Jolley et al., 2009; Reilly et al., 2011; Sinderby et al., 1998; Steier

et al., 2009), I have shown that age is an independent predictor of inspiratory muscle sEMG which cannot be fully corrected with normalisation of sEMG in children. Also, it may be insufficient to perform only one maximal inspiratory manoeuvre to obtain the peak sEMG value for normalisation as not all children will achieve their individual peak sEMG with the same maximal inspiratory manoeuvre. I have also demonstrated that postural differences may affect the sEMG magnitude of scalene but not parasternal or diaphragm muscles, indicating the importance of consistency in posture when comparing inspiratory muscle sEMG in children with and without respiratory conditions. These confounding factors need to be taken into account when evaluating inspiratory muscle sEMG from healthy children to establish a normal reference range, and also when designing future clinical studies comparing NRD between children with and without respiratory conditions.

7.4 Potential translational impact of thesis

The potential translational impact of the thesis for the field of paediatric sleep medicine was touched on in Chapters 3 and 4. The increased NRD in children with OSA and also increased work of breathing compared to healthy snorers indicates an imbalance between the increased upper airway obstructive load and the children's respiratory muscle capacity. The linear relationship identified in Chapter 5 between sEMGdi%max and mouth (hence respiratory muscle) pressure changes confirmed the higher sEMGdi in children with OSA compared to primary snorers reflect increased respiratory effort in children with OSA. sEMG of inspiratory muscles can potentially be used as an alternative quantitative index of respiratory effort to Poes when assessing children's breathing during sleep. Future studies validating inspiratory muscle sEMG

against Poes recordings would help confirm this. The feasibility of introducing sEMGdi as an index of NRD into standard clinical care in sleep medicine based on the results from my study is also recognised by experts in the field (Burns & O'Halloran, 2019). The introduction of more time-efficient automated methods to quantitatively assess inspiratory muscle sEMG, such as those that are incorporated into the NAVA ventilators (NAVA®, Maquet, Germany), would help fast track the inclusion of inspiratory muscle sEMG as an additional quantitative respiratory variable recorded during overnight sleep studies (Baudin et al., 2015; Kallio, Peltoniemi, Anttila, Pokka, & Kontiokari, 2015).

The poor correlations between OAHI and sEMGdi in children with and without OSA in Chapter 3 highlights the limitation of basing the diagnosis of OSA solely on OAHI. Children with significantly increased upper airway resistance may have pathologically elevated respiratory effort during sleep associated with sleep fragmentation and daytime symptoms without scorable apnoeas or hypopnoeas on PSG (Berry et al., 2013; Guilleminault, Stoohs, Clerk, Cetel, & Maistros, 1993; Guilleminault, Winkle, Korobkin, & Simmons, 1982). Recent evidence of cardiovascular and neurocognitive complications even in children who are primary snorers further highlights the shortcoming of OAHI as the main diagnostic criteria of sleep-disordered breathing (Blunden, Lushington, Kennedy, Martin, & Dawson, 2000; Loffredo et al., 2015; Nisbet, Yiallourou, Walter, & Horne, 2014). Daytime behaviour and cognitive assessment in snoring children with increased NRD that does not fulfil the current criteria of OSA should be evaluated and compared to those of non-snoring healthy children. If the prevalence of neurocognitive complications is increased in snoring children with increased NRD it would confirm the importance of assessing NRD in overnight clinical sleep studies and justify interventions such as nasal

corticosteroid, CPAP, or surgery. Whether children with increased work of breathing and elevated NRD subsequently develop obstructive apnoea and hypopnoea if their upper airway obstruction is not treated will need to be determined in future longitudinal cohort studies.

In Chapter 4, I demonstrated the potential use of inspiratory muscle sEMG as an alternative method of assessing efficacy of PAP support in a real-life clinical cohort of children with obstructive and non-obstructive sleep-disordered breathing. sEMGdi was able to detect the efficacy of PAP in unloading diaphragm muscle in children with OSA and nocturnal hypoventilation; it also suggested in one child that the titrated pressure may have been inadequate with lack of improvement in sEMGdi despite improvement in OAHI and gas exchange parameters. This finding echoes previous studies in both children and adults where the pressure required to unload respiratory muscles (as measured by Poes swing) was higher than the pressure required to minimise apnoea or improve gas exchange (Khirani et al., 2013; Montserrat et al., 1995; Sforza et al., 1995). With the increasing use of PAP support in children, not only for nocturnal sleep-disordered breathing, but also for children with other causes of airway obstruction including laryngomalacia, bronchiolitis, and cystic fibrosis, inspiratory muscle sEMG may be an alternative non-invasive respiratory variable to titrate pressure to instead of OAHI, Poes, and gas exchange parameters (Essouri et al., 2011; Fauroux, Hart, & Lofaso, 2002; Fauroux et al., 2001; Khirani et al., 2013).

Another translational impact of the work undertaken in this thesis is the investigation into establishing reference ranges and factors affecting inspiratory muscle tidal sEMG in healthy children. There is little information on the reference range of tidal inspiratory muscle sEMG in healthy children at rest and no information on healthy non-

snoring children during sleep (Chuang et al., 2017; Maarsingh et al., 2000; MacBean, Jolley, et al., 2016). The data in this thesis on tidal sEMGdi during sleep in healthy snoring children (Chapter 2) was included in the European Respiratory Society's (ERS) statement on respiratory muscle testing at rest and during exercise as the only available reference sEMGdi values from children (Laveneziana et al., 2019). As mentioned before, if the field is to move forward, reference values of inspiratory sEMG from healthy children awake and asleep need to be developed in order to incorporate tidal inspiratory muscle sEMG testing as a non-volitional lung function test. Based on the work conducted in this thesis the reference values will need to take factors including age and BMI into account. Standardisation of sEMG recording and evaluation methodologies will allow international collaborative efforts to establish such reference values, similar to the development of spirometry reference equation by the Global Lung Initiative.

7.5 Future directions

Respiratory muscle sEMG recordings provides a quantitative measure of the pattern and magnitude of respiratory muscle electrical activity at rest and in circumstances of altered load: capacity ratio of the respiratory system. This thesis focused on the assessment of the obligatory inspiratory muscles' (scalene, parasternal intercostal, and diaphragm muscles) activity in healthy children and children with sleep-disordered breathing, addressed some of the potential physiological factors that need to be considered when recording inspiratory muscle sEMG in children, and highlighted the potential clinical applications of inspiratory muscle sEMG and hence NRD as a complementary index of assessing breathing when awake and asleep in children.

Currently major challenges that need to be addressed in inspiratory sEMG research are the methodological issues, including lack of standardization for electrode positioning, lack of knowledge on the effect of electrode position on the magnitude of inspiratory muscle sEMG in children, inconsistency in reporting of data as non-normalized or normalized EMG, and inconsistency in the respiratory manoeuvre performed for normalisation. Once these methodological questions are addressed through future studies as discussed above, inspiratory muscle sEMG as a non-invasive index of NRD potentially have an important role in the diagnosis and management of respiratory conditions in children ranging from babies to adolescents, especially for those who are unable to perform spirometry reproducibly.

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