ERS Statement on Respiratory Muscle Testing at Rest and during Exercise

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1. Respiratory muscle function

1.1 Airway opening, oesophageal and gastric pressures: technical considerations

1.1.1 Airway opening

In most individuals, changes in airway pressure ($P_{ao}$) accurately reflect the corresponding changes of alveolar pressure ($P_{alv}$) generated by respiratory muscle contractions, even during dynamic manoeuvres when it is necessary to have a fast transmission of $P_{alv}$ to the airway opening [1]. The speed of this pressure transmission is affected by the flow resistance of the airways and by the compliance of the extrathoracic airways, and the compliance of the equipment [1]. In practice, however, compressibility of gas in the extra-thoracic airways does not pose a real obstacle to the transmission of $P_{alv}$ to the airway opening [1]. In patients with severe airway obstruction the delay in pressure transmission to the airway opening may cause underestimation of $\Delta P_{alv}$ [2].

Measurements of $P_{ao}$ during brief inspiratory occlusions (typically 0.1 second) applied without warning before the individual recognizes the occlusion and reacts to it (i.e. $P_{0.1}$) are a useful index of respiratory centre motor output [3]. $P_{0.1}$ has three determinants: (1) the neural command, (2) the conduction of the neural signal to the inspiratory muscles and (3) the pressure-generating capacity of the inspiratory muscles. Accordingly, a high value of $P_{0.1}$ always indicates intense neuroventilatory activity whereas low values may be difficult to interpret. $P_{0.1}$ is measured at the airway opening, accordingly, in presence of intrinsic positive end expiratory pressure (PEEP), $P_{0.1}$ may underestimate respiratory centre motor output.

Being generated by inspiratory efforts, $P_{0.1}$ values represent negative pressures, yet they are usually reported in positive units. In healthy subjects, the values of $P_{0.1}$ usually range between 0.5 and 1.5 cmH$_2$O during resting breathing [4]. The corresponding values in stable patients with chronic obstructive pulmonary diseases (COPD) range between 2.5 and 5 cmH$_2$O [4]. $P_{0.1}$ has been used to monitor respiratory centre motor output at rest and during exercise in young and elderly healthy subjects [5], in ambulatory children with cystic fibrosis (CF) [6], in ambulatory patients with COPD [7] and heart failure [8], in patients with neuromuscular disorders (NMD) [9], during titration of ventilator support [10, 11], weaning from mechanical ventilation [12] and to predict post extubation respiratory failure [13].
1.1.2. Oesophageal and gastric pressures

Oesophageal pressure (P_{oes}) and gastric pressure (P_{ga}) recordings provide valuable data on respiratory mechanics and respiratory muscle activity [14]. For instance, tidal changes in pleural pressure (∆P_{pl}) are accurately tracked by tidal changes in P_{oes} (∆P_{oes}) even in the supine position [1]. Swings in P_{oes} are obtained recording inspiratory (P_{oes,insp}) and expiratory (P_{oes,exp}) oesophageal pressures as the most negative and positive pressures during tidal breathing, respectively. The tidal P_{oes} swing (P_{oes,tid}) is the amplitude of the waveform between these two points (see figure 3). Other computations of respiratory muscle effort include work of breathing (WOB), pressure time product (PTP) and tension-time index (TTI) (see 1.4.2 Indices of respiratory muscle effort).

P_{oes} measured in supine critically ill patients is often greater than what many assume to be likely pleural pressures [15]. Factors that contribute to this finding may include the weight of mediastinal contents and a concurrent variable elevation of pressure within the coelomic cavity [15]. These mechanism, however, have been put into question by recent experimental findings in lung-injured pigs and human cadavers [16].

Simultaneous recordings of P_{oes} and P_{ga} can be plotted against each other to obtain the so called “pleural pressure-abdominal pressure diagram” [17]. With this diagram, it is possible to estimate the relative contributions of the diaphragm, rib cage inspiratory muscles, and abdominal muscles to tidal breathing [18]. The diaphragm also indicates how these muscles are coordinated during ventilation under different conditions [18]. In addition, simultaneous measurements of P_{oes} and P_{ga} permit the calculation of transdiaphragmatic pressure (P_{di} = P_{ga} - P_{oes}) [18]. Measurement of P_{di} is especially helpful in the diagnosis of severe weakness or paralysis of the diaphragm (see below). The ratio of P_{ga} over P_{di} is an estimator of diaphragm contribution to the tidal breathing [1]. A negative ratio suggests diaphragm dysfunction.

Despite data showing its usefulness in critically ill patients, P_{oes}, P_{ga} and P_{di} are still hardly used in the clinical setting [2]. This is partially due to technical issues, such as the insertion and proper placement of the catheters, the feasibility of obtaining accurate measurements, and the interpretation of the measurements.

1.2 Voluntary tests of respiratory muscle strength
1.2.1 Maximal static inspiratory (PImax) and expiratory (PEmax) mouth pressure

Measurements of maximal static respiratory pressures during forceful inspiratory (PImax) and expiratory (PEmax) efforts against an occluded airway reflect global inspiratory and expiratory muscle strength [18]. When the airway is occluded and the glottis is open, mouth pressure equals \( P_{alv} \) and reflects the pressure across the entire respiratory system [1].

PImax and PEmax vary with lung volume. This is because of the force–length relationship of the respiratory muscles and the varying contribution of passive elastic recoil pressure of the respiratory system [18]. Other sources of PImax and PEmax variability include the type of mouthpiece used, the pressure being evaluated (peak or plateau) and the number of trials performed. To standardize the measurement of PImax and PEmax, it has been recommended to measure the former at or close to residual volume (RV) and the latter at or close to total lung capacity (TLC), although measuring PImax at functional residual capacity (FRC) can also be an option to assess maximal inspiratory muscle strength close to operational lung volume [19].

Recordings of PImax and PEmax should be obtained by an experienced operator, who should strongly urge subjects to make maximum inspiratory (Mueller manoeuvre) and maximal expiratory (Valsalva manoeuvre) efforts. During testing, subjects are normally seated. They need coaching to prevent air leaks around the mouthpiece. Once the operator is satisfied, the maximum value of three inspiratory manoeuvres or three expiratory manoeuvres that vary by less than 10% are recorded. The system requires a small air leak (approximately 2-mm internal diameter and 20–30 mm in length) to prevent glottic closure during the PImax manoeuvre and to reduce the use of buccal muscles during the PEmax manoeuvre.

Ideally, inspiratory and expiratory pressures must be maintained for at least 1.5 s. This allows to record and report the maximum pressure sustained for 1 s. Pressure transducers should be connected to a computer screen to give visual feedback to the subject being tested through the display of the pressure-time curves and for the computations of the 1-sec plateau pressure. For clinical use, flanged mouthpieces are recommended even though they result in somewhat lower pressure values, especially for PEmax [19].

Reliability of the test is good if at least 5 attempts are performed, and better after an initial warm-up of the respiratory muscles [19-21]. Peak values should typically be achieved after 5-6 efforts for PEmax [21], and after 9 efforts for PImax [20].
In the previous ATS/ERS statement on respiratory muscle testing, a PImax of less than \(-80\) cmH\(_2\)O was proposed as a practical threshold to exclude clinically important inspiratory muscle weakness \([19]\). Alternatively, weakness can be defined based on the lower limit of the normal PImax using specific equations. In such a case, the presence or absence of respiratory muscle weakness is critically dependent upon the specific predictive equation being used \([22]\). For example, in a study of more than 1500 subjects, Rodriques et al. \([22]\) reported that the prevalence of weakness ranged from 33.4 to 66.9% according to the reference equation being used. In addition, the investigators noted how some predictive equations do relate better to clinical and physiologic indicators of respiratory muscles weakness. These observations suggest that there are specific predictive equations that might be particularly useful in screening patients for advanced respiratory neuromuscular assessment (see tables S2 and S4 for more details) \([22]\).

PImax can be affected by specific training \([23]\). Learning effects need to be acknowledged and sufficient baseline trials (at least 5 manoeuvres) have to be performed. Exercise of relatively short duration (<30 min) at high or maximal intensity (>75% VO\(_2\)max) results in reductions in PImax both in trained and in untrained individuals \([24, 25]\). These observations have been interpreted as a sign of exercise-induced respiratory muscle fatigue. Reductions in PImax have also been reported following marathon running in non-elite athletes who typically run for more than 3 h at a moderate exercise intensity (<70% VO\(_2\)max) \([26]\).

**Paediatrics.** Measurements of PImax and PEmax are restricted to cooperative children older than 6-8 years of age (table S14). Alternative techniques (airway pressure during crying as a surrogate for both PImax and PEmax and mouth whistle pressure as a surrogate for PEmax) are described below. The minimal number of measurements has not been validated in children. As children may be unable to comply with technical quality standards, peak inspiratory and expiratory pressures may then be used as simpler tests, and have shown their usefulness to predict severe chest infection in children with NMDs \([27]\).

In children, maximal pressures increase with age, and, as in adults, they are greater in males than in females \([28]\). Normal values have been established in large series of children of different ethnicities \([28-32]\). By 11-12 years of age, adult PImax values are reached in both sexes. Maximal pressures measured in infants and children are surprisingly high compared to adults. This seems to be related to the small radius of curvature of the rib cage, diaphragm, and abdomen, that according to the Laplace relationship, converts small tensions into relatively high
pressures [33]. Recordings of PImax and PEmax have a limited value in children with NMDs disease (Duchenne) because they are too difficult to perform [34].

In infants, mouth pressures generated during crying may provide an index of global respiratory muscle strength [35, 36]. The firm application of a rubber cushion mask against the face of an infant is generally sufficient to provoke crying efforts. An artificial leak in the mask prevents glottic closure. Only peak pressures can be recorded during crying. Mean peak crying PImax was -118 ± 21 cmH\textsubscript{2}O in a large group of healthy infants between the age of one month and two years and was independent of age and sex [36]. In some studies, mean peak crying PEmax was 125 ± 35 cm H\textsubscript{2}O and was related to body weight [36]. The main advantage of this test is its simplicity. Moreover, it is valuable in the assessment of infants with NMDs [35].

Mouth whistle pressure (PmW) is a simple and reproducible test to evaluate expiratory muscle strength in patients with amyotrophic lateral sclerosis (ALS) without bulbar dysfunction [37]. In children, PmW has the great advantage of its simplicity, audible feedback, playfulness and non-invasiveness. Aloui et al. [38] recently reported that PmW was closely related to oesophageal whistle pressure and gastric whistle pressure in children with NMDs. This observation confirms that noninvasive mouth pressure is a reliable reflection of P\textsubscript{oex} and P\textsubscript{ga} measurements in children and young adults with NMDs. PEmax and PmW were also highly related although with wide limits of agreement, mainly due to greater differences for the highest values. Indeed, a good agreement between the two tests was found to detect expiratory muscle weakness with 92% of the children being diagnosed as having muscle weakness by both tests.

ICU. Bendix and Bunker were among the first investigators to suggest that MIP might provide a useful reflection of respiratory reserve [39]. Patients who generate a PImax of -20 cmH\textsubscript{2}O during a 30-s occlusion of the airway are considered to display sufficient recovery from neuromuscular blockade to tolerate transfer to the recovery room. In a classic study, Sahn and Lakshminarayan [40] reported that all patients with a PImax more negative than -30 cmH\textsubscript{2}O were successfully weaned, whereas all patients with a PImax less negative than -20 cmH\textsubscript{2}O failed a weaning trial. Unfortunately, the accuracy of PImax in predicting weaning outcome varies considerably among studies. This is not surprising considering that studies differ in the technique for PImax recording (duration of occlusion), design (prospective, retrospective), methods of weaning (T-tube, pressure support, intermittent mandatory ventilation), definition of weaning success and failure. In patients requiring short-term mechanical ventilation, PImax commonly does not
differentiate between weaning success and weaning failure patients [12, 41-44]. Measurements of PEmax are not routinely used in intubated patients.

Voluntary manoeuvres are not always possible in the ICU due to poor patient cooperation. Other parameters may therefore be considered:

*Airway pressure contour.* Airway pressures of mechanically ventilated patients are continuously monitored. Any deviation from the relaxed configuration may indicate active contraction of inspiratory muscles.

*Breathing pattern.* Tidal volume (VT), respiratory rate (RR) and minute ventilation are easy to measure in intubated patients, and their values are continuously displayed on virtually all modern ventilators. Rapid shallow breathing is common in critically ill patients. Several challenges are susceptible to induce rapid shallow breathing including increased respiratory load, chemoreceptor stimulation, altered neuromechanical transmission, anxiety, fear and cortical influence. In the context of separation from mechanical ventilation, rapid shallow breathing is more likely to appear in patients failing a weaning attempt. Accordingly, RR/VT ratio is used as a predictor of weaning failure [41].

### 1.2.2 Maximal sniff nasal inspiratory pressure (SNIP)

The amplitude of a SNIP is not specific of diaphragm contraction because sniffing results from the coordinated action of several inspiratory muscles [45]. The high correlation between SNIP and Poes usually reported in healthy individuals [46, 47] is reduced in patients with airflow obstruction [46, 47] or individuals with nasal obstruction [48]. SNIP measurements in different populations are reproducible and, compared to PImax, are less prone to learning effects [49, 50]. In healthy subjects [51] and in patients with COPD [52], SNIP values have good within subject and between occasion repeatability.

The agreement between SNIP and PImax is variable. It has been suggested that when evaluating patients suspected of having inspiratory muscle weakness these two tests should be regarded as complementary and not interchangeable [48, 53]. SNIP is often recorded in the seated position. To avoid air leaks, one nostril is completely occluded by the pressure sensor (plug), while the other nostril is kept open. Often both nostrils are tested with 1 to 3 SNIP runs and the nostril conducive to the higher values is used for further testing.
While at FRC, subjects are instructed to make a short and fast sniff such that the peak pressure is not sustained. The duration of the sniff should be < 500 ms. Usually, 10 trials are sufficient to reach a plateau in SNIP values – and the highest value is selected [49]. More than 10 tests might be necessary when the SNIP value is below normal or to follow disease progression. SNIP have been successfully recorded in healthy individuals [50, 51, 54, 55], in patients with a variety of disease process including patients with COPD [52, 56, 57] and patients with NMDs [58-60]. The precision of SNIP to reflect swings in P_{oes} is good in healthy individuals [61, 62] and, as already noted, is reduced in patients with airflow obstruction [46, 47] and in patients with nasal obstruction [48]. The repeatability of SNIP, even in patients with COPD, is good [52]. There is a lack of studies on the prognostic role of SNIP in respiratory diseases. In a retrospective study in patients with severe COPD, SNIP was a better predictor of mortality than inspiratory capacity (IC)/TLC [57]. This topic merits further studies, considering that even patients with mild COPD display a reduced SNIP [56].

SNIP is less frequently used than PImax. In one study conducted in patients with moderate to severe COPD, inspiratory muscle training improved the perception of well-being and PImax, but not SNIP [63]. Lung volume reduction surgery results in improvements in SNIP one month after surgery; improvements in SNIP, however, do not correlate with improvements in exercise capacity, dyspnoea and lung function [64].

Four groups of investigators have published SNIP reference values for healthy adults [54, 55, 65, 66], and one group has published SNIP reference values for healthy children [67] (see table S9). Higher SNIP values were found in males and, in most studies, there is a positive correlation with age [54, 55, 66, 67]. The lower limit of a normal SNIP is around -70 cmH₂O in males and -60 cmH₂O in females, which was in agreement with the previous ATS/ERS statement [19]. The lower limits of a normal SNIP are significantly less in Japanese and Taiwanese individuals [55, 65]. Accordingly, when assessing a given individual, reference values obtained from the individual’s population of origin should be used.

**Paediatrics.** SNIP is a natural and simple manoeuvre that most children > 2 years of age can easily perform [67-70]. SNIP values in healthy children (> 6 years old) are similar to those recorded in healthy adults [67]. In healthy children and in children with inspiratory muscle weakness, SNIP provides a reasonable estimate of the inspiratory muscle strength [71].

As in adults, the main limitation of SNIP in children is the underestimation of inspiratory muscle...
strength in case of nasal obstruction (e.g., enlarged adenoids, nasal polyps), severe respiratory muscle weakness and airway obstruction (e.g., cystic fibrosis) [68].

Because of its simplicity, SNIP should be part of the routine evaluation of muscle strength in children with NMDs. SNIP was one of the 4 respiratory lung or muscle parameters that declined significantly with age in boys with Duchenne muscular dystrophy [34]. In these boys, SNIP declines earlier than peak expiratory flow (PEF) [72].

ICU. SNIP measurements are not possible in intubated patients, since there is no communication between the airway and the nostril (see below on the use of flap valves connected to the endotracheal tube to mimic sniff testing in intubated patients [73]).

1.2.3 Peak cough flow (PCF)

Peak cough flow (PCF) – also known as cough peak expiratory flow – has been described as early as 1966 [74]. The effectiveness of mucus clearance depends, among other factors, on an adequate PCF [75]. The act of coughing consists of the following steps: (1) inhalation ranging from 50% of Vt to 50% of VC [76], (2) tight glottic closure, (3) contraction of the expiratory muscles with the attendant rise in intrathoracic pressure to around 70 cmH2O to 400 cmH2O [76], 4) glottic reopening with biphasic turbulent air blast – with an initial peak (i.e., PCF) occurring within 30-50 ms after the glottic opening, followed by a flow-plateau phase of 200-500 ms when airflow is approximately 50% or less of PCF [75, 77].

PCF is usually measured with a hand-held, portable peak flow meter (PFM) [78]. While sitting up straight [78], subjects are instructed to inhale maximally and place the PFM mouthpiece in their mouth and seal their lips and teeth tightly around the mouthpiece. Then, subjects are instructed to cough as hard as they can. Usually, subjects repeat the procedure until they generate three PCF readings with <5% of each other. The highest of these three values is then reported [78].

There is a strong correlation and narrow limits of agreement between PCF values assessed via the pneumotachograph of a spirometer and the PCF values recorded with a portable PFM [78]. When PCF is <270 l·min\(^{-1}\), peak flow can be overestimated by the PFM [78].

PCF has been proposed to monitor expiratory muscle weakness and potential bulbar involvement in patients with NMD [79]. PCF <270 l·min\(^{-1}\) have been associated with increased of pulmonary complications during respiratory tract infections in patients with NMDs [78, 80]. In these
patients, the indication of manual / mechanical exsufflator/insufflator therapy is – among other criteria – based on PCF values.

Precise threshold values for PCF are not available [78]. Healthy subjects have been reported to reach mean PCF values of approximately 468 to 588 l·min\(^{-1}\) (significantly lower values for women than men); patients with NMDs achieve lower PCF values according to the type and stage of the disease [78, 81].

**Paediatrics.** Reference PCF values are available for children [82] (table S14). As for PImax and PEmax, children with NMDs can find it difficult to perform PCF manoeuvres – this is why in children with NMDs PCF values not necessarily correlate with age [34]. The PCF threshold for successful mucus expectoration in children with NMD has been reported to be >160 l·min\(^{-1}\) [27].

**ICU.** Intubated patients cannot close their glottis. Accordingly, intubated patients cannot properly cough. This means that in these patients it is impossible to measure PCF. Intubated patients, however, can huff [83]. The strength of a huff can be quantified measuring PEF during the huff. Cooperative patients can generate huffs. In non-cooperative patients (e.g., delirious, psychiatric conditions etc.), huffing can be induced using aerosolized normal saline solution [83].

In intubated patients, PEF less than 35 l·min\(^{-1}\) [84], 60 l·min\(^{-1}\) [85], 70 l·min\(^{-1}\) [86], or 80 l·min\(^{-1}\) [87], have been associated with extubation failure. This wide range of PEF thresholds plus the fact that all studies were single-centred and were carried out in specific patient population prevent the widespread adoption of this measurement in clinical decision making in intubated patients.

### 1.3 Voluntary manoeuvres with oesophageal and gastric pressures

Recordings of P\(_{\text{o}}\) and P\(_{\text{g}}\) signals during voluntary manoeuvres such as a sniff and a cough are useful in assessing respiratory muscle strength when non-invasive measures fail to provide clinically meaningful information due to anatomical, functional or behavioural causes. P\(_{\text{o}}\) recordings during a sniff are particularly useful when SNIP yields suspiciously low values such in patients with upper airway obstruction (hypertrophy of the adenoids, rhinitis, polyps) or lower airway obstruction (children with CF) [68]. P\(_{\text{g}}\) recordings during a cough are needed, for example, when the glottis function is compromised [88] such as in patients with bulbar ALS [89].
In patients without the above specific impairments, assessment of $P_{oes}$ and $P_{ga}$ may not be necessary as SNIP and PCF correlate well with these measures [90]. However, intra-thoracic and abdominal pressures may be used to refine the diagnosis [21, 53]. In many subjects the value of sniff-$P_{di}$ is greater than that of $P_{Imax}$ [91] and the value of cough-$P_{ga}$ is greater than that of $P_{Emax}$ [21].

For both sniff and cough, strong patient encouragement is required to achieve maximal performance. Multiple attempts with adequate breaks (30 s) are needed to reach a plateau of these measurements [92]. It is advisable to perform more than 3 and up to 10 attempts after a plateau is reached. Visual feedback on a computer screen is a simple and engaging tool to motivate subjects, particularly children [68].

A sniff is usually reported as the pressure difference between baseline and peak pressure. In addition, it is possible to calculate the sniff’s maximal relaxation rate (MRR) – i.e., maximal decrease in pressure or $dP/dt$. The sniff’s MRR can give information on respiratory muscle function including early fatiguing state, selective fiber recruitment, muscle function in patients with thyroid diseases and in malnourished patients [58, 93]. Because MRR is pressure-dependent, the MRR is normalized by dividing by $dP/dt$ by peak pressure [94]. This allows to compare the MRR of sniffs of varying intensity [94].

In adults, the average within-subject, between-occasion CV is higher for sniff-$P_{di}$ (11%) [92] than for cough-$P_{ga}$ (6.9%) [21] (table 2 in main text). No such values are available for children. For sniff-MRR, only individual within-subject, between-occasion CVs have been reported and they range from 6% to 26% [95].

In a study of 64 subjects (37 males, 27 females), Man et al. [21] reported reference values for sniff-$P_{oes}$, $P_{ga}$, and $P_{di}$. No reference values for cough-$P_{ga}$ are yet available. The range between individuals is quite large and reported ranges in healthy subjects are given in table 2 (main text). In many disease states, pressures produced during a sniff (e.g., sniff-$P_{oes}$) and during a cough (i.e., cough-$P_{ga}$) are lower than normal both in adults [21, 53, 90] and children [38, 70, 96], as are non-invasive measures of static ($P_{Imax}$, $P_{Emax}$) or dynamic (SNIP, PCF) respiratory muscles strength and function. Thus, it is important to know what additional diagnostic information on respiratory muscle function can be obtained by measuring pressure signals using balloon (or other pressure-tip) catheters. For example, patients with multiple sclerosis [90] generate a significantly lower cough-$P_{ga}$ (109±46 cmH$_2$O) than controls (150±34 cmH$_2$O). In turn, cough-
$P_{ga}$ can be predicted by measuring PCF. This favours the use of the less invasive technique (PCF) than the more invasive one (cough-$P_{ga}$).

In patients with lung disease, including patients with COPD [95], pulmonary fibrosis [95], CF [68] and patients with systemic lupus erythematosus who develop ‘shrinking’ lung syndrome [95], sniff-$P_{oes}$ recordings help avoid underestimation of inspiratory muscle strength by use of SNIP or Plmax only. In patients with heart failure, a reduced sniff-$P_{di}$ (103±21 cmH$_2$O) correlates with reduced cardiac output [97].

Sniff-$P_{oes}$ may underestimate diaphragm dysfunction such as in patients with bilateral diaphragm paralysis [98]. This is because these patients increasingly recruit rib cage and neck muscles during a sniff manoeuvre. In patients with unilateral diaphragm paresis, Verin et al. [99] observed a significant reduction in sniff-$P_{oes}$. This reduction was positively correlated with the time elapsed from onset of symptoms to respiratory muscle testing [99].

In a large cohort of patients with mixed diagnoses (156 NMD, 94 dyspnoea of unknown origin, 45 COPD, 37 rheumatologic disease and 81 other diseases), Steier et al. [53] assessed to which extent adding $P_{oes}$ or $P_{ga}$ would improve diagnosis of respiratory muscle weakness. These authors reported that using a single test such as Plmax or PEmax, tends to overdiagnose respiratory muscle weakness. Measuring both, Plmax and SNIP, reduced the false-positives by 20%, while adding sniff-$P_{oes}$ did not significantly improve the rate of false-positive tests. When diagnosing expiratory muscle weakness, adding cough-$P_{ga}$ to PEmax decreased false-positives by 30% [53].

In accordance with the previous study, Tzani et al. [81] reported that adding cough-$P_{ga}$ (53% positive) to PEmax (51% positive) and PCF (27% positive), reduced positive cases to 20%.

Man et al. [21] have assessed the value of adding cough-$P_{ga}$ measurement in a mixed group of 99 patients with respiratory muscle weakness. They found that cough-$P_{ga}$ had a much better positive predictive value (94%) than PEmax (58%). In fact, 43% of patients with low PEmax had normal cough-$P_{ga}$ while of 105 patients with low cough-$P_{ga}$, only 6% had normal PEmax. These results suggest that cough-$P_{ga}$ may be useful to exclude expiratory muscle weakness in patients with reduced PEmax.

Voluntary manoeuvres are not always sufficient to elucidate the pathophysiological mechanisms. For example, compared to healthy controls, in patients with hemispheric stroke [100], sniff-$P_{oes}$ (58±37 vs. 109±29 cmH$_2$O), sniff-$P_{di}$ (63±41 vs. 121±39 cmH$_2$O) and voluntary cough-$P_{ga}$ (99±62 cmH$_2$O vs. 209±62 cmH$_2$O) are lower than normal. In contrast, $P_{ga}$ during a reflex cough
is not significantly different (179±78 vs. 208±77 cmH₂O) than normal. These results suggest that the voluntary initiation of muscle contraction contributing to lower values.

The value of sniff-P_{di} and cough-P_{ga} as prognostic tools has been marginally studied. In 98 ALS patients, Morgan et al. [60] assessed whether SNIP and sniff-P_{di} could predict the risk of desaturation during sleep and the hazard ratio for death. Sniff-P_{di} correlated well with SNIP (r=0.9, p>0.01) and SNIP values <40 cmH₂O were associated with desaturation during sleep (no such correlation for FVC or PImax). The hazard ratio for death in this group of patients was 9.1 (95% CI, 4 – 20.8%). These results suggest that it is unnecessary to record sniff-P_{di} in ALS patients to assess their prognosis.

Polkey et al. [59] reported that sniff-P_{di}, sniff-P_{oes} and transdiaphragmatic twitch pressure elicited by magnetic stimulation of the phrenic nerves (P_{di,tw}) could predict ventilation-free survival in a group of 78 ALS patients – i.e., for 3-yr ventilation-free survival, sniff-P_{di} cut-off was 108.5 cmH₂O with a sensitivity of 0.85 and a specificity of 0.98. In the same study, PEmax and particularly P_{di,tw} were predictors of survival [59].

Sniff-P_{di}, sniff-P_{oes} and P_{di,tw} and cough-P_{ga} are seldom used to assess response to interventions. After lung volume reduction surgery, for example, sniff-P_{di} [64, 101], SNIP [64], PImax [64, 101] and P_{di,tw} [102] increased significantly. In patients with COPD completing an exhaustive treadmill walk, sniff-P_{oes} did not change from pre-exercise values [103]. Sniff-P_{oes}-MRR, however, decreased by 42%, and recovered within 5 min of rest [103]. This transient decrease in sniff-P_{oes}-MRR suggests development of inspiratory muscle fatigue.

**Paediatrics.** The measurement of P_{oes} and P_{ga} during voluntary manoeuvres such as sniff and cough is particularly useful in children (table S14). As noted, SNIP can underestimate inspiratory muscle strength in patients with upper airway obstruction (hypertrophy of the adenoids, rhinitis, polyps) and lower airway obstruction (children with CF) [68]. Accordingly, in these patients, a low SNIP value should prompt measurement of sniff-P_{oes}.

The measurement of P_{oes} and P_{ga} may also suggest diaphragmatic dysfunction as reported in children with collagen type VI (ColVI) myopathies [96] and selenopathies [104].

In children as in adults, expiratory muscle strength can be measured during a maximal cough (P_{ga}-cough) [21, 38, 70]. Visualization of the P_{ga} signal during the cough on a computer screen is a simple and playful tool to motivate a child to perform a maximal manoeuvre [21, 38, 70].

**ICU.** In intubated patients instrumented with oesophageal and gastric balloons it is possible to
record maximal P_{oes} (P_{oes,max}) and maximal transdiaphragmatic pressure (P_{di,max}) during forceful inspiratory efforts. P_{oes,max} can be used to evaluate global inspiratory muscle strength while P_{di,max} can be used to evaluate diaphragmatic strength. In most patients the contribution of P_{ga} to P_{di} during a maximal inspiratory effort is minimal-to-none (see figure 4 in Laghi et al. [105]). Accordingly, in these patients, a simple P_{Imax} can give similar information afforded by P_{oes,max} and P_{di,max}.

Intubated patients cannot sniff naturally because the upper airway is bypassed by the endotracheal or tracheostomy tube. To overcome this obstacle, Goldstone et al. [73] used a flap valve in 19 alert and cooperative intubated patients. The flap valve occluded flow during inspiration thereby allowing patients to generate a "sniff-like" inspiratory waveform. In the study, 14 out 19 patients were able to perform sniff-like manoeuvres. Unfortunately, the investigators did not report the sniff-P_{oes} or sniff-P_{di} values recorded in the study nor the intra or inter-observer reproducibility of sniff pressures. What role sniff manoeuvres may play in the assessment of respiratory muscle function in intubated patients remains to be determined.

1.4 Respiratory muscle-related mechanics of breathing

1.4.1 Pressure-related measurements during inspiratory capacity (IC)

IC is the maximal volume of air that can be inhaled to TLC after a quiet exhalation to end-expiratory lung volume (EELV). The main determinants of resting IC in patients with COPD include the magnitude of the resting EELV (inverse relation), the strength of the inspiratory muscles, and the combined elastic properties of the lung and chest wall [106-123]. Resting IC is an indirect measure of lung hyperinflation only in patients with COPD whose TLC is not decreased to less than the lower limit of normal; e.g. no coexistent inspiratory muscle weakness, lung or chest wall restriction. In patients with milder airway obstruction and in some patients with very advanced COPD, TLC and EELV may rise in tandem to a similar extent thus preserving IC [124]. In patients with COPD and moderate obesity, expiratory reserve volume and EELV are diminished to a greater extent than TLC leading to preservation or increase in IC compared with normal weight individuals with similar forced expiratory volume in 1 second (FEV\textsubscript{1}) [125].

IC represents the operating limits for V\textsubscript{T} during exercise in elite athletes and patients with
respiratory disorders. In obstructive lung disorders (COPD), TLC and IC are reduced “from below” with an increase in EELV. In restrictive lung disorders (interstitial lung diseases, inspiratory muscle weakness, chest wall restriction), TLC and IC are reduced “from above” without an increase in EELV. Regardless of the underlying disorder, and in the absence of inspiratory muscle weakness, a reduced IC indicates close proximity of VT to TLC – at the upper less compliant reaches of the respiratory systems s-shaped pressure-volume relaxation curve where the inspiratory muscles are under a significant disadvantage [126]. In these patients early mechanical constraints (and high VT/IC ratios) are evident at relatively low exercise intensity and correlate with increased ratings of dyspnoea [115, 117-119, 127]. In patients with asthma [108, 112], pulmonary arterial hypertension (PAH) [109, 111] and in patients with chronic heart failure (CHF) [114], airway dysfunction with the resultant progressive reduction in IC during exercise (dynamic lung hyperinflation) have important mechanical and sensory consequences [119].

IC’s repeatability and reliability, its predictive, discriminative and evaluative value and its minimal clinical important difference (MCID) have been extensively described elsewhere [122]. The accuracy and construct validity of serial IC measurements during exercise to track change in EELV has been confirmed in a number of studies in COPD. One large retrospective analysis examined test re-test repeatability of IC during rest and exercise in 463 patients with moderate to severe COPD entered in multi-center, multi-national clinical trials designed to test efficacy of bronchodilators. Within-subject coefficient of variation (CV) for IC at rest, at a standardized time during exercise and at peak exercise was 9.5%, 10.8% and 11.6%, respectively. Intra-class correlation (with 95% confidence interval) for IC at rest, standardized time and peak exercise was 0.89 (0.87- 0.91), 0.88 (0.86-0.9) and 0.87 (0.85-0.89), respectively. While IC measurements have been shown to be robust in diverse international clinical research settings, data are lacking on its reliability as an evaluative instrument in clinical practice.

In patients with COPD, the ratio of IC to TLC can predict respiratory and all-cause mortality and the risk and severity of exacerbations [128-130]. There is strong evidence that lung hyperinflation and attendant reduction in IC is closely linked to the severity of dyspnoea experienced by patients with COPD during physical activity. Although exercise limitation in these patients is multi-factorial, respiratory mechanical factors are undoubtedly important. In this context, resting IC is a good predictor of peak ventilatory capacity and peak oxygen uptake in
COPD. Moreover, therapeutic reversal of lung hyperinflation, with improvement of IC, has been shown to be associated with improved dyspnea and exercise endurance [122]. Cross-sectional studies have confirmed that significant differences in IC are discernable across quartiles of severity of airway obstruction based on spirometry. Thus, IC recordings provide additional information about the individuals exercise capacity and dyspnea beyond simple spirometry [122].

In multiple clinical trials, bronchodilators of all classes and duration of action have been shown to increases IC [122]. Generally, bronchodilator-induced improvements in resting IC range from 0.2- 0.4L or 10-15% of the baseline value. Besides bronchodilator therapy, any intervention that reduces inspiratory neural drive and thus breathing frequency such as hyperoxia, helium-oxygen, or exercise training (by delaying metabolic acidosis) has the potential to reduce the rate of increase of EELV during exercise (by prolonging expiratory time), thereby improving dyspnea by delaying the onset of mechanical limitation [122]. Changes in IC during exercise have also mirrored improvement in dynamic respiratory mechanics following lung volume reduction procedures in patients with COPD and following bi-ventricular pacing in patients with CHF [122].

IC measurement is simple, safe and easy to perform. IC manoeuvres are carried out at the end of a steady-state resting baseline period (approximately 3 minutes) until at least 2 reproducible efforts are achieved (i.e., ±100 mL or within approximately 10% of the largest acceptable value). IC measurements at rest should not be performed closer than 1 minute apart, and measurements should not be repeated until breathing has returned to the pre-manoeuvre pattern [122].

As is the case with other spirometric and plethysmographic indices, no minimal clinically important difference value has been clearly established for indirect measurements of lung hyperinflation such as IC. From experience to date, a post-intervention change in IC of ~0.2L at rest or at a standardized time during exercise (or ~15% predicted) could be considered clinically meaningful [122]. More specifically, such changes have been consistently associated with increased exercise endurance time by at least 60 seconds or in the order of 20-30% in patients with moderate to severe COPD. The IC manoeuver is reliable in evaluating dynamic hyperinflation during cardiopulmonary exercise testing (CPET) in patients with COPD, asthma, PAH and CHF. This is because TLC does not change during exhaustive cycle exercise neither in healthy subjects [131] nor in patients [113, 116, 132, 133].
A dynamic decrease in IC during exercise can be caused by true dynamic hyperinflation or by impaired inspiratory muscle performance (weakness/fatigue). Previous studies have assessed the reliability of IC manoeuvres by comparing inspiratory $P_{oes}$ values during IC manoeuvres, and clearly demonstrated that IC-$P_{oes}$ values were remarkably preserved during exercise and independent of exercise intensity and ventilation in COPD [117, 118, 134], CHF [132] and PAH [116] (figure 3). The contention that these patients were able to inhale to a lung volume close or equal to TLC was bolstered by the evidence that end-exercise static lung compliance was remarkably preserved compared with pre-exercise static lung compliance. This finding suggests that in healthy subjects [135], and in patients with COPD [134], PAH [116], and CHF [132] the elastic recoil pressure of the lung does not change during exercise. All these observations suggest that exercise-induced changes in EELV can indeed be reliably monitored with IC manoeuvres. Exercise-induced inspiratory muscle fatigue, if present, does not seem to be sufficient to contribute to decreases in IC during exercise because of the stable IC-$P_{oes}$ during CPET (figure S1) and identical sniff-$P_{oes}$ values pre/post-exercise in these patients. Although IC-$P_{oes}$ and sniff-$P_{oes}$ measures during CPET can rule out a potential inspiratory muscle fatigue, their predictive, discriminative and evaluative value along with their respective MCID have not yet been described. Further studies are therefore needed in this regard.

1.4.2 Indices of respiratory muscle effort

Computation of tidal swings in $P_{oes}$ and $P_{di}$, WOB, PTP and TTI can be used to assess the pressure output of the respiratory muscles [136]. In common usage, the expression WOB is often understood to be synonymous with breathing effort [137]. WOB, however, is technically defined in the mechanical and not biologic terms. Mechanical work (W) occurs when pressure (P) changes the volume (V) of matter: $W = P \times V$. In the case of the respiratory system, mechanical (external) WOB can be calculated by measuring the generation of intrathoracic pressure (i.e., change in $P_{pl}$) due to contraction of the respiratory muscles and the displacement of gas volume [136]. In spontaneously breathing subjects, tidal changes in $P_{pl}$ can be estimated measuring changes in $P_{oes}$[136].

PTP is calculated as the time integral of the area between $P_{oes}$ and the recoil pressure of the chest wall (PTP$_{oes}$) or as the time integral of the area between baseline $P_{di}$ (resting end-expiratory $P_{di}$) and $P_{di}$ during the inspiratory effort (PTP$_{di}$) [137]. PTP$_{oes}$ reflects the effort done by all of the
respiratory muscles and PTP$_{di}$ reflects mostly the effort done by the diaphragm [138, 139].

TTI is an estimate of inspiratory effort relative to respiratory muscle strength. Generally speaking, TTI is calculated as the product of respiratory duty cycle (inspiratory time divided by the time of a total respiratory cycle or T$_{i}$/T$_{TOT}$) and mean inspiratory pressure per breath divided by the maximum inspiratory pressure [137]. The effort done by all of the respiratory muscles, or TTrc, is calculated as the product of mean inspiratory P$_{oes}$ divided by P$_{oes,max}$ and T$_{i}$/T$_{TOT}$: TTrc = (P$_{oes}$/P$_{oes,max}$) x (T$_{i}$/T$_{TOT}$). The effort done by the diaphragm, or TTdi, is calculated as the product of mean inspiratory P$_{di}$ divided by P$_{di,max}$ and T$_{i}$/T$_{TOT}$: TTdi = (P$_{di}$/P$_{di,max}$) x (T$_{i}$/T$_{TOT}$).

Measurements of WOB and PTP have been used to estimate the energy dissipated or consumed by the respiratory muscles [139]. Measurements of WOB, however, underestimate respiratory oxygen consumption during isometric contractions [142]. In addition, WOB does not account well for the duration of muscular contraction and for the decreased efficacy of respiratory muscle contraction and the chest wall distortion under loaded conditions. PTP circumvents some of these limitations of WOB [140]. PTP estimates derived from measurements of P$_{di}$ (PTP$_{di}$) seem to better reflect oxygen cost of breathing than PTP estimates derived from measurements of P$_{oes}$ (PTP$_{oes}$) [141]. PTP has been used to quantify respiratory muscle effort in healthy subjects [143, 144] and patients with respiratory disorders [105, 145-147].

When computing WOB and PTP, it is recommended to include that portion of WOB and PTP necessary to expand the chest wall. This can be accomplished directly by measuring the compliance of the chest wall or indirectly by assuming that the compliance of chest wall amounts to 4% of the predicted vital capacity (VC) per cmH$_2$O [15]. Because the pressure necessary to expand the chest wall during tidal breathing is usually small, some investigators ignore its contribution to WOB or PTP.

PTP can be separated into several components: effort made to overcome intrinsic PEEP such as in patients with COPD, effort to inflate the chest and, in the specific case of mechanically ventilated patients, the effort to trigger the ventilator [136]. An important technical challenge in the computation of PTP is the accurate identification of the beginning of inspiratory effort, particularly when intrinsic PEEP is present [2]. This because patients with intrinsic PEEP often recruit the expiratory muscles during exhalation [148]. Sudden relaxation of the expiratory muscles at the end of exhalation causes a sudden decrease in P$_{oes}$ that can be easily confused with the sudden decrease in P$_{oes}$ that accompanies inspiratory muscle contractions [136]. To correct
for expiratory muscle contribution to intrinsic PEEP and to better define the beginning of
inhalation, it is useful to record $P_{ga}$ [148].

During resting breathing, healthy subjects generate a $PTP_{oes}$ of around 100 cmH$_2$O·sec·min$^{-1}$ [137]. The $PTP_{oes}$ of patients with COPD at rest is twice that in healthy subjects [149]. The average $PTP_{oes}$ of patients in acute respiratory failure can be 4 to 6 times higher than in healthy subjects [137, 150]. During spontaneous breathing trials, increases in respiratory effort reflected by increases in $PTP_{oes}$ are predictive of weaning failure [150].

A simplified strategy to monitor global inspiratory effort consists in measuring $P_{oes,tid}$ (figure 3) [151]. $P_{oes,tid}$ can be expressed as an absolute value or relative to $P_{oes,max}$ ($P_{oes,tid}/P_{oes,max}$). $P_{oes,tid}$ is less precise than measurements of $PTP_{di}$ yet, it has been successfully applied as bedside monitoring tool in sleep studies [152], and during weaning trials [153]. $P_{oes,tid}$ (in analogy with the $PTP_{oes}$) showed larger changes over the course of a failed weaning trial than breathing pattern parameters (rapid shallow breathing index) [150, 153]. $P_{oes,tid}$ can also serve as a useful index of global respiratory muscle effort during exercise in patients with chronic respiratory disease [154]. During exercise, increases in $P_{oes,tid}$ swings relative to stable tidal volume responses are related to the perception of dyspnoea [117, 154].

$TT_{rc}$ and $TT_{di}$ have been used to identify potentially fatiguing contractions of the respiratory muscles [155]. Healthy subjects cannot sustain indefinitely respiratory loads that require the generation of a $TT_{rc}$ greater than 0.30 [156] or a $TT_{di}$ greater than 0.15, 0.18 [157] (In healthy subjects, a sustained increase in $TT_{di}$ above 0.15 leads to diaphragmatic fatigue even before task failure [158]). Reliable calculation of $TT_{rc}$ and $TT_{di}$ is critically dependent on an accurate measurement of $P_{oes,max}$ and $P_{di,max}$, respectively [157]. Unfortunately, in critically ill patients, ‘maximal’ inspiratory voluntary efforts often underestimate maximum respiratory muscle strength [105]. This is because critically ill patients are unable to activate completely the diaphragm during ‘maximal’ voluntary inspiratory maneuvers [105]. Such underestimation of $P_{di,max}$ necessarily produces an overestimation of $TT_{di}$ – one of the reasons why patients who fail a trial of weaning from mechanical ventilation not develop contractile fatigue of the diaphragm despite generating $TT_{di}$ above 0.15 [105].

Variable resistive and elastic unloading of the respiratory muscles by either continuous positive airway pressure or inspiratory pressure support can reduce inspiratory effort. Reduced effort has been associated with reduced dyspnea and improvements in exercise capacity in patients with
COPD [127, 145, 159]. Reductions in the resistive WOB and dynamic hyperinflation by helium-hyperoxia also reduce inspiratory effort and dyspnea [127, 160]. Finally, breathing exercises that promote slow and deep breathing can reduce elastic resistance during breathing and thereby reduce inspiratory effort [161-163]. In addition, changes in inspiratory duty cycle (decreased Ti/Ttot) induced by these breathing techniques can further reduce PTP by reducing inspiratory time per minute [164].

1.4.3 Flow-Volume Loop to Evaluate Expiratory Flow Limitation

The flow-volume loop technique permits the evaluation of expiratory flow limitation (EFL) at rest [165] and during exercise [166]. Evaluation of the expiratory limb of the maximum flow-volume loop can also be used to assess expiratory muscle weakness. Severe expiratory muscle weakness is suggested by a sudden decrease in maximum expiratory flow toward RV [89].

EFL can be assessed by positioning the resting and the exercise tidal flow-volume loops within the pre- or the post-exercise maximum flow-volume loop taking care that the tidal flow-volume loop starts from EELV [166]. EFL is present when the expiratory limb of the tidal flow-volume loop encroaches or exceeds the expiratory limb of the resting maximum flow-volume loop [165]. The severity of EFL can be quantified calculating the percentage of VT that encroaches or exceeds the expiratory limb of the maximum flow-volume loop.

Performing forced expiratory manoeuvres to generate the maximum flow-volume loop is safe in the general population [167, 168].

Although the flow-volume loop technique is widely used, there are many methodological limitations that can impact the validity of the technique. These include thoracic gas compression artifacts [169, 170], presence of exercise-induced bronchodilation [169] or exercise-induced bronchoconstriction [171], differences in the time and volume history preceding maximal and tidal exhalations [172-174], time-constant inequalities [175, 176], and poor patient cooperation and effort. Accurate alignment of the tidal and maximal expiratory flow-volume loops during exercise also depends on the accuracy of IC manoeuvres. Studies in obstructive pulmonary diseases show that EFL is over-estimated using the flow-volume loop method compared to the negative expiratory pressure (NEP) technique (see below) [177-182].
1.4.4 Negative Expiratory Pressure (NEP)
The NEP technique allows the evaluation of EFL at rest and during exercise [183]. This technique can also assist in the evaluation of patients with suspected increased upper airway collapsibility [184, 185]. During normal exhalation, a negative pressure is quickly applied at the airway. If EFL is present, the resulting expiratory flow is not greater than the expiratory flow of the preceding tidal exhalation [181, 183, 186, 187]. EFL can be quantified using the 3-point score or the 5-point score system [188]. EFL can also be quantified by examining the percentage overlap between the NEP breath and the preceding control breath(s) [189]. In patients with COPD, EFL is poorly reproducible when it is assessed using the percentage overlap NEP method [179, 188]. In contrast, EFL is highly reproducible in patients with and without COPD when EFL is assessed using the 3-point score or 5-point score NEP method [188, 190]. NEP has been validated against direct measurement of iso-volume, flow-pressure relationships in mechanically ventilated patients [189]. The NEP technique is a safe, non-invasive procedure that causes no discomfort [183].

The use NEP has been primarily limited to the research setting. The presence of EFL identified using the NEP technique has been associated with dyspnoea on exertion in patients with COPD [183], exercise limitation [180], and as an indicator of worsening COPD [191]. NEP has also been used in healthy infants [1932], in children with asthma and CF [193], in patients with CHF [4], obstructive sleep apnoea syndrome (OSAS), in obese patients without OSAS [195], in obese subjects during exercise [197], in spinal cord injured patients [196], in patients with restrictive disorders [174, 198], in elite athletes [174, 198] and in ICU patients [189].

NEP might help determine whether maximal expiratory flow has been achieved, i.e., decreased maximal expiratory flows may be suggestive of expiratory muscle weakness or lack of coordination of expiratory muscles but there is no data of NEP being used this way.

1.4.5 Inspiratory Flow Reserve (on the flow volume loop)
Maximal inspiratory flow-volume curves are universally recorded during standard pulmonary function testing, a standard procedure with minimal safety concerns [19, 199].

Maximal inspiratory flow-volume curves can be reduced as a result of extrathoracic upper airway obstruction and, in the effort-dependent regions of the curve, as a result of respiratory muscle weakness, poor effort, and poor performance of the manoeuvre [19, 200]. Only a small
percentage of tests indicate abnormalities including inspiratory muscle weakness [201]. The latter observation does not detract from the fact that visual examination of the maximal inspiratory flow-volume loop may still suggest weakness [19, 202]. Maximal inspiratory flow-volume loops have greater variability than the VC manoeuvres and reference values for inspiratory flow may present problems with interpretation [19].

Inspiratory flow oscillations (saw-tooth) may suggest upper airway and surrounding muscular abnormalities [19, 203] including neuromuscular diseases, Parkinson’s disease, laryngeal dyskinesia, pedunculated tumours of the upper airway, tracheobronchomalacia and upper airway burns [201]. Inspiratory flow oscillations have also been reported in snorers without OSAS, in patients with OSAS and in about 10% of healthy individuals [201]. Unfortunately, inspiratory flow-volume curves are not sufficiently sensitive to diagnose upper airway obstruction. This because lesions must narrow the tracheal lumen to less than 8 mm (i.e., a reduction of the tracheal by at least 80 percent) before abnormalities in the flow-volume curves can be detected [201]. By examining PEF, the slope of ascending limb of the maximal expiratory curve, the drop in forced expiratory flow near residual volume, and the inspiratory flow at 50% VC, better predictability may be achieved [202].

As already noted, the quality of maximal inspiratory flow-volume curves depends on patient motivation and cooperation. Moreover, there are more specific tests of muscle weakness than maximal inspiratory flow-volume curves including recordings of VC, SNIP and Pdi,tw [204, 205]. In clinical practice it is useful to use a battery of respiratory muscle tests (PImax, PEmax) and pulmonary function tests (VC, FVC, and less so maximal voluntary ventilation -MVV) to assess respiratory muscles weakness [206, 207].

1.4.6 Maximum Voluntary Ventilation (MVV)

MVV (l·min⁻¹) is produced voluntarily in 12 to 15 s during standard pulmonary function testing as described in the ATS/ERS statement [200] or estimated as forced-expiratory volume in 1 s (FEV₁) × 35 or 40 [19]. *(For additional discussion of the MVV manoeuvre please see section 1.6 Respiratory muscle endurance testing).*

1.5 Evoked manoeuvres
Measurements of transdiaphragmatic pressure elicited by electrical or magnetic stimulation of the phrenic nerves or $P_{di,tw}$ can be used to evaluate diaphragmatic contractility. When the glottis is open, swings in $P_{oes}$ are evident in the upper airway (i.e. the mouth or, if the patient is intubated, the endotracheal tube). Phrenic nerve stimulation is usually done using magnetic stimulators unless patients have pacemakers or other implanted electronic devices [208]. In these patients, electrical stimulation is preferred [209].

Several important factors need consideration since they may change the amplitude of $P_{di,tw}$

i. Nerve stimulation must be supramaximal to be reliable and needs special consideration in obesity (insulation) or with muscle activity (the activation threshold of motor nerve axons increases after minutes of repetitive use [210, 211]). Confirming supramaximality requires accurate measurement of the electromyographic signal, or the amplitude of $P_{di,tw}$ as stimulation output is increased.

ii. Prior contractile activity may lead to a ‘falsely high’ $P_{di,tw}$ through a phenomenon known as twitch potentiation [212]. For this reason, stimuli should be delivered after a minimum rest period of 10 to 20 min. If changes in diaphragmatic contractility immediately after an activity such as exercise are of interest [213], one option is to assess potentiated $P_{di,tw}$ both before and just after the exercise. This, however, bears the risk of ‘incomplete’ potentiation if the voluntary inspiratory contractions used to elicit potentiation are less than 60% of $P_{di,max}$ [214].

A change in lung volume affects diaphragm length and pressure output - e.g. hyperinflation leads to diaphragm shortening and a reduction in $P_{di,tw}$ [209, 215], mainly through a reduction in the oesophageal pressure twitch ($P_{oes,tw}$) [216]. Thus, to obtain reproducible measurements, the subject should be relaxed at consistent EELV. Also, interpreting changes of $P_{di,tw}$ over time should be done with caution if a patient has received interventions which might change lung volume [217]. Typically, a change of 0.3 cmH₂O/unit % VC may be considered a reasonable correction factor [218].

iii. Several magnetic stimulation techniques for the phrenic nerves are available. These include cervical stimulation [219], anterior mediastinal stimulation [220], and uni- [221] or bilateral anterior simulation [221]. Most specialist units prefer the latter as it more often results in supramaximal stimulation of the phrenic nerves than the other approaches.
Magnetic stimulation of the phrenic nerves may also be applied as single [144] or paired [222] stimuli of different stimulation frequencies (both lung volume and fatigue can affect the magnitude of $P_{di, tw}$ elicited as a function of interstimulus interval [218, 222]).

iv. Reliability: In healthy subjects, the mean between-occasion variability in $P_{di, tw}$ is $20 \pm 11\%$ and CV $11\%$ [92]. The limits of agreement of the difference of $P_{di, tw}$ recordings is $\pm 6$ cmH$_2$O [92].

A non-invasive estimate of $P_{di, tw}$ may be obtained by measuring pressure change in the upper airway or mouth ($P_{mo, tw}$), reflecting $P_{oes, tw}$ quite closely [42, 223-225]. Whilst this obviates the need to pass oesophageal and gastric balloons, most investigators have found it necessary to instruct non-intubated patients to make a small inspiratory or expiratory effort to ensure the glottis is open [224, 226]. This may be conveniently done using an electronically triggered valve that briefly closes the airway during phrenic nerve stimulation [223, 227]. In interpreting $P_{mo, tw}$ results, it should be recalled that the overall signal of origin (i.e., $P_{oes, tw}$) is smaller (typically $P_{oes, tw}$ is around 50% of $P_{di, tw}$) and the ‘noise’ (e.g. due to cardiac contraction) is constant. Furthermore, $P_{oes, tw}$ (and therefore $P_{mo, tw}$) is more influenced by lung volume than $P_{di, tw}$ [218].

In several disease states, muscle weakness is seen with $P_{di, tw}$ values below 18 cmH$_2$O, the purported cut-off value suggested to diagnose diaphragm weakness [53]. At the end of exhaustive exercise, diaphragm fatigue (defined as $\geq 10\text{-}15\%$ reduction in $P_{di, tw}$), can be documented in about 70% of healthy subjects. In contrast, diaphragm fatigue has been reported in some patients (COPD [228], low back pain [229]) but not in other patients (COPD [230], CF [231], CHF [232], interstitial lung disease - ILD- [233]). Although pre-exercise respiratory muscle fatigue impairs exercise performance [234, 235], development of fatigue is not directly related to performance in healthy subjects [236] and it does not predict outcomes in the clinical setting [105].

Stimulation of the thoracic nerve roots with simultaneous measurement of $P_{ga}$ can be used to evaluate the main expiratory muscles, i.e. the abdominal muscles. Supramaximal stimulation of the thoracic nerve roots is difficult to obtain using currently available technologies. Abdominal muscle contractility is assessed mainly in the context of development of expiratory muscle fatigue during exercise. Resting gastric twitch pressure ($P_{ga, tw}$) values have a slightly higher variability (CV 9-10%) [237] than $P_{di, tw}$ (6%) [237]. $P_{ga, tw}$ decreases by up to 20% after expiratory muscle activity such as volitional heavy breathing [143, 237] or exercise [238] in the
healthy. In patients, results are less uniform with some studies showing a $P_{ga,tw}$ decrease after exercise (ILD [233]) while others do not (COPD [228]).

**Paediatrics.** Like in adults, the recording of $P_{di,tw}$ in children is a straightforward and non-volitional strategy to test the strength of the diaphragm [70, 239-243]. Magnetic stimulation most easily depolarizes large nerve fibres, therefore in small humans (i.e. children and neonates) it may be difficult to ensure supramaximal stimulation (table S14). Normal values of $P_{di,tw}$ are available for neonates [241], infants [239] and children [244]. $P_{di,tw}$ has been measured in infants with abdominal wall defects, congenital diaphragmatic hernia [245, 246] and in children after liver transplantation [243].

Lung hyperinflation and a poor nutritional status have been associated with low $P_{di,tw}$ values in children with CF [240]. Similarly, low $P_{di,tw}$ values have been reported in neonates with diaphragmatic paralysis [242], in children with NMDs [70].

**ICU.** In critically ill, intubated patients, it is possible to assess diaphragmatic contractility measuring $P_{di,tw}$ elicited by electrical or magnetic stimulation of the phrenic nerves [247]. The use of electrical stimulation in intubated patients, however, is fraught with technical limitations and, for all practical purposes, it has been abandoned [247]. Glottic closure cannot arise in a patient with an endotracheal tube. Accordingly, in ventilated patients, there is a good correlation between twitch airway pressure ($P_{ao,tw}$) and $P_{di,tw}$ [42, 248]. The limits of agreement between the two measurements, however, are wide, meaning that a particular $P_{ao,tw}$ is a poor predictor of $P_{di,tw}$ [42, 248]. Yet, measurements of $P_{ao,tw}$ are reproducible and, therefore, might be used to track changes in diaphragmatic contractility in ventilated patients [42].

Measurements of $P_{ao,tw}$ and $P_{di,tw}$ elicited by magnetic stimulation of the phrenic nerves have given insights on diaphragmatic contractility in the critical care setting. These include the objective demonstration that intubated patients cannot maximally recruit the diaphragm during ‘maximal’ voluntary inspiratory manoeuvres, that weaning failure is not caused by diaphragmatic fatigue and that patients who require mechanical ventilation develop profound diaphragmatic weakness [105, 249-254].

Experimental evidence suggests that in critically ill patients acquired diaphragm weakness variably defined as a $P_{ao,tw} < 11 \text{ cmH}_2\text{O}$ [249] or $P_{di,tw} \leq 10 \text{ cmH}_2\text{O}$ [253] might be associated
with excess morbidity and mortality [249-254]. Duration of mechanical ventilation has been reported as a risk factor for diaphragm weakness by some [250, 251, 252] but not all investigators [254]. Similarly, sepsis and severity of underlying disease are risk factors for diaphragm weakness according to some but not other investigators [105, 249, 25, 254]. There are also contrasting results on the impact of diaphragm weakness on weaning outcome and duration of mechanical ventilation [106, 249-254]. Investigators have reported an association between diaphragm weakness and mortality in mechanically ventilated patients [249, 251, 254, 256]. Lack of standardization with twitch recordings hinders the comparison of the results of different studies. Whether diaphragmatic weakness is marker of disease severity or it is causally related to worse outcomes in critically ill patients remains to be determined [257].

1.6 Respiratory muscle endurance testing

1.6.1 Inspiratory muscle endurance to an external load

External loading protocols are frequently used to measure respiratory muscle endurance. The load can be incremental or constant, and is to be sustained until symptom limitation – endurance time or Tlim [258]. The load itself can be (1) flow resistive, in which the pressure required of the muscles is dependent on the flow rate across the resistance; (2) threshold load in which a finite pressure is required to open a valve that allows flow – i.e., the pressure at the airway opening generated by the respiratory muscles is relatively constant and independent of both volume and flow [258, 259]; or (3) hybrid between flow resistive loads and threshold loading (tapered flow resistive loading) [260]. During the latter type of loading, an initial threshold load has to be overcome. Then, the pressure is flow-dependently tapered down to accommodate length tension characteristics of the respiratory muscle. The result is an iso-flow (i.e. iso-velocity) contraction.

Muscle performance is influenced by breathing pattern (both timing components and inspiratory volumes). Accordingly, some investigators recommend controlling these parameters during testing [258, 259]. The control of breathing pattern, however, adds complexity to the procedure. This is why measurements of inspiratory muscle endurance have been regarded, until recently, to be beyond the scope of routine clinical practice [260]. Some investigators, however, argue that the need to control breathing pattern during endurance testing might be overcome by simply recording mouth pressure, flow and inspiratory volumes during the test [19]. WOB performed during the test (integrated from inspiratory volumes and mouth pressure) has been put forward as
the most important determinant of Tlim, regardless of the pattern of breathing [19]. With the introduction of new handheld devices that record continuously flow, volume, and pressure responses, it is now easier to monitor breathing pattern and WOB during endurance tests [260, 263]. This has opened the possibility to implement well controlled endurance tests into standard clinical evaluation of different interventions.

Generally speaking, inspiratory muscle endurance tests to external loads require development of large pressures swing [19]. Concurrently, ventilatory requirements remain unchanged or modestly increase [19]. Such conditions are similar to those of weight lifting, with relatively low velocities of muscle shortening. In contrast, hyperpnoea endurance tests (see section 1.6.2) are more like activities of running with large velocities of shortening.

One technique of inspiratory muscle endurance testing is the so-called incremental threshold loading technique first described in the late 1980s [264, 265]. This technique was designed to resemble a Bruce protocol, which is popular for incremental, whole body exercise testing. Before the threshold loading test, the subject's PImax is measured by standard techniques. Then the subject inhales against an external threshold load set at approximately 30–40% of PImax. Every 1 to 2 min, the load is increased by approximately 5–10% of PImax, until the load cannot be tolerated. The maximum inspiratory mouth pressure sustained for the last full 1-min or 2-min interval is considered the peak pressure (Ppeak).

The incremental threshold load test holds strong appeal as a measure of inspiratory muscle function because it is well tolerated and provides a clear outcome variable. Moreover, it is sensitive to disease states and clinical treatment [266, 267]. Unfortunately, the extent to which the results from this test represent endurance or strength is not clear. Strictly speaking, the incremental threshold load test has not been proven to be a direct measure of endurance, just as an incremental exercise test is not generally considered an endurance test.

During incremental threshold loading tests, peak external work reaches its highest value somewhere during the first stages of the test and then falls precipitously before attaining Ppeak, while oxygen consumption and pressure development are still rising [264]. This means that efficiency and ability to generate inspiratory flow and inspiratory volume are falling during the final stages of the test. Peak external work performed during the test may be a useful measurement of the dynamic capacity of the muscles of the chest wall to perform external work. This value might be as important clinically as the measure of endurance by Ppeak.
In contrast, during a constant load test patients are asked to breathe against a constant submaximal inspiratory load until Tlim. It has recently been shown that if inspiratory loads are selected that result in a Tlim of less than 7 min at baseline, post-intervention test durations can be limited to 15 min without important ceiling effects [258, 260]. Data from a recent multicentre RCT demonstrated a large effect size in Tlim (0.77) measured with this constant load protocol in response to inspiratory muscle training (figure S2) [268]. Longer baseline Tlim data have previously been shown to result in ceiling effects when the post-test was limited to 15 min [258]. This lowered effect sizes of a constant load test (small to medium effect size of 0.44) in comparison to an incremental test (medium to large effect size of 0.68) in response to inspiratory muscle training [258]. Based on these data it seems reasonable to choose an external load that limits baseline test duration to 5-10 min in order to subsequently be able to limit post-test duration to 15-20 min without important ceiling effects. Standardized breathing instructions should be provided and post intervention tests should be repeated using an identical load. Improvements in Tlim and total external work performed during the tests can be recorded as main outcomes of the test.

Typical changes in breathing parameters observed during breathing against external loads after training interventions include the following: patients are able to generate higher inspiratory flow rates against equivalent external loads resulting in shorter inspiratory time, as well as being able to increase inspiratory volume and work per breath [263]. While shortening inspiratory time could be interpreted as a breathing pattern adopted to reduce the load on the muscles it also reflects the ability of the muscle to perform faster contractions against high external resistances (i.e. improvements in muscle power). The increases in inspiratory volume and external work are also suggestive of a breathing pattern that increases load on the respiratory muscles.

1.6.2. Hyperpnoea endurance test

Hyperpnoea endurance tests consist in reproducing hyperpnoea as induced by intense physical exercise, without the addition of any inspiratory or expiratory load. Of the hyperpnoea endurance tests that address endurance of in- and expiratory muscles, the easiest to perform is the MVV since this test is of brief duration where the naturally occurring hypocapnia can be tolerated while all other tests need specific equipment assuring normocapnia.

**Maximum voluntary ventilation (MVV).** During the MVV manoeuvre the breathing rate...
should be between 70 and 110 breaths·min\(^{-1}\) using a tidal volume of approximately 50% FVC [200]. Subjects are asked to breathe usually for 12 s (sometimes 10 s or 15 s), and the value of MVV is then given in l·min\(^{-1}\).

Improvements to MVV reported in the context of respiratory or whole-body exercise training are likely to include a large task-learning component. For example, respiratory muscle training shown to improve MVV by 14% [269] to 184% [270]. For athletes, the main determinants of MVV are gender, FEV\(_1\) and PEF [271] while the amount of physical training does not contribute to MVV.

The MVV manoeuvre as a test of endurance has several limitations. From a physiological point of view a 12-second test is far too short to assess intramuscular processes associated with endurance. Mechanical aspects of chest wall and lung tissue can affect the MVV value other than respiratory muscle function (i.e., stiff chest wall or lung-restrictive components, and obstruction of airways) [205]. Further, MVV has poor specificity, is highly effort dependent, and uncomfortable for patients to perform [200]. The test depends on motivation and can be tiring for some patients [19]. Accordingly, MVV is no longer recommended in the evaluation and management of patients with respiratory muscle weakness or for inspiratory and expiratory respiratory muscle endurance testing [19, 200].

**Maximal sustainable ventilation (MSV).** Testing the maximal ventilation that can be sustained for an extended period of time or MSV, is a more meaningful measure of respiratory muscle endurance than MVV. Unfortunately, there is no standardized protocol on how to perform MSV testing. Theoretically, several MSV tests with decreasing levels of ventilation should be performed up to exhaustion to determine an intensity that could be sustained for an ‘infinite duration’, in analogy to critical power for whole-body endurance testing. For a detailed description of the methodologies used, the reader is referred to the previous ATS/ERS statement [19]. In brief, after assessment of MVV [272], subjects are asked to breathe at 70-90% MVV with visual feedback and adjustment of the intensity in the first minutes of testing such that a maximal ventilatory level can be sustained for 10-15 min [273-275].

MSV tests based on prolonged hyperpnoea require special equipment to provide normocapnic conditions and visual feedback; of note, different resistances of these systems can affect test results [269, 274-276].

Using MSV testing, investigators have reported decreased respiratory muscle endurance in
patients with CHF [277] compared to healthy subject. In addition, when conducting MSV testing using the same protocol and equipment, it is possible to document improvements in respiratory muscle endurance following hyperpnoea training in healthy subjects [278, 279], in patients with CHF [280] and in patients with COPD [276]. Finally, compared to MSV at sea-level, Forte et al [281] have reported an increase in MSV at high altitude in healthy subjects. This result is likely mediated by a decrease in air density at high altitude [281]. When compared to changes in MVV after respiratory muscle training, the size effect of changes in the MSV is larger than that of MVV [282].

**Maximal incremental hyperpnoea.** Loading the respiratory muscles by hyperpnoea of stepwise incremental intensity is gaining popularity. Usually subjects are instructed to breathe at 20% MVV for 3 min and then to increase ventilation by 10% MVV every 3 min up to the highest %MVV that can be sustained for 3 min [19]. Ventilation achieved during maximal incremental hyperpnoea testing are of similar magnitude of those recorded during traditional MSV tests [277, 280]. After hyperpnoea training, respiratory muscle endurance assessed with maximal incremental hyperpnoea improves by 52% in obese subjects (range ~19 to ~28 min) [283] and by 12% in spinal cord injury patients (range from ~15 min to ~18 min) [284]. Maximal incremental hyperpnoea testing requires special equipment yet, in recent years, commercial devices have become available and normal values have been established in a large study of 160 healthy subjects from young to old age [285].

**Maximal constant-load hyperpnoea.** Respiratory muscle endurance can be tested with constant-load hyperpnoea ranging from 40% MVV in spinal cord injured patients to 70% MVV in healthy individuals. Large inter-subject differences, even in healthy subjects, can lead to very different Tlim for a given level of constant-load hyperpnoea. Tlim following hyperpnoea training increases by 168-630% in healthy subjects [270, 286-288], 65-250% in patients with COPD [289, 290], 103% in CF patients [291], 256% in spinal cord injured patients [292], and 265% in obese subjects [293]. Figure S3 shows the improvement in Tlim during constant-load hyperpnoea following respiratory muscle endurance training based on a meta-analysis of nine studies.

**Paediatrics.** Endurance indexes are rarely calculated on a routine basis in children despite the fact that they may be informative. Diaphragmatic and oesophageal tension-time index decline significantly as boys with Duchenne muscular dystrophy get older [34].
2. Respiratory muscle neurophysiology

The respiratory muscles contract phasically throughout life to maintain ventilation. Their precise neural control is important for co-ordinated activity of ‘pump’ muscles that generate intrathoracic pressures and ‘valve’ muscles that maintain airway patency (figure 4).

During resting breathing, rhythmic inputs that arise from pacemaker cells in the medulla [294] are transmitted to respiratory motoneurones in the spinal cord. This automatic control for ventilation is sensitive to increased carbon dioxide (CO$_2$) levels such as during exercise. Additional inputs arising from cortical networks including motor and premotor areas [295] also act on respiratory motoneurones, for example, via corticospinal pathways.

Respiratory muscle neurophysiological testing can be achieved with the use of (1) EMG to measure the output of the respiratory motoneurones, (2) electroencephalography (EEG), which tests the involvement of motor and premotor areas and (3) transcranial magnetic stimulation (TMS) which assesses the neural pathways to the respiratory muscles.

2.1 Electromyography (EMG)

The validity of respiratory EMG recordings depends on the (1) ability of the EMG technique to accurately reflect the activity in the target respiratory muscle and (2) that the activity recorded accurately reflects the neural control of the respiratory muscles.

Respiratory muscle EMGs are usually contaminated with electrocardiogram (ECG). This artifact is usually eliminated with the gating technique [296, 297] and, less often, with other techniques including subtraction of the ECG template from the EMG signal [297]. Respiratory muscle EMGs, especially those recorded with surface electrodes in the ICU, are also subject to power line and electro-magnetic interference [19, 298, 299]. An additional common problem with surface EMGs is signal contamination from adjacent muscles and artefactual changes due to changes in lung volume or posture [19]. Diaphragm EMG recorded via a multi-pair oesophageal electrode is less susceptible to lung volume and posture artefacts than diaphragm EMG recorded via surface electrodes [300, 301]. Intramuscular recordings are much less susceptible, but not immune [302], to contamination from neighbouring muscles and thus are superior to surface
recordings to reflect respiratory neural drive. A major benefit of the single motor unit technique is that recordings do not need to be normalized to maximal efforts.

Respiratory EMG can be used to monitor neuromuscular function and changes in respiratory motor output after interventions such as CO₂ inhalation, drugs, exercise and change in respiratory load. There is linear relationship between $P_{di,tw}$ and compound muscle action potential (CMAP) recorded from oesophageal or surface electrodes [303].

Quantification of diaphragm EMG with a multi-pair oesophageal electrode [303, 304] is a superior measure of respiratory motor output than pressure measurements, as the latter, can be affected by muscle length changes and, in the case of the $P_{ao}$ signal, by airway resistance [107]. Given that surface and oesophageal respiratory EMG values change with electrode placement and subject dimensions, they are usually standardised to a maximal value (see table 4), a reasonable strategy if subjects can achieve maximal or near maximal voluntary muscle recruitment [e.g. 305].

For intramuscular recordings when assessing respiratory motor output using the single motor unit technique, the caveats include (1) regional differences in activity, for example between the dorsal and ventral regions of the dorsal external intercostal muscle [306] and (2) that muscle force is achieved via both rate coding and motor unit recruitment, and the balance between these two processes of increasing force can vary between respiratory muscles [307]. For evoked responses, normal values of phrenic nerve conduction time are well established with both electrical and magnetic stimulation of the phrenic nerve [19, 298, 308, 309].

Tables S10 and S11 summarise the results for studies that have evaluated peak EMG (typically standardised to a maximal value) during breathing at rest and during exercise in cardiorespiratory disorders. The amplitude of the CMAPs of the diaphragm elicited by phrenic nerve stimulation are usually reduced in most NMDs (e.g. motoneurone disease), latencies, however, are prolonged in only some NMDs (e.g., demyelination) [see 303]. Spectral analysis of respiratory EMG to detect respiratory muscle fatigue [19, 298, 310] is usually limited to the research setting.

**ICU.** In the ICU, recording of the electrical activity of the crural diaphragm (EAdi) using a dedicated nasogastric tube with EMG electrodes has greatly facilitated bedside monitoring of diaphragm activity in paediatric [311] and adult patients [312]. The ratio of tidal change in EAdi to maximum EAdi (EAdi,max) can be used to estimate the patient’s effort to breathe [313]. EAdi is a promising tool to monitor diaphragm activity especially during weaning from mechanical
ventilation [314]. Of note, EAdi,max varies widely between subjects so that there is no clear reference range for EAdi. Neurally adjusted ventilatory assist (NAVA), is a novel ventilator mode that synchronizes ventilation to EAdi [315, 316].

2.3 Transcranial magnetic stimulation (TMS)

Depending on the selected coil, current amplitude, duration, and direction, a focal or non-focal magnetic field will depolarize neurons or their axons. TMS can be applied with different paradigms (i.e. single-pulse, paired-pulse and the neuroplasticity-inducing repetitive TMS (rTMS)) to obtain measures that explore distinct neurobiological and neurochemical processes (table 5).

TMS does not appear to cause long-term adverse neurological, cardiovascular, hormonal, motor, sensory, or cognitive effects in healthy subjects. Delivering a single-pulse (<1 Hz) of TMS to the brain is very safe [317] (table 5, table S12).

The validity of TMS depends on the appropriate location of EMG electrodes and control of background muscle activity and noise. In the laboratory, single and paired-pulse TMS have been used to study the diaphragm’s corticospinal pathways in healthy subjects. Insights into the role of the cerebral control of breathing at rest, during exercise and inspiratory loading have been obtained using single-pulse TMS [318, 319] and by repetitive pulse TMS [320, 321]. Clinically, TMS is used to document respiratory muscle involvement in various disease states such as extention of diaphragm’s response to TMS on the paralyzed side in stroke patients [322], abnormalities of diaphragm’s response to TMS in patients with multiple sclerosis [323] or ALS [324] (see also table S13). In patients with acute ischemic stroke, respiratory muscle function, assessed by measuring changes in P\textsubscript{es} induced by TMS, represents a simple bedside technique to assess airway clearance and evaluate aspiration risk [325].

Widespread disease-related alteration of corticomotor excitability (as documented by changes in a hand muscle first dorsal interosseous) have been documented in cardiorespiratory diseases including OSAS [328] and COPD [329]. Plasticity-related TMS measures in OSAS patients have given conflicting results concerning changes in the amplitude of motor evoked potential (MEP) of upper airway muscles (increase in genioglossus or decrease in submental muscle) in response to rTMS trains [326, 327, 337, 338] and widespread lack of response in first dorsal interosseous MEPs characteristics after the application of high-frequency rTMS [336] or
continuous theta-burst stimulation [339].

In OSAS, a reduction of cortical excitability (i.e. MEP amplitude and latency, cortical silent period (CSP) duration) recorded in first dorsal interosseous was related to the metabolic changes induced by OSAS [330, 331]. Although some TMS studies indicate an increase in cortical-motoneuronal excitability in some upper airway and respiratory muscles [326, 327, 332, 333], no difference or a wide-spread defect in the conductivity or excitability of the corticomotor system was documented in hand muscles [330, 331, 334-336] in OSAS patients during wakefulness. During acute exacerbation of COPD, motor threshold (MT) and central motor conduction time (CMCT) recorded in first dorsal interosseous was increased compared to controls [340], with conflicting results in CSP duration [329, 340]. Some TMS parameters recorded in first dorsal interosseous correlated with variables of pulmonary function and arterial blood gases [340]. Intracortical inhibition (ICI) of first dorsal interosseous was less pronounced in the acute-exacerbation COPD group compared to controls [329].

Effects of some interventions on TMS outcomes are summarized in table S13. In awake healthy or OSAS subjects, hyperoxic CO₂-induced hyperventilation was associated with heightened chest wall/diaphragm corticomotor activation, as evidenced by decreased motor threshold and increased MEP amplitude, without modulating GG or abductor pollicis brevis MEP responses or scalene CSP duration [341-344].

Inspiratory resistive breathing facilitates the diaphragm response to TMS while it does not increase the automatic drive to breathe [345]. In healthy subjects, non-invasive ventilation can depress diaphragm motor cortex excitability [346]; whereas in COPD patients, an acute effect of non-invasive ventilation was observed with a reduction of diaphragm MEP amplitude, with no effect on ICF or ICI, implying an effect of neuromechanical feedback at brainstem or spinal level [347]. Compared to the responses during acute-exacerbations of COPD, 3 to 4 months of O₂ therapy normalize motor threshold (resting and active) and ICI impairment and prolong CSP duration in first dorsal interosseous [329].

3. Respiratory muscle imaging

3.1 Ultrasound
**Paediatrics.** In children, diaphragmatic movement can be assessed by either fluoroscopy or ultrasound. The latter is gaining popularity as it does not require radiation and its bedside availability [348]. Normal, impaired, missing, or even paradoxical diaphragmatic motion can be visualized in real time by ultrasound [349]. For neonates and infants, a subxiphoid transverse view is preferred as both right and left hemi-diaphragms can be seen at the same time. Moreover, with the subxiphoid transverse view it is easy to evaluate paradoxical diaphragm movement. For older children, each hemi-diaphragm is evaluated separately with either a more lateral subcostal approach or an intercostal approach. Ultrasound is also used to evaluate lobulated-shaped hemi-diaphragms [349, 350] to assess whether the finding results from focal diaphragmatic eventration, a potential diaphragmatic hernia, or a rare thoracic kidney/spleen. Most cases of diaphragmatic hernia can be diagnosed by plain radiographs. But when the herniated viscera do not contain air, ultrasound can be used to show herniated fluid-filled bowel loops or herniated liver with the ‘waist’ sign through the diaphragmatic defect.

### 3.2 Optoelectronic plethysmography (OEP)

OEP is an established technique to measure the variations of the volume of the chest wall and its compartments during breathing [349, 350].

Contraction of the diaphragm expands the rib cage (RCa) compartment and the abdominal (AB) compartment. Rib cage muscles, including intercostals, parasternals, scalenes and the neck muscles, mostly act on the pulmonary rib cage compartment (RCp) and are both inspiratory and expiratory. The abdominal muscles act on RCa and AB and are expiratory. In disease states, when each muscle group contracts alone or the contraction of one group is predominant compared to the contraction of other groups result in asynchronies between compartments or complete ‘paradoxical’ motion. These abnormal movements can be quantified by discernible phase shifts between compartments ($V_{RCp}$, $V_{RCA}$, and $V_{AB}$) [351-355].

The accuracy of OEP has been evaluated by comparing chest wall volume with lung volume variations measured by a spirometer or integrating a flow measurement at the airway opening. These assessments have been performed in a variety of conditions including newborns at rest [355] (accuracy −2.0%), quiet breathing, slow VC manoeuvres [351], incremental exercise on a
cycle ergometer [357] (CV of the two signals < 4%), during cycling exercise [358] (CV 2.4±3.9%), in patients with respiratory muscle dysfunction [359]. Intra-observer and inter-observer reliability were evaluated at rest and during exercise [360], with intraclass correlation coefficient values >0.75 and error values <10%. The possibility to track changes in EELV by OEP on a breath-by-breath basis has been evaluated during incremental exercise (compared with simultaneous measurements of IC [361], with a mean difference of 7.0±5.8% or 35±24 ml) and in mechanically ventilated patients at different levels of external PEEP [362]. OEP has been extensively used to evaluate total and compartmental volume variations during exercise in patients with COPD. Patients with more severe COPD experience dynamic hyperinflation during incremental exercise, but other patients, specifically those with a greater expiratory flow reserve at rest, adopt at least two significantly different patterns of change in end-expiratory volume of the chest wall [361, 363, 364]. Some patients experience a progressive and significant increase in end-expiratory volume of the chest wall (“early hyperinflators”) and other experiencing an increase in end-expiratory volume only at higher levels of exercise (“late hyperinflators”). Three distinct patterns of breathing and chest wall volume changes to cope with chronic respiratory failure have been recorded in patients with severe COPD, interstitial pulmonary fibrosis, and CF [365].

OEP has been also used in the study of patients with several NMDs. In awake patients with Duchenne muscular dystrophy, abdominal motion during resting breathing while supine is an important indicator of the degree of respiratory muscle impairment, of disease progression and an early indicator of nocturnal hypoxemia [366]. A reduced abdominal contribution to VT during resting breathing is also associated with inefficient cough [367]. In addition, a reduced abdominal contribution to VT is a specific and early marker of diaphragm weakness in adolescent and adult patients with Duchenne muscular dystrophy who present either no sign or only mild nocturnal oxygen desaturation [368]. Mild initial modifications of thoraco-abdominal motion have been described in Limb-girdle muscular dystrophy, Becker muscular dystrophy, facioscapulohumeral dystrophy [369] and in ALS [370]. A negative or reduced contribution of VRCP to VT indicative of inspiratory ribcage muscle weakness, is a distinctive feature of spinal muscle atrophy type1 and type 2 since infancy [371].

4. Respiratory muscle structure, perfusion and metabolism
4.2 Oxygen cost of breathing

Oxygen cost of breathing (VO$_{2RM}$) is an index of the energy required for ventilation. During rest, respiratory muscles use 1-2% of the total body oxygen uptake (VO$_2$) resulting in an approximate VO$_{2RM}$ of around 2.5 ml·min$^{-1}$. During exercise, ventilation and WOB increase in proportion to the metabolic demands [372]. VO$_{2RM}$ during maximal exercise represents $\approx 10\%$ of whole-body maximal oxygen uptake [373-375] or even $>15\%$ in endurance-trained men [373]. VO$_{2RM}$ in humans is mostly measured using indirect methods by measuring ventilation and VO$_2$ at rest followed by an increase in ventilation (voluntarily, by CO$_2$ breathing or by the addition of dead space) [376]. By extrapolating the changes observed in VO$_2$ and ventilation, VO$_{2RM}$ is estimated [377]. However, different approaches were used to estimate VO$_{2RM}$ in the past with considerable variability [378]. As ventilation rises, WOB also increases. Using a wide range of ventilation values, the CV of the method ranges to estimate VO$_{2RM}$ from 4.3 to 5.7% [379].
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Table S1. Summary of relative contraindications and the main reasons to avoid respiratory muscle testing

<table>
<thead>
<tr>
<th>Contraindication</th>
<th>Reason to avoid respiratory muscle testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent surgery (thoracic / abdominal / brain / ear, nose, throat)</td>
<td>Rupture site of injury, avoid pain, discomfort</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>Worsen pneumothorax, avoid discomfort and pain</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Induce further infarction leading to cardiac arrest</td>
</tr>
<tr>
<td>Ascending aortic aneurysm</td>
<td>Rupture of aneurysm, catastrophic/fatal event</td>
</tr>
<tr>
<td>Haemoptysis</td>
<td>Pulmonary emboli or myocardial infarction</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>Death, hypoxia leading to respiratory failure</td>
</tr>
<tr>
<td>Acute diarrhoea / stress incontinence</td>
<td>Discomfort, embarrassment, infection risk</td>
</tr>
<tr>
<td>Severe hypertension (systolic &gt;200 mmHg, diastolic &gt;120 mmHg)</td>
<td>Risk of blackout/collapse, rupture of cerebral blood vessels, etc.</td>
</tr>
<tr>
<td>Confused/demented patients</td>
<td>Tests are volitional and need full patient cooperation</td>
</tr>
<tr>
<td>Patient discomfort</td>
<td>Vomiting, diarrhoea, cold sores, common cold</td>
</tr>
<tr>
<td>Infection control issue</td>
<td>Contagious infections (norovirus, tuberculosis, flu)</td>
</tr>
</tbody>
</table>

Based on Cooper [380]
## Table S2. Reference values for maximal inspiratory pressure (PImax) measurements performed at residual volume

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Reference values (equation), cmH&lt;sub&gt;2&lt;/sub&gt;O</th>
<th>LLN (Lower Limit Normality), cmH&lt;sub&gt;2&lt;/sub&gt;O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclauser Pessoa et al. [381]</td>
<td>Based on 22 studies (n=840; 426 men, 414 women see Tables S7 and S8 for details) that measured PImax in accordance with 2002 ATS/ERS statement [19] (either flanged or tube mouthpiece)</td>
<td>-</td>
<td>See table S3</td>
</tr>
<tr>
<td>Rodrigues et al. [22]</td>
<td>Based on 6 most cited publications of reference values for PImax [32, 381-385]. 3 references providing higher normal values are recommended [381, 382, 385] (see below for details of three recommended studies).</td>
<td>See table S4</td>
<td></td>
</tr>
<tr>
<td>Bruschi et al. [383]</td>
<td>n=669 subjects (290 male / 379 female) from 18-70 years.</td>
<td>LnPImax = 4.02 – (0.26 x sex) – (0.004 x age) + (0.47 x body surface area)</td>
<td></td>
</tr>
<tr>
<td>Neder et al. [386]</td>
<td>n=100 subjects (50 male / 50 female) from 20 to 80 years.</td>
<td>Male = 155 – (0.8 x age)</td>
<td>Female = 110 – (0.49 x age)</td>
</tr>
<tr>
<td>Black and Hyatt. [382]</td>
<td>n=120 subjects, (60 male / 60 female) from 20 to 74 years.</td>
<td>Male = 143 – (0.55 x age)</td>
<td>Female = 104 – (0.51 x age)</td>
</tr>
<tr>
<td>Evans et al. [387]</td>
<td>Based on 5 studies [383, 387-390] using 2002 ATS/ERS statement [19] and flanged mouthpiece</td>
<td>Male = 120 – (0.41 x age)</td>
<td>Female = 108 – (0.61 x age)</td>
</tr>
</tbody>
</table>
Table S3. Reference values for maximal inspiratory pressure (Plmax) measurements obtained at residual volume for different age groups

<table>
<thead>
<tr>
<th>Age group, years</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Studies, n/sample size, n</td>
<td>Plmax, cmH2O, mean (95% CI)</td>
</tr>
<tr>
<td>18–29</td>
<td>6/96</td>
<td>128.0 (116.3–139.5)</td>
</tr>
<tr>
<td>30–39</td>
<td>6/69</td>
<td>128.5 (118.3–138.7)</td>
</tr>
<tr>
<td>40–49</td>
<td>6/72</td>
<td>117.1 (104.9–129.2)</td>
</tr>
<tr>
<td>50–59</td>
<td>5/61</td>
<td>108.1 (98.7–117.6)</td>
</tr>
<tr>
<td>60–69</td>
<td>5/65</td>
<td>92.7 (84.6–100.8)</td>
</tr>
<tr>
<td>70–83</td>
<td>5/63</td>
<td>76.2 (66.1–86.4)</td>
</tr>
</tbody>
</table>

From Sclauser Pessoa et al. [381]
Table S4. Absolute maximal inspiratory pressure (PImax) values obtained at residual volume associated with “higher” likelihood of inspiratory muscle weakness, by sex and age

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Men*</th>
<th>PImax (cmH2O)</th>
<th>Women*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>63</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>55</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>61-80</td>
<td>47</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>&gt; 80</td>
<td>42</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

* n = 164 (< 40 y), 302 (40-60 y), 365 (61-80 y), and 35 (> 80 y). + n = 140 (< 40 y), 293 (40-60 y), 387 (61-80 y), and 43 (> 80 y). From Rodrigues et al. [22]
Table S5. Reference values for maximal expiratory pressure (PEmax) measurements performed at total lung capacity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Reference values (equation), cmH\textsubscript{2}O</th>
<th>LLN (Lower Limit Normality), cmH\textsubscript{2}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans et al. [387]</td>
<td>Based on 4 studies [32, 203, 383, 385] using 2002 ATS/ERS statement [19] and flanged mouthpiece</td>
<td>Male = 174 – (0.83 x age)Female = 131 – (0.86 x age)</td>
<td>Male = 117 – (0.83 x age)Female = 95 – (0.57 x age)</td>
</tr>
<tr>
<td>Neder et al. [386]</td>
<td>n=100 subjects (50 male / 50 female) from 20 to 80 years. Flanged mouthpiece.</td>
<td>Male = 165 – (0.81 x age)Female = 115 – (0.61 x age)</td>
<td></td>
</tr>
<tr>
<td>Black and Hyatt. [382]</td>
<td>n=120 subjects, (60 male / 60 female) from 20 to 74 years. Tube mouthpiece.</td>
<td>Male = 268 – (1.03 x age)Female = 170 – (0.53 x age)</td>
<td></td>
</tr>
<tr>
<td>Bruschi et al. [383]</td>
<td>n=669 subjects (290 male / 379 female) from 18-70 years. Tube mouthpiece.</td>
<td>LnPEmax = 4.54 – (0.35 x sex) – (0.003 x age) + (0.24 x body surface area)</td>
<td></td>
</tr>
</tbody>
</table>
Table S6. Reference normal ranges for maximal expiratory pressure (PEmax) measurements performed at total lung capacity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Numbers</th>
<th>PEmax (cmH2O)</th>
<th>Mouthpiece</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Ringqvist et al. [39]</td>
<td>106</td>
<td>94</td>
<td>239 ± 46</td>
</tr>
<tr>
<td>Black and Hyatt [38]</td>
<td>60</td>
<td>60</td>
<td>233 ± 42</td>
</tr>
<tr>
<td>Rochester and Arora [393]</td>
<td>80</td>
<td>121</td>
<td>215 ± 45</td>
</tr>
<tr>
<td>Bruschi et al. [383]</td>
<td>290</td>
<td>379</td>
<td>140 ± 30</td>
</tr>
<tr>
<td>Enright et al. [384]</td>
<td>244</td>
<td>292</td>
<td>175 ± 46</td>
</tr>
<tr>
<td>Leech et al. [394]</td>
<td>325</td>
<td>50</td>
<td>154 ± 82</td>
</tr>
<tr>
<td>Wilson et al. [32]</td>
<td>80</td>
<td>480</td>
<td>147 ± 34</td>
</tr>
<tr>
<td>Neder et al. [386]</td>
<td>50</td>
<td>87</td>
<td>141 ± 22</td>
</tr>
<tr>
<td>Vincken et al. [202]</td>
<td>46</td>
<td>60</td>
<td>140 ± 38</td>
</tr>
</tbody>
</table>

Table adapted from previous 2002 ATS/ERS statement [19] adding data from studies included in review from Evans et al. [387]
Table S7. Characteristics of participants from studies included in the review from Sclauer Pessoa et al. (PImax n=22; PEmax n=17) [380]

<table>
<thead>
<tr>
<th>Reference</th>
<th>Pressures</th>
<th>Age, years (range)</th>
<th>Height, cm (mean±SD)</th>
<th>Weight, kg (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cook et al., [33]; n=32 (23M/9F)</td>
<td>PImax and PEmax</td>
<td>M: 18–64; F: 18–32</td>
<td>M: 179; F: 164</td>
<td>NR</td>
</tr>
<tr>
<td>Ringqvist et al. [392]; n=200</td>
<td>PImax and PEmax</td>
<td>18–83</td>
<td>Reported according to age</td>
<td>Reported according to age</td>
</tr>
<tr>
<td>Black and Hyatt [382]; n=120,</td>
<td>PImax and PEmax</td>
<td>20–74</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Leech et al. [394]; n=595 (252M/343F)</td>
<td>PImax and PEmax</td>
<td>15–35</td>
<td>Reported according to age</td>
<td>Reported according to age</td>
</tr>
<tr>
<td>Wilson et al. [32]; n=135 (48M/87F)</td>
<td>PImax and PEmax</td>
<td>18–49 &gt;50</td>
<td>M: 179±6; F: 163±7</td>
<td>M: 74.5±8.5; F: 61.4±9</td>
</tr>
<tr>
<td>Camelo et al. [395]; n=60 (30M/30F)</td>
<td>PImax and PEmax</td>
<td>20–49</td>
<td>M: 170±7.7; F: 160.2±6.2</td>
<td>M: 70.0±10.8; F: 56.0±9.1</td>
</tr>
<tr>
<td>Vincken et al. [202]; n=106 (46M/60F)</td>
<td>PImax and PEmax</td>
<td>16–79</td>
<td>M: 172±7; F: 160±7</td>
<td>M: 74±9; F: 59±10.0</td>
</tr>
<tr>
<td>McElvaney et al. [396]; n=104</td>
<td>PImax and PEmax</td>
<td>&gt;55</td>
<td>M: 174±7; F: 161±6</td>
<td>M: 107±10; F: 112±12</td>
</tr>
<tr>
<td>Bruschi et al. [383]; n=669</td>
<td>PImax and PEmax</td>
<td>18–70</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Enright et al. [384]; n=2871</td>
<td>PImax and PEmax</td>
<td>&gt;65</td>
<td>M: 173.2±6.59; F: 158.8±6.30</td>
<td>M: 79.5±11.6; F: 66.2±12.5</td>
</tr>
<tr>
<td>Enright et al. [397]; n=228 (112M/176F)</td>
<td>PImax and PEmax</td>
<td>&gt;65</td>
<td>M: 171.25; F: 157</td>
<td>M: 79.7; F: 65.7</td>
</tr>
<tr>
<td>Johan et al. [398]; n=452 (277M/175F)</td>
<td>PImax and PEmax</td>
<td>20–80</td>
<td>M: 164–167; F: 155–157</td>
<td>M: 64.1–67.21; F: 53.6–</td>
</tr>
<tr>
<td>Pande et al. [399]; n=273 (153M/120F)</td>
<td>PImax</td>
<td>20–65</td>
<td>M: 165.6±6.1; F: 153.5±5.2</td>
<td>M: 62.5±11.7; F: 57.5±10.9</td>
</tr>
<tr>
<td>Harik-Khan et al. [385]; n=267</td>
<td>PImax</td>
<td>&lt;40 – &gt;75</td>
<td>M: 164.2±7.3; F: 177.6±6.6</td>
<td>M: 64.7±11.9; F: 81.7±13.3</td>
</tr>
<tr>
<td>Neder et al. [386]; n=100 (50M/50F)</td>
<td>PImax and PEmax</td>
<td>20–80</td>
<td>M: 168.4±6.2; F: 157.1±7.1</td>
<td>M: 73.8±10.7; F: 62.5±10.8</td>
</tr>
<tr>
<td>Hautmann et al. [388]; n=504</td>
<td>PImax</td>
<td>18–82</td>
<td>M: 176.9±6.82; F: 164.9±6.37</td>
<td>M: 78.3±10.9; F: 66.4±10.8</td>
</tr>
<tr>
<td>Wohlgemuth et al. [391]; n=252</td>
<td>PImax and PEmax</td>
<td>18–80</td>
<td>Reported according to age</td>
<td>Reported according to age</td>
</tr>
<tr>
<td>Windisch et al. [390]; n=490</td>
<td>PImax</td>
<td>10–90</td>
<td>M: 179.5±7.7; F: 166.4±7.0</td>
<td>M: 77.9±11.2; F: 66.0±10.9</td>
</tr>
<tr>
<td>Sachs et al. [400]; n=1755</td>
<td>PImax</td>
<td>45–84</td>
<td>M: 172; F: 158</td>
<td>M: 80.45; F: 68.18</td>
</tr>
<tr>
<td>Simões et al. [401]; n=140 (70M/70F)</td>
<td>PImax and PEmax</td>
<td>20–89</td>
<td>Reported according to age</td>
<td>Reported according to age</td>
</tr>
<tr>
<td>Costa et al. [402]; n=120 (60M/60F)</td>
<td>PImax and PEmax</td>
<td>20–80</td>
<td>Reported according to age</td>
<td>Reported according to age</td>
</tr>
<tr>
<td>Gopalakrishna et al. [403]; n=250</td>
<td>PImax and PEmax</td>
<td>20–70</td>
<td>M: 165.70±7.56; F: 155.99±5.81</td>
<td>M: 64.62±9.73; F:</td>
</tr>
</tbody>
</table>

NR, not reported; M, male; F, female
Table S8. Technical aspects in 22 studies that influence maximal respiratory pressures (PImax (n=22) from residual volume and PEmax (n=17) from total lung capacity) [380]

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mouthpiece</th>
<th>Small leak (size)</th>
<th>Pressure evaluated</th>
<th>Time of PImax</th>
<th>Trials, n</th>
<th>Criterion for stopping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cook et al. [33]</td>
<td>Tube</td>
<td>NR</td>
<td>Peak (PImax and PEmax)</td>
<td>Without control</td>
<td>Min of 2</td>
<td>NR</td>
</tr>
<tr>
<td>Ringqvist et al. [392]</td>
<td>Tube</td>
<td>Yes (2 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Max 1.5 s</td>
<td>Min of 5</td>
<td>Highest value</td>
</tr>
<tr>
<td>Black and Hyatt [382]</td>
<td>Tube</td>
<td>Yes (2 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 2</td>
<td>Highest value</td>
</tr>
<tr>
<td>Leech et al. [394]</td>
<td>NR</td>
<td>Yes (0.90 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>NR</td>
<td>Max of 3</td>
<td>Highest value</td>
</tr>
<tr>
<td>Wilson et al. [32]</td>
<td>Flanged</td>
<td>Yes, size NR</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 3</td>
<td>2 identical readings</td>
</tr>
<tr>
<td>Camelo et al. [395]</td>
<td>Tube</td>
<td>Yes (2 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 4</td>
<td>Highest value</td>
</tr>
<tr>
<td>Vincken et al. [202]</td>
<td>Flanged</td>
<td>Yes (1.27 mm)</td>
<td>Plateau (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 4</td>
<td>Highest 2 values within 5% difference</td>
</tr>
<tr>
<td>McElvaney et al. [396]</td>
<td>Tube</td>
<td>Yes (0.6 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1s</td>
<td>Min of 3</td>
<td>Highest 3 values within 5% difference</td>
</tr>
<tr>
<td>Bruschi et al. [383]</td>
<td>Tube</td>
<td>Yes (1.06 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 5</td>
<td>Highest value</td>
</tr>
<tr>
<td>Enright et al. [384]</td>
<td>Tube</td>
<td>Yes (1 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>2 s</td>
<td>3–5</td>
<td>Highest 2 values within 10% difference</td>
</tr>
<tr>
<td>Enright et al. [397]</td>
<td>Tube</td>
<td>Yes (1 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>2 s</td>
<td>5</td>
<td>Highest 2 values within 10% difference</td>
</tr>
<tr>
<td>Johan et al. [398]</td>
<td>Flanged</td>
<td>Yes, size NR</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>3–5</td>
<td>Highest value of 3 similar trials</td>
</tr>
<tr>
<td>Pande et al. [399]</td>
<td>NR</td>
<td>Yes (1.27 mm)</td>
<td>Peak (PImax)</td>
<td>Min of 2 s</td>
<td>NR</td>
<td>Highest value</td>
</tr>
<tr>
<td>Harik-Khan et al. [385]</td>
<td>Tube</td>
<td>Yes (1 mm)</td>
<td>Peak (PImax)</td>
<td>2 s</td>
<td>Max of 5</td>
<td>Highest 2 values within 10% difference</td>
</tr>
<tr>
<td>Neder et al. [386]</td>
<td>Flanged</td>
<td>Yes, size NR</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>3–5</td>
<td>Highest value. &lt;10% of 3 trials</td>
</tr>
<tr>
<td>Hautmann et al. [388]</td>
<td>NR</td>
<td>Yes, size NR</td>
<td>Plateau (PImax)</td>
<td>Min of 2 s</td>
<td>Min of 7</td>
<td>Highest value</td>
</tr>
<tr>
<td>Wohlgemuth et al. [391]</td>
<td>Face mask</td>
<td>Yes (2 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 3</td>
<td>Highest value varying 5%</td>
</tr>
<tr>
<td>Windisch et al. [390]</td>
<td>Flanged</td>
<td>Yes (2 mm)</td>
<td>Peak and plateau (PImax)</td>
<td>Min of 1 s</td>
<td>Min of 7</td>
<td>Highest 2 values within 10% difference</td>
</tr>
<tr>
<td>Sachs et al. [400]</td>
<td>Tube</td>
<td>NR</td>
<td>Plateau (PImax)</td>
<td>Min of 1 s</td>
<td>5</td>
<td>Highest 2 values within 10% difference</td>
</tr>
<tr>
<td>Simões et al. [401]</td>
<td>Tube</td>
<td>Yes (2 mm)</td>
<td>Plateau (PImax and PEmax)</td>
<td>About 1 s</td>
<td>Min of 3</td>
<td>Highest value &lt;10% of all trials</td>
</tr>
<tr>
<td>Costa et al. [402]</td>
<td>NR</td>
<td>Yes (2 mm)</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 3</td>
<td>Highest value &lt;10% of 2 trials</td>
</tr>
<tr>
<td>Gopalakrishna et al. [403]</td>
<td>NR</td>
<td>NR</td>
<td>Peak (PImax and PEmax)</td>
<td>Min of 1 s</td>
<td>Min of 3</td>
<td>Highest 2 values within &lt;10% difference</td>
</tr>
</tbody>
</table>

NR, nor reported
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Reference values (equation), cmH₂O</th>
<th>LLN (Lower Limit Normality), cmH₂O</th>
<th>Mean LLN, cmH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araujo et al. [54]</td>
<td>243 healthy individuals (20-80 years), Brazil</td>
<td>(Males) SNIP = -0.47(age) + 135.6</td>
<td>(Males) LLN = -0.47(age) +135.6 – 44.9</td>
<td>(males) = 69.2 (females) = 61.9</td>
</tr>
<tr>
<td>Kamide et al. [55]</td>
<td>223 healthy Japanese (20-70y)</td>
<td>(males) SNIP = -0.67 (age) + 104.65</td>
<td>(males) LLN = -0.67 (age) + 104.65 – 43.78</td>
<td>(males) = 32.9 (females) = 28.8</td>
</tr>
<tr>
<td>Uldry and Fitting [66]</td>
<td>168 healthy subjects (20-80 y), Europe</td>
<td>(males) SNIP = -0.42 (age) + 126.8</td>
<td>(males) LLN = -0.42(age) + 126.8 – 39.0</td>
<td></td>
</tr>
<tr>
<td>Huang et al. [65]</td>
<td>119 healthy volunteers (18-69y), Taiwan</td>
<td>(males) SNIP = 21.10 + 1.24(body weight)</td>
<td>(males) LLN = 21.10 + 1.24(body weight) – 50.16</td>
<td>(males) 60.33 cmH₂O (females) 52.05 cmH₂O</td>
</tr>
<tr>
<td>Stefanutti et al. [67]</td>
<td>180 healthy children (6-17y), Europe</td>
<td>(Boys) SNIP = 3.3(age) + 70</td>
<td>(Boys) SNIP = 3.3(age) + 70 – 39.8 cmH₂O</td>
<td>(Boys) 93 ± 23 cmH₂O</td>
</tr>
</tbody>
</table>

**Table S9. Reference values maximal sniff nasal inspiratory pressure (SNIP)**
Table S10. Summary of prognostics, discriminative, clinical meaningful difference and evaluative information of endorsed EMG techniques at rest in cardiorespiratory disease.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Variable</th>
<th>Reference</th>
<th>Subject Characteristics</th>
<th>Protocol</th>
<th>Prognostic information</th>
<th>Discriminative</th>
<th>Minimal clinically important difference</th>
<th>Evaluative: pharmacological interventions</th>
<th>Evaluative: non-pharmacological interventions</th>
<th>Cautions</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>SMUdi</td>
<td>De Troyer et al. [404] Gandevia et al. [405]</td>
<td>Severe COPD patients Vs. Controls</td>
<td>Resting breathing</td>
<td>↑ peak discharge rate. In COPD, 79% of SMUdi discharged at &gt;15Hz compared to &lt;5% of SMUdi for controls</td>
<td></td>
<td></td>
<td></td>
<td>Elevated discharge rate of di SMUs 'recovers' towards normal following LVRS [406]</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>iEMGscal</td>
<td>De Troyer et al. [407]</td>
<td>Severe COPD</td>
<td>Resting breathing</td>
<td>Strong insp activity in scal, but minimal in SCM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>iEMGabdo</td>
<td>Ninane et al. [408]</td>
<td>COPD Vs. Controls</td>
<td>Resting breathing</td>
<td>Degree of activity related to airflow obstruction (i.e. FEV1)</td>
<td></td>
<td></td>
<td></td>
<td>Exp activity in TA in COPD but not controls</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Jolley et al. [304]</td>
<td>COPD Vs Controls</td>
<td>Resting breathing</td>
<td>Degree of activity related to airflow obstruction (i.e. FEV1 %pred, VC and IC)</td>
<td></td>
<td></td>
<td></td>
<td>↑EMGdi % max in COPD</td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>sEMGpara%</td>
<td>Murphy et al.</td>
<td>COPD</td>
<td>Resting</td>
<td>Discriminated</td>
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<tr>
<td>Condition</td>
<td>sEMG parameter</td>
<td>Study Ref.</td>
<td>Group Comparison</td>
<td>Test Description</td>
<td>Findings</td>
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<tr>
<td>Obesity</td>
<td>oesEMGdi %max</td>
<td>Steier et al. [411]</td>
<td>Obese subjects versus Controls</td>
<td>Resting breathing in different postures</td>
<td>Degree of muscle activity thought to be related to PEEPi as PEEPi and EMGdi %max ↓ with CPAP ↑EMGdi % max in obese, worsened by supine posture (cf seated)</td>
<td></td>
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<tr>
<td>Asthma</td>
<td>sEMGpara %max</td>
<td>Steier et al. [412]</td>
<td>Controlled asthmatics versus Uncontrolled asthmatics versus Controls</td>
<td>Resting breathing, awake and asleep</td>
<td>Low predictive value for AHI in sleep ↑EMGpara %max in uncontrolled asthma cf to controlled asthma. ↑EMGpara % max in wakefulness and sleep and more variable cf controls.</td>
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<tr>
<td>CF</td>
<td>sEMGpara %max</td>
<td>Reilly et al. [413]</td>
<td>Cystic Fibrosis versus Controls</td>
<td>Resting breathing (and exercise)</td>
<td>sEMGpara %max and oesEMGdi %max related to degree of airway obstruction, hyperinflation, dynamic lung compliance ↑sEMGpara% max and ↑oesEMG% max cf to controls</td>
<td></td>
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<tr>
<td>OSA</td>
<td>oesEMGdi %max</td>
<td>Steier et al. [414]</td>
<td>OSA versus Controls</td>
<td>Resting breathing</td>
<td>↑oesEMGdi % max cf to</td>
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</tbody>
</table>

Effect may be, in
<table>
<thead>
<tr>
<th>Condition</th>
<th>Measure</th>
<th>Authors</th>
<th>Description</th>
<th>Controls</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSA</td>
<td>oesEMGdi %max</td>
<td>He et al. [415]</td>
<td>during sleep</td>
<td>controls</td>
<td>part, due to ↑BMI [410]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xiao et al. [416]</td>
<td>OSA</td>
<td>Hypopnoeic and apnoeic events during sleep</td>
<td>No different in oesEMGdi % max for events with and without arousal. ↑oesEMGdi % max at end of hypopnoeic cf apnoeic events.</td>
</tr>
<tr>
<td></td>
<td>oesEMGdi %max</td>
<td>Faisal et al. [156]</td>
<td>Mild-mod ILD versus COPD versus Controls</td>
<td>Resting breathing</td>
<td>↑oesEMGdi % max in ILD and COPD cf to controls</td>
</tr>
</tbody>
</table>

COPD: chronic obstructive pulmonary disease; SMUs: single motor units; di: diaphragm; Para: parasternal intercostal muscles in the second space; Scal: scalene; SCM: sternocleidomastoid muscle; i: intramuscular; EMG: electromyography; multi: multiunit recordings; Abdo: abdominal muscles; LVRS: lung volume reduction surgery; inspiratory: inspiratory; exp: expiratory; TA: transversus abdominis; CPAP: continuous positive airway pressure; OSA: obstructive sleep apnoea; ILD: interstitial lung disease; oes: oesophageal; max: maximal.
Table S11. Prognostics, discriminative, clinical meaningful difference and evaluative information of EMG techniques tested during exercise in cardiorespiratory disease.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Variable</th>
<th>Reference</th>
<th>Subject Characteristics</th>
<th>Exercise protocol(s)</th>
<th>Prognostic information</th>
<th>Discriminative</th>
<th>Minimal clinically important difference</th>
<th>Evaluative: pharmacological interventions</th>
<th>Evaluative: non-pharmacological interventions</th>
<th>Cautions</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>oesEMGdi</td>
<td>Qin et al. [417]</td>
<td>Severe COPD versus control</td>
<td>Constant load treadmill</td>
<td>EMGdi similar at end of both types of exercise</td>
<td></td>
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</tr>
<tr>
<td>COPD</td>
<td>oesEMGdi</td>
<td>Luo et al. [418]</td>
<td>Mod-severe COPD</td>
<td>Constant versus incremental treadmill</td>
<td>↑EMGdi % max in COPD cf controls</td>
<td></td>
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<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Guenette et al. [419]</td>
<td>Mild COPD versus Controls</td>
<td>Incremental cycle</td>
<td>↑EMGdi in those patients who stopped because of breathlessness and not leg fatigue</td>
<td></td>
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</tr>
<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Jolley et al. [420]</td>
<td>Severe COPD patients</td>
<td>Cycle to exhaustion</td>
<td>EMGdi % max related to breathlessness</td>
<td></td>
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</tr>
<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Jolley et al. [421]</td>
<td>Severe COPD patients</td>
<td>Incremental cycle and treadmill</td>
<td>Exertional breathlessness related to EMGdi %max</td>
<td></td>
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</tr>
<tr>
<td>Condition</td>
<td>oesEMGdi %max</td>
<td>Study</td>
<td>Description</td>
<td>Exercise Test</td>
<td></td>
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<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Qin et al. [422]</td>
<td>Severe COPD +/- inhaled tiotropium (muscarinic receptor antagonist)</td>
<td>Constant cycle</td>
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<td></td>
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<td></td>
<td>↓EMGdi at rest with tiotropium, improved ‘efficiency’ of neural respiratory drive during exercise and prolonged exercise duration</td>
<td></td>
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<tr>
<td>COPD</td>
<td>oesEMGdi %max</td>
<td>Qiu et al. [423]</td>
<td>COPD (range of severities)</td>
<td>Constant rate treadmill versus Tai Chi</td>
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<td></td>
<td></td>
<td>Similar EMGdi in both forms of exercise</td>
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<tr>
<td>ILD/COPD</td>
<td>oesEMGdi %max</td>
<td>Faisal et al. [154]</td>
<td>Mild-mod ILD versus COPD versus Controls</td>
<td>Incremental cycle</td>
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<td></td>
<td></td>
<td>Similar ↑EMGdi in ILD and COPD at rest and during exercise cf controls</td>
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<tr>
<td>Non-COPD smokers</td>
<td>oesEMGdi %max</td>
<td>Elbehairy et al. [424]</td>
<td>Smokers (normal lung function) Versus Controls</td>
<td>Incremental cycle</td>
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<td></td>
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<td></td>
<td></td>
<td>↑EMGdi % max cf controls, mainly due to lower EMGdimax in smokers</td>
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<tr>
<td>CF</td>
<td>sPara % max</td>
<td>Smith et al. [425]</td>
<td>Cystic Fibrosis Versus Controls</td>
<td>Incremental shuttle walk test</td>
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<td></td>
<td>sEMGpara%max related to exercise performance (VO2 peak), but not as strongly as lung gas transfer.</td>
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<tr>
<td>CF</td>
<td>sPara % max oesEMGdi %max</td>
<td>Reilly et al. [413]</td>
<td>Cystic Fibrosis Versus Controls</td>
<td>Incremental cycle exercise test to exhaustion</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>sEMGpara %max and oesEMGdi %max related to breathlessness</td>
<td></td>
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<tr>
<td>Obesity COPD</td>
<td>oesEMGdi %max</td>
<td>Ciavaglia et al. [426] Obese with moderate COPD</td>
<td>Incremental cycle</td>
<td>Versus treadmill</td>
<td>Similar oesEMGdi %max in 2 exercise types, but resulted in different transdi pressures.</td>
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</tbody>
</table>

Abbreviations: as for Table S10
**Table S12.** Conditions that may increase the risk of adverse effects of transcranial magnetic stimulation (relative contraindications of TMS) [427]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy (effects on pregnant women are unknown);</td>
<td>Personal or family history of seizures, including febrile seizures as an infant;</td>
</tr>
<tr>
<td>Metal implants in the head;</td>
<td>Previous brain neurosurgery;</td>
</tr>
<tr>
<td>Cardiac pacemakers;</td>
<td>Unstable major medical conditions;</td>
</tr>
<tr>
<td>Poorly-controlled migraine headaches;</td>
<td>Medications that lower seizure threshold;</td>
</tr>
<tr>
<td>History of major head injury;</td>
<td>Neurological disorders;</td>
</tr>
<tr>
<td>History of stroke;</td>
<td>Major psychiatric disorders.</td>
</tr>
<tr>
<td>Disease</td>
<td>Reference</td>
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<tr>
<td>---------</td>
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<tr>
<td>OSAS</td>
<td>Demoule et al. [428]</td>
</tr>
<tr>
<td></td>
<td>Civardi et al. [330]</td>
</tr>
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<td></td>
<td>Grippo et al. [331]</td>
</tr>
<tr>
<td></td>
<td>Wang et al. [429]</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
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</tr>
<tr>
<td>Sériès et al.</td>
<td>Healthy subjects</td>
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<td></td>
<td>OSAS patients vs.</td>
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<td>Controls</td>
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<td>OSAS patients vs.</td>
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<tr>
<td></td>
<td>Controls</td>
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<td>OSAS patients vs.</td>
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<td>Controls</td>
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<tr>
<td>Borel et al.</td>
<td>Healthy subjects</td>
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<tr>
<td>Study</td>
<td>Group 1</td>
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<tr>
<td>Opie et al. [336]</td>
<td>OSAS patients 13 (11M)</td>
</tr>
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<tr>
<td>Das et al. [339]</td>
<td>OSAS patients 13 (10M)</td>
</tr>
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<tr>
<td>Melo-Silva et al. [326]</td>
<td>OSAS patients 14 (11M)</td>
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<tr>
<td>Melo-Silva et al. [327]</td>
<td>OSAS patients 10 (9M)</td>
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<tr>
<td>Study</td>
<td>Group</td>
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<tr>
<td>Lanza et al. [334]</td>
<td>OSAS patients vs. RLS patients vs. Controls</td>
</tr>
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<tr>
<td>Rousseau et al. [337]</td>
<td>OSAS patients</td>
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<tr>
<td>Rousseau et al. [338]</td>
<td>OSAS patients</td>
</tr>
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<tr>
<td>Borel et al. [344]</td>
<td>OSAS patients vs. Controls</td>
</tr>
<tr>
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<tr>
<td>Oliviero et al. [329]</td>
<td>AE-COPD patients vs. Controls</td>
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<tr>
<td>Study</td>
<td>Population</td>
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<tr>
<td>Hopkinson et al. [431]</td>
<td>COPD (stable outpatients) vs. Controls</td>
</tr>
<tr>
<td>Sharshar et al. [346]</td>
<td>Healthy subjects 6 (5M)</td>
</tr>
<tr>
<td>Locher et al. [345]</td>
<td>Healthy subjects 6 (4M)</td>
</tr>
<tr>
<td>Study</td>
<td>Group</td>
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<tr>
<td>Mohamed-Hussein et al. [340]</td>
<td>AECOPD vs. Controls</td>
</tr>
<tr>
<td>Hopkinson et al. [347]</td>
<td>Unventilated COPD outpatients vs. Ventilated COPD outpatients</td>
</tr>
<tr>
<td>Straus et al. [342]</td>
<td>Healthy subjects</td>
</tr>
</tbody>
</table>

A reduction of DI MEP during NIV, without change in response to paired stimuli.

Increasing the ventilatory neural drive through CO2 inhalation facilitates the response of the DI to TMS.
<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Mean Age (Range)</th>
<th>Coil Type</th>
<th>Protocol</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luu et al. [343]</td>
<td>Healthy subjects</td>
<td>29.2±7.1</td>
<td>Circular coil (non-focal); Scalenes and parasternal intercostal muscle;</td>
<td>Single-pulse TMS; Awake; End-tidal CO₂ stimulation;</td>
<td>1. CSP: NS by end-tidal CO₂.</td>
</tr>
<tr>
<td>Duguet et al. [432]</td>
<td>Central respiratory paralysis patients (ICU)</td>
<td>22.9±16</td>
<td>Circular coil (non-focal); DI/ APB; Vertex.</td>
<td>Single-pulse TMS; PNMS; Awake and free of sedative and psychotropic drugs;</td>
<td>1. TMS failed to elicit DI EMG and ABP EMG responses in the 11 patients; 2. No (DI/ APB) response of TMS in 6 subjects who had not recovered any ventilator activity at 1 year; 3. A (DI) EMG response to TMS was recorded in 9/10 cases (with usual latencies) who exhibited spontaneous ventilator respiration at 1 year.</td>
</tr>
<tr>
<td>Harraf et al. [325]</td>
<td>Acute ischemic stroke patients vs. Controls</td>
<td>68.9±9.8</td>
<td>Double cone coil (non-focal); DI (costal and crural); Vertex.</td>
<td>Single-pulse TMS; PNMS; Awake; Gastric (P&lt;sub&gt;gas&lt;/sub&gt;) and esophageal (P&lt;sub&gt;oes&lt;/sub&gt;) pressures were measured simultaneously with MEP by TMS.</td>
<td>1. TMS P&lt;sub&gt;gas&lt;/sub&gt;: ↓following TMS at injured compared with uninjured hemisphere in stroke patients; 2. Correlations between PCFR and P&lt;sub&gt;gas&lt;/sub&gt; or PEmax. 3. Correlations between P&lt;sub&gt;gas&lt;/sub&gt; and PEmax in stroke patients.</td>
</tr>
</tbody>
</table>

**Abbreviations:** ABP: abductor pollicis brevis muscle; ADM: abductor digit minimi muscle; AECOPD: acute exacerbation of COPD; AHI: apnea-hypopnea index; CMCT: central motor conduction time; CMS: Cervical magnetic stimulation; COPD: chronic obstructive pulmonary disease; CSP: cortical silent period; DI: diaphragmatic muscle; FDI: first dorsal intersosseus muscle; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in one second; GG: genioglossus muscle; ICF: intracortical facilitation; ICI: intracortical inhibition; IS<sub>I</sub>: interstimulus interval; ICU: intensive care unit; LCW: lower chest wall; M: male; M1: primary motor cortex; MEP amp: MEP amplitude; MEP lat: MEP latency; MEP: motor evoked potentials; MT: motor threshold; NIV: isocapnic volume cycled ventilation delivered noninvasively; NS: not significant; OSAS: obstructive sleep apnea syndrome; PCFR: peak voluntary cough flow rates; PEmax: maximum
static expiratory pressure; PNMS: phrenic nerve magnetic stimulation; RLS: restless legs syndrom; rMT: resting motor threshold; rTMS: repetitive transcranial magnetic stimulation; SD: standard deviation; TBS: theta burst stimulation; TMS Pgas: TMS gastric pressure twitch.
### Table S14. Advantages and limitations of the different respiratory muscle tests in children (adapted from [38, 433]).

<table>
<thead>
<tr>
<th>Volitional / Non volitional</th>
<th>Specificity of the test for a specific (type of) muscle</th>
<th>Advantages (+) / Limitations (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non invasive tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breathing pattern</td>
<td>NV No</td>
<td>(+) Can be performed at any age; (-) Requires quiet breathing (sleep in infants)</td>
</tr>
<tr>
<td>Lung volumes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital capacity (sitting and supine)</td>
<td>V Inspiratory and expiratory</td>
<td>(+) Easy to perform, largely used in children &gt; 4–8 years old; sensitive for assessing progress; (-) Requires cooperation; poor specificity for the diagnosis of respiratory muscle weakness</td>
</tr>
<tr>
<td>Residual volume</td>
<td>V Expiratory</td>
<td></td>
</tr>
<tr>
<td>Total lung capacity</td>
<td>V Inspiratory</td>
<td></td>
</tr>
<tr>
<td>Maximal static pressures</td>
<td>V Inspiratory (PImax) Expiratory (PEmax)</td>
<td>(+) Simple but difficult to perform, largely used in children &gt; 6–8 years old; (-) Requires full cooperation</td>
</tr>
<tr>
<td>Sniff nasal inspiratory pressure (SNIP)</td>
<td>V Inspiratory</td>
<td>(+) Natural maneuver, easy to perform in children &gt; 2 years old, playful, visual feedback; (-) Requires cooperation; glottic closure or airway characteristics may prevent adequate equilibration; not reliable in case of rhinitis or hypertrophy of the adenoids</td>
</tr>
<tr>
<td>Peak cough flow / peak expiratory flow</td>
<td>V Expiratory</td>
<td>(+) Easy to perform, largely used in children &gt; 4–8 years old; (-) Requires cooperation</td>
</tr>
<tr>
<td>Mouth pressure during a maximal whistle</td>
<td>V Inspiratory and expiratory</td>
<td>(+) Natural maneuver, easy to perform in children &gt; 2 years old, playful, audible feedback; (-) Requires cooperation, lack of reference values</td>
</tr>
<tr>
<td>Crying mouth pressure</td>
<td>V Inspiratory and expiratory</td>
<td>(+) Easily to perform in the newborn; (-) High variability; glottic closure should be prevented</td>
</tr>
<tr>
<td>Tension time index</td>
<td>V Inspiratory muscles (TImus)</td>
<td>(+) Evaluates muscle endurance; (-) Requires the measurement of occlusion pressure (P0.1), PImax/SNIP and breathing pattern</td>
</tr>
<tr>
<td><strong>Invasive tests</strong></td>
<td></td>
<td></td>
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<tr>
<td>Breathing pattern with Poes and Pga</td>
<td>NV Diaphragm</td>
<td>(+) Can be performed at any age; (-) Mildly uncomfortable, requires quiet breathing (sleep in infants)</td>
</tr>
<tr>
<td>Poes and Pga during a maximal sniff</td>
<td>V Inspiratory and diaphragm</td>
<td>(+) Natural maneuver, easy to perform in children &gt; 2 years old, playful, visual feedback; (-) Mildly uncomfortable; requires cooperation; values may be less than maximal static values because of...</td>
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<tr>
<td>Procedure</td>
<td>Volition</td>
<td>Notes</td>
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<tr>
<td>$P_{\text{gas}}$ during a maximal cough</td>
<td>V</td>
<td>Expiratory $P_{\text{ga}}$ during a maximal cough&lt;br&gt;$$P_{\text{oes}}$$ and $P_{\text{gas}}$ during a maximal whistle&lt;br&gt;Crying $P_{\text{di}}$&lt;br&gt;Tension time index&lt;br&gt;Stop study&lt;br&gt;Polysomnography / polygraphy</td>
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V: volitional, NV: non volitional, $P_{\text{oes}}$: esophageal pressure, $P_{\text{gas}}$: gastric pressure, $P_{\text{di}}$: transdiaphragmatic pressure, $P_{\text{Imax}}$: maximal static inspiratory pressure, $P_{\text{Emax}}$: maximal static expiratory pressure, $T_{\text{mus}}$: non invasive tension time of the respiratory muscles, $P_{0.1}$: pressure generated in the first 100 milliseconds of inspiration against an occluded airway, SNIP: Sniff nasal inspiratory pressure, TTDi: tension time index of the diaphragm, TTes: tension time index of the inspiratory muscles.
Figures

Figure S1. Tracings of lung volume (Volume) and oesophageal pressure (P_{oes}) from inspiratory capacity (IC) manoeuvres taken during resting breathing, at 60 watts (iso-WR) and peak-exercise from one representative PAH patient who reduced IC (or increased end-expiratory lung volume, i.e., EELV) during exercise (PAH-H, upper left panel) and one who increased IC (or reduced EELV) (PAH-NH, lower left panel). Please note that, regardless of changes in IC during exercise, dynamic peak inspiratory P_{oes} recorded during IC manoeuvres (P_{oes,IC}) is remarkably preserved in both PAH-H (upper left panel) and PAH-NH (lower left panel). Maximal and tidal flow-volume loops (average data) are shown at rest and at peak-exercise in PAH-H (upper right panel) and PAH-NH (lower right panel). Tidal flow-volume loops are provided at rest (solid line) and at peak-exercise (dashed line). Note a significant decrease in dynamic inspiratory capacity during exercise in PAH-H compared with PAH-NH. Abbreviations: TLC=total lung capacity.
**Figure S2.** Improvements in inspiratory muscle endurance capacity assessed by incremental (Pmax) or constant load (Tlim) tests in response to inspiratory muscle training (IMT) in patients with chronic obstructive pulmonary diseases. MTL = mechanical threshold loading, TFRL = tapered flow resistive loading.
Figure S3. Changes in respiratory muscle endurance measured by a constant-load hyperpnoea test following respiratory muscle endurance training (RMET) or a control period (CON, no training) in healthy subjects. From [434].
Figure S4. Dyspnoea score perceived during cycling according to maximal inspiratory pressure (PImax) and forced expiratory volume in 1 second (FEV1) based on data collected in 550 subjects exercised for clinical purposes and grouped [435].