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Chapter 4

Relative impact of respiratory muscle activity on tidal flow and end expiratory volume in healthy neonates

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Introduction

Evidence from the literature shows that in young infants, end-expiratory volume level is not only determined by passive mechanical factors such as thoracic and pulmonary elastic properties, but also by active control mediated through intercostal and diaphragmatic muscle activity\(^1\). This is mainly due to the high compliance of the infantile chest wall and the resulting low passive elastic equilibrium volume being close to closing volume\(^2,3\). To avoid alveolar and airway collapse, neonates actively elevate their end-expiratory level by the complex interaction between upper airway resistance, breathing frequency and post-inspiratory muscle activity\(^4\). Most of this knowledge has been gained using measurements of EMG and relative changes of volume measured at the airway opening, rather than in comparison to the direct measurement of the end-expiratory level.

Recent advances in the development of diagnostic infant lung function tests have made it possible to study tidal flow and volume as well as end-expiratory volume and ventilation inhomogeneities during spontaneous unsedated sleep\(^5\). Furthermore, there is increasing interest in the relation between respiratory muscle activity and resulting flow and volume in the field of intensive care, since first attempts to trigger mechanical ventilation in infants based on the EMG of their own respiratory muscle activity have been published\(^6\).

However, little is known about the temporal dynamic interaction of intercostal and diaphragmatic muscle activity with the resulting flow pattern as well as the lung volume and ventilation inhomogeneities in spontaneously sleeping infants. It is important to understand these interactions since neonates have a high potential to dynamically adapt to new environmental conditions\(^7,8\). A comprehensive picture of the changes in lung mechanics in disease or after exposure to environmental toxins or drugs can only be achieved when lung mechanics are measured in relation to changes in muscle activity. To study such dynamic interactions in infants, non-invasive ways to monitor muscle activity and lung function need to be used. Transcutaneous electromyography of the respiratory muscles (rEMG) has been used in the past to study breathing patterns in neonates\(^1,3,9-13\), and has been validated as an estimate of lung function changes in older
children with asthma. However, no systematic feasibility and repeatability studies or comparison studies to flow and independently measured lung volumes in neonates exist where measurement conditions adhere to the recently defined standards of infant lung function testing.

After establishing feasibility and variability of rEMG and the influence of the face mask in neonates, we aimed to determine the quantitative temporal correlation of intercostal and diaphragm EMG with tidal airflow and to study their impact on lung volume in spontaneously breathing infants during natural quiet sleep. We were particularly interested to determine if lung mechanical function in the infant changes dynamically on a breath-to-breath basis, and thus focussed on the breath-by-breath variations and the relative fluctuating impact of intercostal and diaphragmatic muscle activity on end-expiratory level.

Methods

Study design

The study was performed in two parts: first, a methodological part investigating feasibility, repeatability and variability of the rEMG technique in comparison to standardised tidal flow and volume. We also studied the influence of the elastic and resistive load of the face mask on rEMG activity, and determined the correlation of the tidal time and amplitude indices of the rEMG signal with the matched flow measured at the airway opening.

In the second, physiological part, we investigated whether the relative contribution of the intercostal and diaphragmatic muscle activity changes breath by breath during normal tidal breathing in infants, and whether the rEMG activity averaged over many breaths is related to end-expiratory lung volume and ventilation inhomogeneity measured with a gas washout method.

Study participants

The twenty term-born infants had a median (range) post-menstrual age of 44.4 (41.9 – 48.1) weeks, and had a body weight and length of 4.1 (3.1 – 6.4) kg and 54.5 (50.0 – 61.3)
cm, respectively. The contribution of the face mask on the respiratory neural drive was determined in a subgroup of 12 of the 20 healthy infants. These healthy infants were recruited for an ongoing birth cohort study in Bern, Switzerland\textsuperscript{20-22}. The study was approved by the Medical Ethics Committee of the University Hospital and the Canton of Berne and written informed consent was obtained from all parents.

**Measuring procedure**

Infants were studied during quiet sleep in supine position with the head in midline, and with the mask (size 1, Homedics, Cham, Switzerland) placed over the mouth and nose according to the standards\textsuperscript{17}. Tidal flow and multi-breath inert gas washout measurements were performed using a modified commercially-available apparatus (Exhalyzer\textregistered D, Eco Medics AG, Duernen, Switzerland). This equipment is based on ultrasonic flow meter technology following the assessment of flow and molar mass at the same time as\textsuperscript{21,23}. The deadspace of the flowmeter (with the size 1 deadspace reducer insert provided by the manufacturer) was measured to be 3.5 mL by water displacement. The deadspace of the mask determined to be 7.5 mL\textsuperscript{22}.

Sleep state was defined clinically using the criteria of Prechtl\textsuperscript{19}, with closed eyes, absence of gross body and limb movements, and regular respiratory pattern. The rEMG measurements were synchronized with the flow signal and recorded simultaneously. The tidal flow-volume loops were checked for leaks and the rEMG signals were examined for quality: presence of clear electrical heart activity (ECG) and absence of mains interference. Thirty breathing cycles of the rEMG-measurement were sampled without the face mask before lung function measurements were started (T0 or baseline). The pre-warmed face mask was then set gently onto the infant’s mouth and nose. The EMG-activity of the first 30 breaths was recorded in parallel to tidal flow measurements at the airway opening (T1), followed by a sequence of 30 breaths after two minutes (T2) and followed by a third sequence of 30 breaths after a time period of 8-10 minutes (T3) whereby the face mask was not moved and the sleep stage did not change throughout this measurement sequence.
Lung function measurements at airway opening

From the flow signal we calculated the mean and SD of tidal breathing parameters associated with respiratory neural drive (respiratory frequency (f), inspiratory time (tI), expiratory time (tE), tI as a fraction of respiratory cycle time (tI/ttot), time to peak tidal expiratory flow (tPEFT)/tE, tidal volume (Vt), minute ventilation (Ve) and maximal inspiratory flow (VImax)) of 30 breaths at time points T1, T2 and T3. In a subgroup of 12 infants, the same equipment was used to obtain triplicate measurements of functional residual capacity (FRC) using the multi-breath SF6 washout technique as previously described21-23. Three series of washout procedures were performed and an average obtained for lung volume, FRC was calculated using an optimized analysis method21.

rEMG recordings

The electrical activity of the diaphragm and intercostal muscles were measured transcutaneously from pairs of single electrodes. To assess diaphragmatic activity, two electrodes were placed bilaterally on the costal margin in the nipple line and two bilaterally on the back at the same height. The activity of the intercostal muscles was obtained from two electrodes, placed in the second intercostal space left and right, 2 cm parasternally. A common electrode was placed at the level of the sternum (Fig 1).

To obtain simultaneous recordings of flow and rEMG activity, an extra isolated analogue output was created for the flow signal of the ultrasonic flow meter. The electrodes were connected to the bipolar electro-physiological inputs (EXG) and the flow signal was connected to a general purpose (AUX) input of a Porti-16 front-end (Twente Medical Systems International (TMSI), Enschede, The Netherlands). The data was digitally pre-processed and recorded by Portilab2 (TMSI, Enschede, The Netherlands). Technical aspects of the measurement and measurement device have been described24 and the technical information and validation of the pre- and processing, sampling rate, filtering algorithm, and signal-to-noise ratio has been described by O’Brien et al25. The accuracy of the signal was tested and the channels were matched according to the standards for data acquisition in infant lung function testing17,18. There was no time
delay between the flow signal and the gated electrical signal of the diaphragm and intercostal muscles (Fig 2).

Figure 1)
Left: frontal view. Right: dorsal view. Ec, common or "ground" electrode. Derivations are as follows: E1-E2, intercostal electromyography (EMG); E3-E4, frontal diaphragmatic EMG; and E5-E6, dorsal diaphragmatic EMG.
Figure 2)
Time relation between flow, gated rEMG of the diaphragm and intercostals muscles. Abbreviations: Flow = flow signal, upwards is inspiration, downwards is expiration; g Int = gated signal of the intercostals muscles; g FDia = gated signal of frontal diaphragm; avg Interc = average intercostol muscle EMG signal; avg FDia = average frontal diaphragm signal. Average output signal showing good interpolation during gating and almost no residual heartbeat components.
Data analysis and statistics

Data are expressed as mean (± SD) and coefficient of variation (CV) unless stated otherwise. Differences were calculated using the Wilcoxon test for paired measurements (relation between T1 and T2 and T2 and T3). Repeatability was assessed by Bland and Altman plots26.

We derived the corresponding timing indices from the averaged rEMG signal and flow. The time between successive maxima of the averaged rEMG signal is called the interval time (\( t_{\text{tot,rEMG}} \)). Time from the lowest activity to the next maximum is the inspiratory time of the rEMG (\( t_{\text{I,rEMG}} \)) and from a maximum to the following lowest activity the expiratory time of the rEMG (\( t_{\text{E,rEMG}} \)). Time from the onset of the expiratory flow to the lowest of the averaged rEMG is called the post-inspiratory time (\( t_{\text{pia}} \)), time from the onset of the averaged rEMG to the start of the inspiratory flow is called the ramp inspiratory activity time (\( t_{\text{ria}} \)), and the time between \( t_{\text{pia}} \) and \( t_{\text{ria}} \) is called \( t_{\text{plateau}} \). The peak of the rEMG is expressed in microvoltage (uV) and reflects maximal electrical activity of the intercostal muscles or diaphragm. To calculate the relative amplitude variations of the EMG signals, the logarithm of the EMG Activity Ratio (logEMGAR) was used15. This described the relative changes in EMG activity, either increasing or decreasing symmetrical around unity.

To investigate the influence of the mask, the mean inspiratory peak of the logEMGAR at T0 was compared with the mean inspiratory peak at T1, T2 and T3.

The correlation between the time-indices of rEMG measurement and tidal flow at the airway opening were calculated using Bland and Altman plots26. Time indices obtained from rEMG measurements of the frontal diaphragm signal were calculated and compared with \( t_{\text{i}} \) and \( t_{\text{e}} \) from the lung function measurement.

The data processing and analysis were done using the data acquisition and processing package Poly 5.0 (Inspektor Research Systems, Amsterdam, The Netherlands). Statistical
Result analysis and graphics were performed with SPSS (SPSS Inc., Chicago, USA) and SigmaPlot (Systat Software Inc., California, USA).

Results

a) Methodological aspects

Feasibility of Measurements
All children tolerated the measurements well and none woke up during the procedures. During tidal breathing measurements no leakage of the face mask was detected. The EMG recordings of the diaphragm and intercostal muscles were successfully performed in 18 of 20 infants (90%) and were free of mains interference. In 2 infants (10%), electrical interference to the EMG signals was caused by contact between the conducting parts of the electrodes; the resulting technical problems necessitated exclusion of this data from analysis.

Repeatability of tidal flow parameters and corresponding tidal rEMG measurements
Mean values (SD) of measurements of the selected tidal flow parameters related to neural drive are summarized in table 1. While most of the parameters showed good repeatability between these three occasions, breathing frequency decreased at T3 and $t_{PTE}/t_E$ significantly increased at T2 and then decreased at T3.

No significant differences were found among the three different measurement time points in $t_{rEMG}$, $t_{E,rEMG}$, $t_{ria}$, $t_{pia}$, $t_{plateau}$ and Peak, indicating good repeatability. Mean values (SD) of measurements of the selected parameters of the rEMG are also summarized in table 1.
Table 1. Tidal flow parameters and indices of rEMG of the diaphragm (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>respiratory rate (min⁻¹)</td>
<td>46.4 (7.6)</td>
<td>47.3 (8.2)</td>
<td>44.0 (8.3)**</td>
</tr>
<tr>
<td>t₁ (ms)</td>
<td>608 (112)</td>
<td>602 (113)</td>
<td>640 (134)</td>
</tr>
<tr>
<td>tₑ (ms)</td>
<td>737 (154)</td>
<td>715 (145)</td>
<td>783 (180)</td>
</tr>
<tr>
<td>iₘ/ₘₑ (%)</td>
<td>45.3 (3.6)</td>
<td>45.7 (4.7)</td>
<td>45.3 (4.5)</td>
</tr>
<tr>
<td>tₚₕₑₚₑ (%)</td>
<td>32.9 (6.55)</td>
<td>37.9 (8.5)*</td>
<td>31.7 (6.7)**</td>
</tr>
<tr>
<td>V₁ (mL)</td>
<td>29.2 (5.3)</td>
<td>29.2 (5.2)</td>
<td>30.4 (5.9)</td>
</tr>
<tr>
<td>Vₚₑ (mL/min)</td>
<td>1331 (264)</td>
<td>1360 (270)</td>
<td>1284 (314)</td>
</tr>
<tr>
<td>Vₚₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑₑطورسومة</td>
<td>tₑₑₑₑₑₑₑₑₑₑₑえばمة</td>
<td>562 (38)</td>
<td>611 (97)</td>
</tr>
<tr>
<td>tₑₑₑₑₑₑₑₑₑₑₑebaةمة</td>
<td>739 (215)</td>
<td>726 (217)</td>
<td>794 (241)</td>
</tr>
<tr>
<td>tₑₑₑₑₑₑₑₑₑₑₑebaةمة</td>
<td>143.9 (38.0)</td>
<td>149.3 (46.6)</td>
<td>168.3 (46.7)</td>
</tr>
<tr>
<td>tₑₑₑₑₑₑₑₑₑₑₑebaةمة</td>
<td>537 (204)</td>
<td>521 (199)</td>
<td>555 (212)</td>
</tr>
<tr>
<td>tₑₑₑₑₑₑₑₑₑₑₑebaةمة</td>
<td>32.8 (10.2)</td>
<td>31.7 (10.4)</td>
<td>34.7 (14.1)</td>
</tr>
<tr>
<td>Peak Dia (mmHg)</td>
<td>7.5 (1.9)</td>
<td>7.5 (1.9)</td>
<td>7.3 (1.9)</td>
</tr>
</tbody>
</table>

* p < 0.05 with Wilcoxon Test of the paired measurements (between T1 and T2)

** p < 0.05 with Wilcoxon Test of the paired measurements (between T2 and T3)
**Influence of face mask on muscle activity**

Fig 3 illustrates the influence of the face mask used in infant lung function testing on the respiratory neural drive. Each line represents one infant (n=12) at the four measurement time points. The electromyographic analysis of the diaphragm showed a significant (p<0.001) increase in logEMGAR when the face mask was placed (T1), compared to baseline (T0). The mean logEMGAR at T1 and T3 were 0.09 and 0.07 respectively, indicating that the peak inspiratory electrical activity of the diaphragm increased at 23% and 17%, respectively.

![Graph](image)

**Figure 3**

Dependence of the amplitude of the diaphragm rEMG signal on the resistive load of the face mask. Three measurement time points “Start (T1), > 2 min (T2) and End (T3)” were compared with baseline (T0), each line represents an infant. The electromyographic analysis of the diaphragm showed a significant (p<0.001) increase in logEMGAR when the face mask was placed (T1), compared to baseline (T0). The mean logEMGAR at T1 and T3 were 0.09 and 0.07 respectively, indicating that the peak inspiratory electrical activity of the diaphragm increased at 23% and 17%, respectively.
Correlation between rEMG and flow indices

Fig 4 shows tidal flow, volume, and intercostals and diaphragmatic muscle activity in one representative infant. Electrical ramp inspiratory activity (ria) of the diaphragm and intercostal muscles was detected prior the onset of inspiratory flow\(^{27}\). In all infants, the intercostal muscles started contracting before the diaphragm. During inspiration (specifically stage II of the inspiratory phase), the diaphragm and intercostal muscles attained their maximal electrical activity. This peak activity occurred on average at 65.3 ms (± 127 ms) after the peak tidal inspiratory flow for the intercostal and 132.2 ms (± 87 ms) for the diaphragmatic activity.

The respiratory muscles were still active during early expiration, the so-called post-inspiratory activity (pia) of the diaphragm or stage I expiration. The pia followed a plateau phase (stage II expiration) for both muscle groups. This muscle activity pattern was found in all infants and the time intervals are summarized in table 1.

The comparable time indices for flow and rEMG showed good agreement. The mean \(t_i\) of the flow signal was 623 ms (± 145 ms), the mean \(t_{i,\text{rEMG}}\) 574 ms (± 90 ms). The mean \(t_e\) and \(t_{e,\text{rEMG}}\) were 726 ms (± 233 ms) and 776 ms (± 295 ms) respectively. A Bland and Altman plot (shown in figure 5 for \(t_i\)) revealed relatively good agreement between the two different methods in inspiration and expiration time. The mean of the difference for inspiratory time and expiratory time were respectively -16.4 ms (95% limits of agreement: -99.0 ms to 131.8 ms, 95% CI of mean difference: -32.1 ms to 0.67 ms (this is almost significant, probably because of the one outlier)) and 9.7 ms (95% limits of agreement: -94.7 ms to 114.1 ms, 95% CI of mean difference: -4.7 ms to 24.1 ms). Only one outlier was present in both measures (Fig 5), and when investigated in detail, showed evidence of braking in the flow signal.

While there was excellent agreement between the timing indices of rEMG and tidal flow, we found no relation between the peak electrical activity (amplitude) of the diaphragm and the tidal breathing parameters \(V_T\), \(V'_\text{Imax}\), associated with respiratory neural drive.
Figure 4)
Time relation between flow, average rEMG of the diaphragm (frontal) and intercostal muscles.
ria: ramp inspiratory activity, pia: post inspiratory activity. Upper panel of the figure: solid line represents flow, dashed line represents volume.
Figure 5
Bland-Altman plot comparing the inspiratory time (ms) calculated with the flow signal and with the rEMG signal of the diaphragm. The mean difference was 20.3 ms (95% limits of agreement: -92.7 to 133.3 ms).

b) Physiological aspects

Variability in the relative contribution of intercostal and diaphragmatic activity to flow
The relative activity of diaphragmatic and intercostals muscle activity can be studied breath by breath since measurement conditions are not likely to change a lot from one breath to the next. Interestingly, the relative contribution of the intercostals and diaphragmatic muscle activity to flow amplitude changed considerably from breath to breath. A representative example shows the relative contribution of diaphragmatic and intercostal muscles to tidal flow in a three-dimensional graph (Fig 6).
The contribution of the intercostal muscles and diaphragm to flow in one infant over 30 breaths, showing dynamical changes breath by breath.

This suggests that flow is dynamically regulated in infants on a breath to breath basis indicating a high contribution of neuro-respiratory control mechanism to the flow waveform.

For the whole group the variability (group mean CV) in the relative contribution of intercostals was 14.2 % (± 6.6 %), for the diaphragm it was 32.9 % (± 9.4 %), indicating much higher breath-to-breath fluctuations in diaphragmatic activity. Interestingly, the variability in the peak inspiratory flow (group mean CV 7.2 % (± 2.5 %)) was lower than in the diaphragm and intercostal muscles.
Even within this small age range, breath-to-breath variability in diaphragmatic muscle activity was dependent on body weight. We observed a significant negative correlation between body weight and the variability in the relative contribution of the diaphragm ($r=-0.67$, $r^2=0.39$, $p=0.007$), but no relationship with the variability in the relative contribution of the intercostals muscle.
To further test whether respiratory control is important for the interaction of respiratory muscle activity and resulting flow, we measured the breath-to-breath variability of muscle activity after the placement of a face mask. The variability in the relative contribution of intercostals (group mean CV 25.6 % (± 8.4 %)) decreased with the elastic and resistive load of the face mask to CV 14.2 % (± 6.6 %). With the diaphragm (group mean CV 38.5 % (± 10.7 %)) this decreased to CV 32.9 % (± 9.4 %). However, these decreases were not significant (p>0.05).

Other studies suggest that post-inspiratory muscle activity during expiration is related to end-expiratory volume. We found a significant linear correlation (r=0.93, r²=0.87, p<0.01) between t_pia and t_EL,REMG (averaged from 30 breaths). In contrast, there was no correlation between t_ria and t_EL,REMG in all infants. We found a weak positive correlation between t_pia / t_tot,REMG (means of 30 breaths) and FRC expressed in mL/kg (r=0.523, r²=0.273, p<0.01) (Fig 7a) and a weak negative correlation between the FRC and the ratio t_ria / t_tot,REMG (r= -0.511, r²=0.261, p<0.01) (Fig 7b). As illustrated in two examples (figure 7c), this means, the longer the post-inspiratory muscle activity and the shorter ramp inspiratory muscle occurs, the higher the FRC (see discussion). There was no correlation between t_tot and weight and FRC, indicating that these relationship were not trivial and related to breathing frequency.
Figure 7a)
Relationship between $t_{pi} / t_{tot,EMG}$ and FRC (mL/kg) ($r=0.523$, $r^2 0.273$, p<0.01).

Figure 7b)
Relationship between $t_{ria} / t_{tot,EMG}$ and FRC (mL/kg) ($r=-0.511$, $r^2 0.261$, p<0.01).
The solid lines represent the flow signal, the dotted lines represent the diaphragm signal. The grey patient has a longer post-inspiratory muscle activity of the diaphragm than the black patient. This results in a higher FRC (corrected for body weight). The respiratory rate of both infants is the same (48 breaths/min).

**Discussion**

The interaction between muscle activity, flow and lung volume in spontaneously sleeping neonates has never been studied according to the recently defined standards of infant lung function testing. We have found good feasibility and repeatability of intercostal and diaphragmatic rEMG in spontaneously sleeping healthy neonates. A poor correlation between rEMG amplitude and peak tidal flows was observed. Moreover, rEMG amplitude appeared to be significantly dependent on the resistive load of the face mask. We found diaphragm and intercostal muscle activity commenced prior to the onset of inspiratory flow and respiratory muscles remained active during the
expiratory cycle. We revealed that diaphragmatic activity showed a breath-to-breath variability twice as large as the variability in intercostal activity. Also, infants with relative longer pia and earlier ria showed higher end-expiratory levels.

Quality and usefulness of rEMG measurements in infants

Technical problems necessitated exclusion of data of two infants from analysis. Once this problem had been recognized it was easily solved by avoiding contact with the electrodes. Altogether, rEMG is an easy to handle and non-invasive way to obtain information about the electrical activity of the diaphragm and intercostal muscles, and correspondingly to obtain indirect information about the respiratory neural drive.

We observed proportionality between the amplitude of rEMG activity and resulting flow amplitude, however the direct correlation (averaged over all infants) was poor. This is mainly due to the complex relative contribution and timing of diaphragmatic and intercostal muscle activity to airflow in infants, and to the fact that transcutaneous monitoring may not represent exactly the centre of the respiratory muscles activity. The amplitude depends on the distance between the activated motor unit to the electrode. Furthermore, the skin thickness may vary form infant to infant resulting in absolute differences in EMG amplitude. The log EMGAR calculation partly compensates for the latter two facts. Furthermore the breath-to-breath relative contribution of the muscle activity is less affected by these limitations. Nevertheless, the rEMG is only a proportional surrogate of the overall muscle activity of these particular muscle groups.

We observed that the rEMG amplitude of the diaphragm was altered by the resistive and elastic load of the face mask. Our data support the hypothesis that tidal airflow analyses depend upon the recording technique, and especially on the face mask. An increase in the resistance of the infant lung function equipment by the face mask affected not only various tidal breathing parameters but also the peak amplitude of the rEMG of the diaphragm. After allowing some face mask adaptation time, the repeatability of the timing indices was nevertheless good and comparable to the repeatability of flow measured at the airway opening.
Relationship between muscle activity and resulting flow

The \( t_{rEMG} \) and \( t_{fEMG} \) were related to \( t_i \) and \( t_e \) of the flow signal respectively and showed a good correlation and no significant difference between two methods. The wide scatter and the presence of a few outliers indicate that other factors influence the susceptibility of the techniques. These factors may comprise physiological variability or technical ones. This demonstrates a good synchronised timing between rEMG cyclic changes and tidal flow changes. Relative to the onset of inspiratory flow in infants, it appears from our findings that the intercostal muscles are first recruited and thereafter the diaphragmatic muscles (Fig 3). For both muscle groups, inspiratory activity starts during expiration of the previous breath, in order to overcome the low intrathoracic pressures in infants\(^{31}\). In healthy adults, Saboisky et al \(^{32}\) recently observed that the earliest recruited muscles were the diaphragm and the third external intercostal muscles. This difference may be explained by a relatively low lung compliance and a highly compliant chest wall, resulting in a low elastic equilibrium volume in infants in comparison to adults\(^{2,33}\). Our data show that the volume of the ribcage increases by first lifting up the thorax via the intercostal muscles, and thereafter the volume further increases by activation of the diaphragm. The onset of inspiratory muscle activity is likely to be different in the presence of increased intrapulmonary pressure, e.g. during pulmonary hyperinflation, making these timing indices potentially useful for detecting alterations in lung mechanics in disease\(^{34}\).

Also during inspiration, the peak rEMG activity occurred shortly after the peak tidal inspiratory flow, first for the intercostals and then for the diaphragm. From our data both intercostal and diaphragmatic activity seemed to persist long into expiration, whereby the relative contribution of this pia became more and more dominated by the diaphragmatic activity.

Variability in the relative contribution of intercostals and diaphragmatic muscle activity to flow

Lung mechanics in infants is a dynamic equilibrium of the complex interaction between control of breathing, respiratory muscle activity and respiratory mechanics. It is likely that such a system shows large fluctuations and behaves like a dynamical nonlinear system\(^{35,36}\). We have shown that the contribution of intercostal versus diaphragmatic
muscle activity to flow changes on a breath-by-breath basis. On average, the relative contribution of the diaphragmatic activity was larger than the relative contribution of the intercostal activity. This may have to do with the mechanics of the thoraco-pulmonary system, changes in the degree of activation of whole muscle groups, and/or variations in the centre of maximal muscle activation relative to the electrode position. We propose that the breath-by-breath fluctuations are an expression of a rapid adaptive process to maintain optimal ventilation of the lung and to keep the peak inspiratory flow constant. Fluctuations in the relative contribution of respiratory muscle activity may be part of the adaptive capacity of the respiratory system to external influences. This is supported by our findings in the response to the onset of the elastic and compliant face mask load, whereby we find a clear decrease in variability in both muscle groups.

The relationship between muscle activity and resulting volume
There has been no study showing the direct comparison between independently measured FRC and muscle activity in vivo in unsedated infants. We showed that the marked pia and an shorter ria of the diaphragm is correlated with end-expiratory volume level or FRC measured by multi breath gas washout. We hypothesise that infants extend their post-inspiratory muscle activity to ensure an actively controlled deflation and avoid alveolar and airway collapse in accordance to previously work\^3,\^4. These studies predated the publication of the new measurements standards\^16-\^18. We hypothesise furthermore that infants with low FRC need to activate the ramp inspiratory muscle activity earlier and longer in order to generate more pressure to reach a critical intrathoracic pressure that generates a flow. We believe this phenomenon is typical in infants who have their elastic equilibrium volume and closing volume close to the residual lung volume, and who have their end-expiratory volume (FRC) dynamically elevated.

The relationship between post-inspiratory muscle activity and FRC measured under natural conditions was weaker than previously anticipated\^3, suggesting that other mechanisms are involved in this process (e.g. laryngeal braking). We hypothesise that a entire network of complex interacting mechanism determine FRC, and it is likely – as our data nicely suggest – that this happens on a breath to breath basis.
Limits of method

Some authors have expressed concern that EMG tracings can be contaminated by activity of other muscles, the so-called cross-talk\textsuperscript{29,37}. In an earlier report we have demonstrated that, with our measurement method, this appeared not to be the case\textsuperscript{38}. This is in line with the observations of others who reported no abdominal muscle activity in healthy infants in the supine position during quiet sleep\textsuperscript{39}. Another critical point of the EMG analysis may be the gating and filtering process, which may lead to problems of measuring the exact time indices in the breathing cycle. However, this new method of cone filtering is fairly robust against time delays, and the excellent correlation of time indices in EMG and flow proves that these filtering procedures are optimal. The relationship between timing indices of rEMG and lung volume is mainly based on cross-sectional data, since intra-individual differences in EMG timing indices and FRC are close to the detection limits of the measurement techniques. The trivial relationship between the used EMG timing indices, weight and age were not significant, thus some inferences to be made based on cross sectional data.

In summary,

Using transcutaneous rEMG in matched comparison to air flow and lung volume measurements, we have found good feasibility and repeatability of intercostal and diaphragm rEMG activity. We have quantified their timing relationship to airflow in spontaneously sleeping healthy term neonates. This enables us to use this technique in future studies of lung disease in spontaneously breathing infants with lung disease. This timing and variability (threshold issues) information is essential for future studies on EMG triggered mechanical ventilation\textsuperscript{6}.

Combining non-invasive rEMG and lung function measurements may help to provide a more comprehensive picture of lung mechanics and its adaptive reaction in disease. The assessment of such adaptive reactions may give important additional information of the diseased lung since naturally breathing unsedated infants have a high capacity to dynamically maintain their lung volume, an observation we have found in our own data and from published work\textsuperscript{40}.


