

## Paired phrenic nerve stimuli for the detection of diaphragm fatigue in humans

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*Paired phrenic nerve stimuli for the detection of diaphragm fatigue in humans. M.I. Polkey, D. Kyrroussis, C-H. Hamnegård, P.D. Hughes, G.F. Rafferty, J. Moxham, M. Green. ©ERS Journals Ltd 1997.*

**ABSTRACT:** The transdiaphragmatic pressure ( $P_{di}$ ) elicited by paired bilateral phrenic nerve stimulation may be viewed as the sum of the  $P_{di}$  values produced by the first ( $t_1$ ) and second ( $t_2$ ) stimulus. The  $P_{di}$  at  $t_2$  ( $P_{di,t_2}$ ) is a function of the interstimulus interval. A reduction in the ratio obtained by dividing  $P_{di,t_2}$  at 10 Hz ( $P_{di,t_2,10}$ ) by  $P_{di}$  at 100 Hz ( $P_{di,t_2,100}$ ) ( $t_{210:100}$ ) has been proposed as a test of low frequency diaphragm fatigue. The aim of the present study was to establish whether this change could also be detected using paired cervical magnetic nerve stimulation (pCMS), and whether  $t_{210:100}$  was influenced by lung volume.

We studied healthy subjects at functional residual capacity (FRC), at 0.5 and 1.0 L below FRC, and at 0.5, 1.0 and 1.5 L above FRC. The subjects were then subjected to a fatiguing protocol (2 min of maximal isocapnic ventilation (MIV)). Studies were repeated at FRC 20 and 60 min after MIV and between these times at 1.0 L below and 1.5 L above FRC.

In the unfatigued state,  $t_{210:100}$  had a negative relationship with increasing lung volume ( $r^2=0.98$ ,  $p=0.002$ ). After MIV there was a fall in the  $P_{di}$  elicited by a single stimulus (mean fall at 20 min 17.9% and at 60 min 14.6%,  $p<0.03$  for both).  $t_{210:100}$  fell by a mean 28.1% after 20 min and mean 22.9% at 60 min ( $p<0.03$  for both). This change was mainly mediated by a fall in the  $P_{di,t_2,10}$ . The  $t_{210:100}$  was not able to distinguish between fatigue and acute hyperinflation.

We conclude that paired cervical magnetic nerve stimulation may be used to detect low frequency diaphragm fatigue but that it remains important to control for lung volume.

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Evidence that low frequency diaphragm fatigue (LFF) contributes to clinical problems is sparse. In part this may be because traditional techniques used in studies of human physiology have not proved easy to apply in environments where LFF is likely. The ability to detect diaphragm fatigue in such situations is thus of great interest to those engaged in clinical physiological studies.

Recently, YAN *et al.* [1] showed, in a rat diaphragm preparation, that the ratio of the force produced by repetitive 10 Hz stimulation to that produced by 100 Hz stimulation was closely related to the ratio of the tension additions elicited by the second of a pair of stimuli with interstimulus intervals 100 ms and 10 ms (the ratio  $t_{210:100}$ ). They further showed, in humans, that the  $t_{210:100}$  decreased after inspiratory resistive loading to task failure and concluded that a single value of  $t_{210:100}$  less than 1.1 indicated the presence of low frequency fatigue.

The purpose of the present investigation was to further evaluate the possibility of using paired phrenic nerve stimuli for the detection of LFF of the diaphragm in clinical situations. Specifically, we wanted to establish whether LFF could also be demonstrated by paired cer-

vical magnetic stimulation (pCMS), since CMS [2] is well tolerated by patients [3, 4]. Secondly, we investigated whether the  $t_{210:100}$  was influenced by lung volume (as an index of muscle length), since this has not been previously addressed.

### Methods

The subjects were seven members of our laboratory staff (six male, one female) without neurological or respiratory disease. The protocol was approved by the Ethics Committee of Kings College Hospital.

The force response of the diaphragm, assessed as transdiaphragmatic pressure ( $P_{di}$ ), was measured using a pair of commercially available latex balloon catheters, 110 cm in length (PK Morgan, Rainham, Kent, UK), placed in the stomach and oesophagus. The catheters were connected to differential pressure transducers (Validyne MP45-1, Validyne, Northridge, CA, USA), carrier amplifiers (PK Morgan, Rainham, Kent, UK), a 12 bit NB-MIO-16 analogue-digital board (National Instruments, Austin, TX,

USA) and a Macintosh Quadra Centris 650 personal computer (Apple Computer Inc., Cupertino, CA, USA) running Labview™ software (National Instruments, Austin, TX, USA).  $P_{di}$  was obtained on-line, by subtraction of oesophageal pressure ( $P_{oes}$ ) from gastric pressure ( $P_{ga}$ ). Pressure and volume signals were sampled at 100 Hz. Hemidiaphragm electromyography (EMG) signals were obtained in four subjects; these were recorded using silver/silver chloride surface electrodes (Arbo Medical, CT, USA) placed in the seventh intercostal space. The signal was carried *via* long leads to a Neuro-sign 100 amplifier (Magstim Co. Ltd, Whitland, Dyfed, UK) and thence to the analogue-digital board; EMG signals were sampled at 2 kHz.

#### Phrenic nerve stimulation

Bilateral paired stimulation of the phrenic nerve roots was performed with the subjects seated wearing a nose-clip with the abdomen unbound, trouser belt undone and with the neck flexed. Twitch potentiation [5] is known to influence the responses obtained with pCMS [6]; to minimize this, subjects were required to breathe quietly for 20 min before and throughout the study. Single stimuli were performed over the neck between the fifth and seventh cervical vertebrae to find the best spot for stimulation; this spot was marked and used for the remainder of the study [7]. Paired stimuli were given from a 90 mm circular coil (P/N 8443) powered by two linked Magstim 200 stimulators (Magstim Co., Whitland, Dyfed, UK). The linking circuitry (BiStim Module, Magstim Co., Whitland, Dyfed, UK) was capable of precisely controlling the interstimulus interval between 1 and 999 ms to an accuracy of within 0.05 ms. All stimuli were given at maximum stimulator output. Three pairs of stimuli were given for each experimental condition at interstimulus intervals of 10, 33, 50, 100 and 999 ms; the corresponding stimulating frequencies were therefore 100, 30, 20, 10 and 1 Hz.

#### Protocol

**Lung Volume.** With the subject rested and relaxed, stimuli were given at functional residual capacity (FRC) over the range of interstimulus intervals, stimuli being given in random order. This protocol was then repeated at the following lung volumes: 0.5 and 1.0 L below FRC; and 0.5, 1.0 and 1.5 L above FRC. To achieve these changes in lung volume, subjects slowly inspired or expired through a mouthpiece connected *via* a closed circuit to a spirometer (Ohio 840, Airco, Houston, TX, USA). When the predetermined lung volume was achieved the subject closed a valve at the mouthpiece and relaxed. Such relaxation was helped by prior practice with the aid of visual feedback. Once the operator considered that the subject had relaxed, as judged by levelling off of  $P_{oes}$  and  $P_{di}$ , the phrenic nerves were stimulated. Great care was taken to avoid generating high transdiaphragmatic pressures (which would have induced potentiation) when achieving high lung volumes. This proved feasible after a little practice (fig. 1) in all but one subject (No. 7); data from this subject were, therefore, obtained only at FRC.

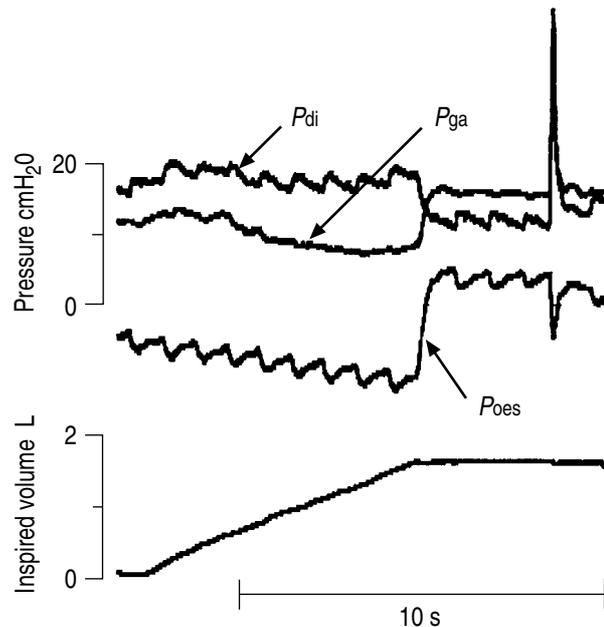


Fig. 1. – Representative trace of a subject achieving inflation and relaxation of a lung volume 1.5 L above functional residual capacity (FRC) without the generation of significant transdiaphragmatic pressure ( $P_{di}$ ), which could have created twitch potentiation.  $P_{oes}$ : oesophageal pressure;  $P_{ga}$ : gastric pressure.

**Fatigue.** Diaphragm fatigue was induced by performing a 2 min period of maximum isocapnic ventilation (MIV). We have previously described the details of the circuit [8] and have shown that this task induces a long-lasting fall in the  $P_{di}$  produced by a single stimulus ( $P_{di,tw}$ ) in highly motivated normal subjects [9]. Twenty minutes after the MIV the stimulation protocol was repeated at FRC and at 1.0 L below and 1.5 L above FRC. Further measurements at FRC were obtained 60 min after the MIV. One very inexperienced subject (No. 1) did not perform a sufficiently vigorous MIV to induce fatigue (arbitrarily defined as a >5% fall in  $P_{di,tw}$ ); data on this subject have been withheld from this portion of the analysis.

#### Data Analysis

For convenience and to facilitate comparison with YAN *et al.* [1] varying interstimulus intervals will be referred to in Hz rather than ms. At no point in the study were more than two twitches used.  $P_{di,tw}$  and the total  $P_{di}$  produced by paired stimuli ( $P_{di,ptw}$ ) were defined as the difference between the baseline pressure immediately before stimulation and the peak pressure immediately afterwards. Twitches were only accepted for analysis if performed with the subject relaxed, as judged by  $P_{oes}$ , and when baseline  $P_{di}$  was similar to that seen at end expiration during normal breathing, indicating relaxation of the diaphragm. The effect of the interstimulus interval on the amplitude of the  $P_{di}$  elicited by the second stimulus ( $P_{di,t2}$ ) was obtained by digitally subtracting the mean  $P_{di}$  waveform of a single twitch from the mean of the paired responses (at a given interstimulus interval). The  $t_{210:100}$  was obtained by division of  $P_{di,t2}$  at 10 Hz ( $P_{di,t2,10}$ ) by the  $P_{di,t2,100}$ . Statistical significance

was tested using Wilcoxon's Signed Rank Test for  $P_{di,t2}$  data and paired t-tests for the ratio  $P_{di,ptw}: P_{oes,ptw}$  (where  $P_{oes,ptw}$  is  $P_{oes}$  produced by paired stimuli) (Statview 4.0, Abacus Concepts, Berkeley, CA, USA). A p-value of less than or equal to 0.05 was taken as statistically significant.

**Results**

Stimulation proved acceptable to all subjects. In the fresh diaphragm the mean (SD) value for  $t_{210:100}$  at FRC was 1.33 (0.36) with a range of 0.67–1.77. During MIV there was a typical [9] decline in minute ventilation from an initial mean (SD) level of 178 (25) L·min<sup>-1</sup> to 110 (16) L·min<sup>-1</sup>. LFF of the diaphragm was observed after MIV in six subjects, as judged by a fall in  $P_{di,tw}$  of greater than 5%. In these subjects the mean fall in  $P_{di,tw}$  at 20 min was 17.9% and at 60 min 14.6%, (p<0.03 for both).  $t_{210:100}$  fell by a mean 28.1% to 0.98 after 20 min and a mean 22.9% to 1.05 at 60 min (p<0.03 for both). In the four subjects in whom EMG data were obtained the mean peak-to-peak amplitude of the surface hemidiaphragm action potential (CDAP) recorded from 1 Hz stimuli after MIV at FRC was 105% (range 98–113%) of values recorded before MIV. The change in  $t_{210:100}$  was mainly mediated by a fall in the  $P_{di,t2,10}$  as shown in table 1. The relationship between fall in  $P_{di,tw}$  and fall in  $t_{210:100}$  was not significant, but both remained reduced for at least 60 min after MIV (fig. 2). The subject (No. 1) in whom the fall in  $P_{di,tw}$  was small displayed equivocal changes in the ratio  $t_{210:100}$  (slightly raised at 20 min and slightly reduced at 60 min).

There was a negative relationship between  $t_{210:100}$  and increasing lung volume (fig. 3), the slope of which was best fitted with a polynomial function ( $r^2=0.98$ , p=0.002). Since data are only available for three lung volumes after MIV, we did not submit them to regression analysis, but as shown in figure 3, the mean values of  $t_{210:100}$  were lower at each lung volume, though this did not reach significance at 1.0 L below FRC. The similarity of reduction in  $t_{210:100}$  produced by both fatigue and hyperinflation was reflected in the shape of the  $t_{210:100}$  force-frequency curve (fig. 4).

The partitioning (judged as the ratio  $P_{oes}$  produced by paired stimuli ( $P_{oes,ptw}$ ):  $P_{di,ptw}$ ) of the signal produced was not influenced by the interstimulus interval;

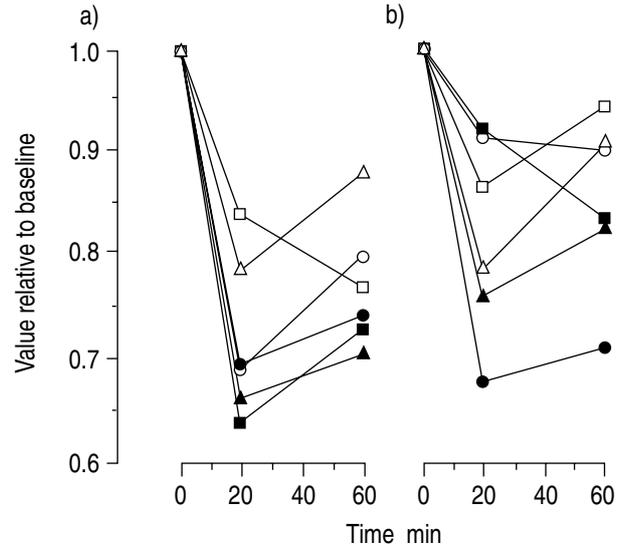


Fig. 2. – Comparison of the effect of diaphragm fatigue on: a) the ratio of the tensions elicited by the second of a pair of stimuli with interstimulus intervals 100 ms and 10 ms ( $t_{210:100}$ ); and b) transdiaphragmatic pressure produced by a single stimulus ( $P_{di,tw}$ ). There is clear and long-lasting fall in both  $P_{di,tw}$  and  $t_{210:100}$ . ●: Subject 1; ○: Subject 3; ■: Subject 4; □: Subject 5; ▲: Subject 6; △: Subject 7.

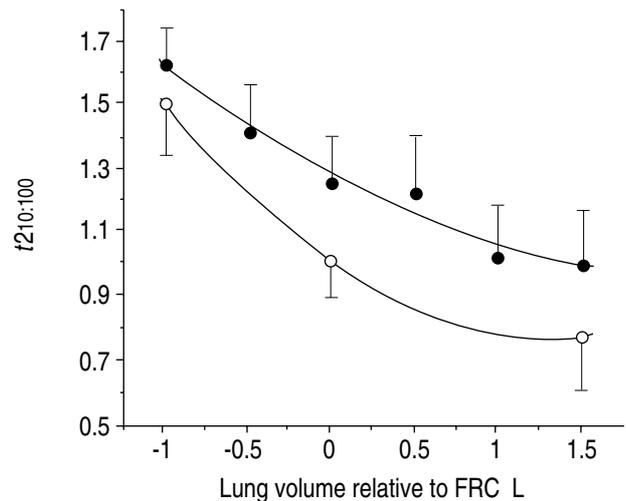


Fig. 3. –  $t_{210:100}$  expressed as a function of lung volume in both unfatigued (●) and fatigued (○) diaphragm (n=6). For definitions see legends to figure 1 and 2.

Table 1. – Individual data for single twitch and  $P_{di,t2}$  at 10 and 100 Hz, 20 min after maximal isocapnic ventilation (MIV)

Subject No.	$P_{di,tw}$ cmH <sub>2</sub> O			$P_{di,t2}$ 10 Hz cmH <sub>2</sub> O			$P_{di,t2}$ 100 Hz cmH <sub>2</sub> O			$t_{210:100}$ Baseline	$t_{210:100}$ After MIV
	Baseline	After MIV	Fall %	Baseline	After MIV	Fall %	Baseline	After MIV	Fall %		
1	28.9	28.1	3	28.1	26.5	6	25.4	21.7	15	1.10	1.22
2	30.3	20.5	32	32.3	21.7	33	24.9	24.1	3	1.30	0.90
3	26.1	23.8	9	19.8	15.2	23	29.6	32.8	-11	0.67	0.46
4	33.9	31.2	8	38.2	26.7	30	23.3	25.4	-9	1.64	1.05
5	21.1	18.3	13	29.9	25.0	16	20.9	20.8	0	1.43	1.20
6	28.2	21.5	24	40.6	24.8	39	23.0	21.2	8	1.77	1.17
7	21.5	17.0	21	23.1	22.1	4	16.6	20.1	-21	1.40	1.10
Mean	27.1	22.9	16	30.3	23.1	21.7	23.4	23.7	-2	1.33	1.01
SD	4.6	5.2	10	7.5	4.0	13.4	4.0	4.4	12	0.36	0.27

$P_{di,tw}$ : transdiaphragmatic pressure produced by a single stimulus;  $P_{di,t2}$ : transdiaphragmatic pressure at the second stimulus.

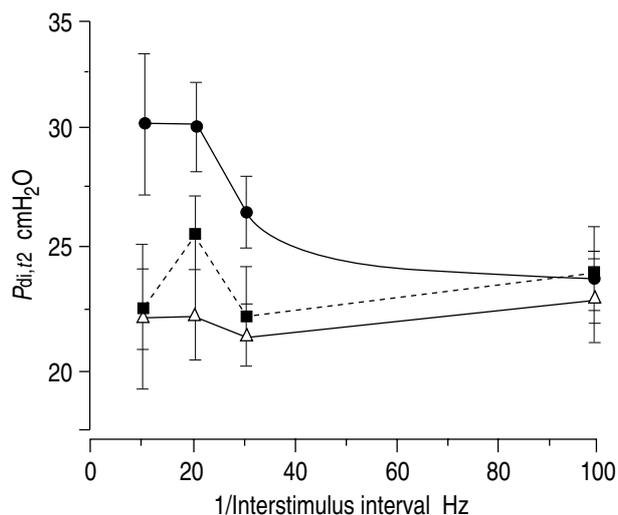


Fig. 4. —  $P_{di,t2}$  force-frequency curves for subjects unfatigued at FRC (—●—;  $n=7$ ), 20 min after MIV at FRC (---■---;  $n=6$ ) and fresh at 1.5 L above FRC (—△—;  $n=6$ ). The effect of fatigue cannot be distinguished from the effect of acute hyperinflation. Values are presented as mean $\pm$ sd. FRC: functional residual capacity; MIV: maximal isocapnic ventilation;  $P_{di,t2}$ : transdiaphragmatic pressure produced by the second stimulus.

however, for each interstimulus interval, a close relationship between mean  $P_{oes,ptw}:P_{di,ptw}$  and lung volume was obtained using a second order polynomial function ( $r^2 \geq 0.97$  and  $p < 0.005$  in each case) (fig. 5). In order to assess the effect of fatigue on partitioning we therefore pooled data from all interstimulus intervals for each study condition in order to compare  $P_{oes,ptw}:P_{di,ptw}$  before and after MIV. After MIV,  $P_{oes,ptw}:P_{di,ptw}$  was significantly reduced at FRC ( $p=0.0003$ ) and at 1.5 L above FRC ( $p < 0.0001$ ) but not at 1 L below FRC ( $p=0.34$ ) (fig. 6).

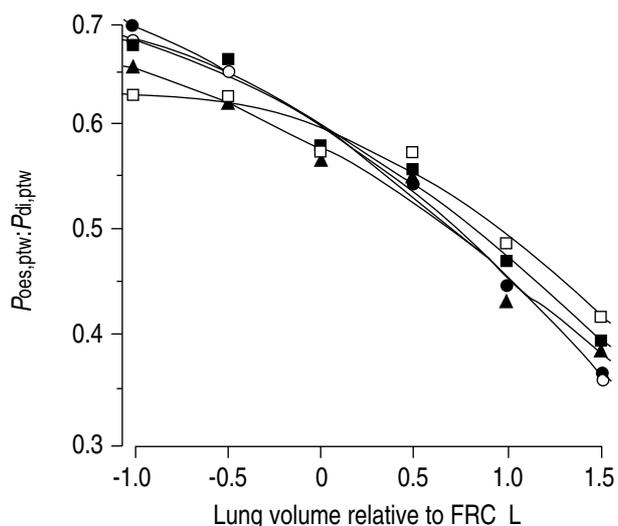


Fig. 5. — Mean  $P_{oes,ptw}:P_{di,ptw}$  plotted as a function of lung volume for each frequency (interstimulus interval). ●: 100 Hz fresh; ○: 30 Hz fresh; ■: 20 Hz fresh; □: 10 Hz fresh; ▲: 1 Hz fresh. FRC: functional residual capacity.  $P_{oes,ptw}$ : oesophageal pressure produced by paired stimuli;  $P_{di,ptw}$ : transdiaphragmatic pressure produced by paired stimuli.

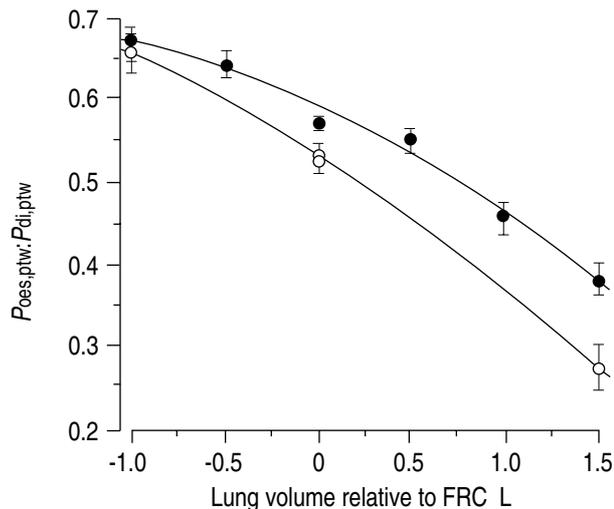


Fig. 6. — Mean  $P_{oes,ptw}:P_{di,ptw}$  (Of all interstimulus intervals pooled) before and after MIV; Error bars show the SEM. ●: fresh; ○: after maximal isocapnic ventilation. For definitions, see legend to figure 5.

## Discussion

The main finding of the present study is that the use of pCMS is feasible for the generation of paired phrenic nerve stimuli. Diaphragm fatigue was shown to influence the shape, as judged by the  $t_{210:100}$  of the  $t_2$  force frequency curve. However, the changes in the shape of the  $t_2$  force-frequency curve induced by fatigue were indistinguishable from those produced by acute hyperinflation.

### Critique of the methods

*Specificity of cervical magnetic stimulation (CMS).* In contrast to electrical stimulation (ES) which is relatively specific for the phrenic nerves, CMS is recognized to activate many muscles of the upper thorax [2, 7, 10]. One consequence of this is that it can be difficult to demonstrate supramaximality by levelling off of  $P_{di,tw}$ ; this is because as power output increases, recruitment of extradiaphragmatic muscles causes an increased  $P_{oes,tw}$  [10, 11] (presumably by stiffening the rib cage) even though this effect is not by itself inspiratory [12]. However, CMS-generated  $P_{di,tw}$  has proved to be closely correlated to diaphragm strength, whether judged by sniff  $P_{di}$ , ES-generated  $P_{di,tw}$  [4], or bilateral anterior magnetic phrenic nerve stimulation [13]. It has also been shown to be practical for use in a variety of patients with respiratory muscle weakness [4, 14, 15]; this is in contrast to ES which has a wide range of normal values [16]. However, given that activation of extradiaphragmatic muscles does contribute to  $P_{oes}$  produced by a single stimulus ( $P_{oes,tw}$ ) produced by CMS, the good relationship between CMS-generated  $P_{di,tw}$  and other indices of diaphragm strength must rely on the assumption that pathological processes usually affect all muscles equally. This assumption is, however, not valid in all clinical scenarios; surgical trauma after cardiac [17] or liver transplant [18] surgery being obvious exceptions. Furthermore, when CMS is used to investigate fatigue it is necessary to bear in mind that different protocols are considered to preferentially load different muscle groups [19], and that  $P_{di,tw}$  can fall without great change in the

ES-generated  $P_{di,tw}$  [20]. For many potential clinical applications the distinction between global inspiratory muscle fatigue and diaphragm fatigue may not be critical; nevertheless this consideration represents a potentially important difference between paired electrical stimulation (pES) and pCMS.

*Constancy of stimulation.* In physiological studies it is usual to demonstrate that the stimulation intensity is constant by showing that it is supramaximal, *i.e.* that further increase in stimulation intensity gives no further response in either (or both) electrical or mechanical response of the muscle. As discussed above, such data is difficult to obtain reliably with CMS and we did not provide it for the present study. We have, however, shown in sham studies that, in both normal subjects and patients, the mechanical response (*i.e.*  $P_{di,tw}$ ) to CMS is stable in the absence of diaphragm fatigue [3, 9, 21]. Thus, although we cannot absolutely exclude the possibility of submaximal stimulation we doubt whether this would have influenced our results, particularly since, if anything, submaximal stimulation tends to underestimate the presence of LFF [22].

*Chest wall configuration.* Despite having an isovolumic system the diaphragm could, especially with long inter-stimulus intervals, have changed configuration by the time of the second stimulus giving rise to the concern that muscle length at that time was shorter than indicated by the spirometer. This is particularly so given that we performed the studies with the abdomen unbound (this was a deliberate choice since it is often not practical to bind the abdomen in clinical studies). Thus we cannot absolutely exclude configurational changes between the twitches but this type of mechanism would not explain changes due to fatigue since contraction time is not influenced by fatigue [23]. Furthermore, the effect of lung volume on the  $P_{di,tw}$  has been shown to be largely independent of chest wall configuration [24], so it seems unlikely that this could wholly explain changes due to lung volume either.

*Why not use tetanic ES?* It is acknowledged that the force-frequency curve was not measured directly in the present study, and, self-evidently, this would have been preferable. However, ES has been available for approximately 20 yrs and to date none of the studies reporting tetanic ES have succeeded in using it bilaterally over the full range of frequencies even at FRC. We therefore doubt that it will ever be feasible for clinical studies of physiology involving patients. By contrast, the use of paired stimulation to deduce the force-frequency relationship in isolated muscle preparations is long established [25, 26], and has been validated in the case of the diaphragm by YAN *et al.* [1]. Preliminary experience also suggests that it is acceptable to patients [27].

#### Significance of the findings

Our data show that calculation of the  $t_{210:100}$  elicited by pCMS is capable of detecting diaphragm fatigue in that it agreed reasonably with the single twitch. However, compared to the single twitch it has some disadvantages: 1) two magnets and the linking module are required, which increases the cost of the technique; 2) relatively complex software is required to analyse the data whereas the height of a single twitch can be derived

from a simple recording system; and 3) although CMS is more acceptable to patients than ES, two twitches are inevitably less acceptable than one. However, pCMS may be of value to investigators specifically investigating phenomena that are a function of frequency, for example high frequency fatigue. Further potential applications would be in areas where the independence of  $t_{210:100}$  from twitch height could be exploited, *i.e.* in which it was hypothesized that changes in the shape of the force-frequency curve might occur simultaneously with changes in the twitch height. Our present volume data are an example of this.

It is established that the force generated by a muscle is influenced by its length [28]; however for the *in vivo* diaphragm this has only previously been demonstrated for the response to single stimuli [24, 29, 30]. No previous studies have addressed the effect of length change on the force-frequency characteristics of the human diaphragm, although it is clearly demonstrated in studies of isolated mammalian muscle [31], isolated diaphragm [32] and human limb muscle *in vivo* [33, 34], that acute shortening causes a disproportionate force loss in response to low frequency stimulation. This leads to a change in the shape of the force-frequency curve which is additive to the previously discussed effect of shortening on twitch tension. This phenomenon could be used to test the hypothesis that compensatory diaphragm adaptation occurs in chronic hyperinflation; the rationale being that if adaptation does not occur, the shape of the  $t_2$  force-frequency curve in patients with chronic obstructive pulmonary disease should resemble the shape observed in normal subjects during acute hyperinflation. Conversely, if it does occur it should be more like that seen in normal subjects at FRC.

Our observation that fatigue is more evident at high lung volumes is expected, since YAN *et al.* [23] previously demonstrated that the fall in ES-generated  $P_{di,tw}$  induced by a fatiguing protocol was greater if the muscle was assessed at high lung volume (*i.e.* short length). This finding is also consistent with observations made on human tibialis anterior *in vivo* [33].

After fatigue, the partitioning, assessed as the contribution of  $P_{oes,ptw}$  to the total  $P_{di,ptw}$ , was reduced at FRC and at 1.5 L above FRC but not at 1.0 L below FRC (fig. 6). These data are of interest since in a study using single bilateral ES, fatigue was not found to affect partitioning [23]. With CMS, and presumably pCMS,  $P_{oes,ptw}$  is enhanced because of co-activation of the upper thoracic muscles [7, 10]. Thus, the likely explanation for this discrepancy is that after MIV there is also fatigue of the muscles of the upper thorax which leads to a relatively smaller  $P_{oes,ptw}$ . That this should be more evident at high lung volumes is consistent with studies showing that the mechanical effectiveness of these muscles is reduced at high lung volume [35, 36]. This observation is not in itself surprising given that MIV produces fatigue of the sternomastoid [37] and abdominal muscles [38] in addition to the diaphragm [9, 13]. However, these data do support the view advanced by some investigators [20] that, at least when used to assess fatigue, CMS could be viewed as a global test of the inspiratory muscles rather than of the diaphragm alone.

In conclusion, our data show a fall in the ratio of the tensions elicited by the second of a pair of stimuli with

interstimulus intervals of 100 ms and 10 ms, elicited by paired cervical magnetic stimulation after maximal isocapnic ventilation which paralleled a fall in transdiaphragmatic pressure produced by a single stimulus confirming that the technique can detect low frequency diaphragm fatigue. However, a similar change results from acute hyperinflation and it therefore remains important to control for lung volume in the clinical investigation of inspiratory muscle fatigue when using this technique.

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