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Transdiapragmatic pressure and contractile properties of the diaphragm following magnetic stimulation

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ABSTRACT

Insufficient normal values exist regarding twitch transdiaphragmatic pressure (twPdi) derived from standardized cervical and cortical magnetic stimulation (MS) of the diaphragm.

Therefore, 63 subjects (24 men, 39 women; 34 ± 13 years) underwent transcortical and posterior cervical MS of the diaphragm with simultaneous recording of twitch oesophageal and gastric pressures (twPes, twPgas).

Following cortical MS at functional residual capacity, twPdi amplitudes showed high intra-individual variability which was markedly reduced when an inspiratory pressure trigger was applied. Lower limit of the 95% confidence interval computed around the mean value (LLN) was 12 cmH_2O, independent of gender or age. Following cervical MS of the phrenic nerves, twPdi amplitudes were well reproducible and unaffected by gender, but age-dependent (age 18–30: LLN 23 cmH_2O; age ≥ 30: LLN 16 cmH_2O; $p < 0.05$).

The inspiratory pathway can be assessed using cervical MS of the phrenic nerves. If transcranial motor cortex stimulation of the diaphragm is also applied, a standardized inspiratory pressure trigger is recommended. Dynamics of diaphragm contraction appear to be age-dependent.

1. Introduction

An American Thoracic Society (ATS) and European Respiratory Society (ERS) statement on respiratory muscle testing identifies phrenic nerve conduction studies (NCS) as a reliable technique for non-volitional evaluation of the inspiratory pathway. In particular, diaphragmatic strength can be assessed by combining magnetic stimulation (MS) of the phrenic nerves with simultaneous recording of the twitch transdiaphragmatic pressure (twPdi). The twPdi can be calculated by subtraction of twitch oesophageal pressure (twPes) from twitch gastric pressure (twPgas), measured transnasally using balloon catheters placed into the stomach and oesophagus. (Gibson et al., 2002).

MS of the phrenic nerves is painless and easy to apply (Similowski et al., 1989; Aubier et al., 1985). Cervical MS (CEMS) of the phrenic nerves results in bilateral contraction of the diaphragm and corresponding changes in twPdi. The twPdi curve can be analysed in greater depth based on amplitude (reflecting diaphragmatic strength) and temporal course. The latter reflects the diaphragm's contraction-relaxation cycle, which is crucial for normal respiration and adaptation to changes in both respiratory load and rate (Esau et al., 1983; Wilcox et al., 1988).

Clinical applications of twPdi recordings include evaluation of critically ill patients and those with neuromuscular disorders or chronic pulmonary disease, in whom diaphragmatic weakness develops or is already present (Esau et al., 1983; Wilcox et al., 1988; Kabitz et al., 2007a; Polkey et al., 1996). Twitch Pdi following CEMS provides non-

Abbreviations: ATS, American Thoracic Society; CEMS, cervical magnetic stimulation; COMS, cortical magnetic stimulation; ERS, European Respiratory Society; FVC, forced vital capacity; FRC, functional residual capacity; LLN, lower limit of the 95% confidence interval computed around the mean value; PImax, maximum inspiratory pressure; PEmax, maximum expiratory pressure; MRC, maximum rate of contraction; MRR, maximum rate of relaxation; MS, magnetic stimulation; Pdi, transdiaphragmatic pressure; Pes, oesophageal pressure; Pgas, gastric pressure; t½, half relaxation time; twPdi, transdiaphragmatic pressure following magnetic stimulation; twPes, oesophageal pressure following magnetic stimulation; twPgas, gastric pressure following magnetic stimulation

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volitional measures of diaphragm strength and fatigue which can be monitored during patient follow-up (Gibson et al., 2002).

Several studies applied twPdi recordings, mostly after CEMS of the phrenic nerves in small cohorts, (Similowski et al., 1989; Wragg et al., 1994a; Mier et al., 1989a; Luo et al., 2002) and transcranial, or cortical, MS (COMS) of the diaphragm has also been utilised. Resulting electrical muscle activity can be recorded bilaterally using surface electrodes placed at the lower costal margins, referred to as diaphragm motor evoked potentials following COMS, and diaphragm compound muscle action potentials following CEMS. While normal values for diaphragm motor evoked potentials and diaphragm compound muscle action potentials amplitude and latency, and central motor conduction time have been established (Zifko et al., 1996; Similowski et al., 1997; Khedr and Trakhan, 2001; Lissens, 1994), normal values for twPdi in larger cohorts are required in order to assess how the central and peripheral portion of the inspiratory pathway contribute to diaphragm performance.

It has been shown that pre-excitatory facilitation of the inspiratory pathway may increase diaphragm motor evoked potentials and diaphragm compound muscle action potentials amplitudes and twPdi following MS. This can be achieved using an inspiratory pressure trigger which standardises the time point of stimulus delivery (Windisch et al., 2005; Kabitz et al., 2007b). In most studies on COMS and CEMS of the diaphragm in healthy individuals, MS was only performed immediately after the onset of a regular breath as determined by visual observation of abdominal movements (Similowski et al., 1989; Wragg et al., 1994a; Mier et al., 1989a; Luo et al., 2002). Because this approach is difficult to standardise, normal values using the inspiratory trigger technique are also required.

The aim of this study was therefore to establish normal values derived from comprehensive and standardized evaluation of twPdi, twPes and twPgas using COMS and CEMS of the diaphragm in a large cohort of healthy subjects.

2. Material and methods

2.1. Study design

This prospective observational study was conducted from November 2017 to July 2018. Ethical approval was obtained from the local ethics committee (Ethikkommission der Universität Münster und der Ärztekammer Westfalen-Lippe ethical approval number: AZ 2016-072-f-S). All participants gave written informed consent. This study was part of a broader project exploring respiratory muscle strength and function in neuromuscular disorders and chronic obstructive pulmonary disease having established according normal values in healthy subjects (NCT03032562).

2.2. Study participants

Healthy adult volunteers with normal spirometric lung function tests based on current reference values (Evans and Whitelaw (2009); Dean (1999)) were included.

2.3. Pulmonary function tests

Tests were performed in the upright position according to standard recommendations (Gibson et al., 2002). Forced vital capacity (FVC) and forced expiratory volume (FEV) were determined in ≥5 consecutive tests until the best result was achieved with < 10% variability to the preceding test. Participants who failed to achieve < 10% variability to the preceding test within the first 5 runs repeated testing until this criterion was fulfilled (which was the case within the three following runs in all subjects, i. e. the maximum number of tests performed was 8). At least three tests were performed for measurement of maximum inspiratory and expiratory pressures (PImax and PEmax) and the best result was recorded when variability to the preceding test was < 10%. As for PImax and PEmax measurements, all participants performed at least 3 tests. In those who failed to achieve < 10% variability to the preceding test within the first 3 runs the test was repeated until this criterion was fulfilled (with the maximum number of runs being 6). Technical devices comprised an electronic spirometer (Vitalograph 3000™, Vitalograph, Hamburg, Germany) and an electronic pressure meter (Micro RPM™, Care Fusion, Baesweiler, Germany).

2.4. Magnetic stimulation

All experiments were preceded by a 20-minute rest period (quiet breathing, no talking). MS was performed using a MagPro Compact™ magnetic stimulator (MagVenture, Willich, Germany) equipped with a 12 cm C-100 circular coil (2.0 T, 0.1 ms square-wave pulses), at functional residual capacity (FRC) or using an inspiratory pressure trigger (see below) with healthy volunteers wearing a noseclip. Diaphragm motor evoked potentials (MEP) and compound muscle action potentials (CMAP) were recorded using surface electrodes with the reference electrode placed 5 cm cranially to the tip of the xiphoid process, and the active electrodes applied to both costal margins 16 cm from the reference electrode. For COMS, the magnetic coil was positioned flatly over Cz´ according to the international 10–20 electroencephalograph system. (Maskill et al. (1991)).

For CEMS, the coil was first placed flatly over C7 and moved towards C6 in a stepwise manner until the highest reproducible twPdi at 100% of the maximum magnetic power output was obtained. The optimal nuchal stimulation site was marked with a pen. Stimulation intensity was then initially set at 60% of the maximum output and gradually increased by 10% until both the highest twPdi amplitude and the highest MEP/CMAP amplitude was achieved. Resulting amplitudes were plotted against stimulus intensity revealing that supramaximality could not reliably be achieved with less than 100% of magnetic output (2.0 T) in many (20 out of 63) subjects. In these cases, twPdi and MEP/CMAP amplitude showed a further increase in amplitude when stimulus intensity was increased from 90% to 100% of maximum magnetic output. Supplemental Fig. S1(A) shows representative CMAP recordings of a subject in whom titration of the magnetic field output from 90% to 100% led to an increase in amplitude > 10% compared to the preceding two stimulations. In contrast, Supplemental Fig. S1(B) shows representative tracings of a subject in whom the increase of stimulus intensity beyond 80% did not lead to a further increment in CMAP amplitude. Thus, ≥5 stimulations at maximum magnetic output were delivered in each coil position thereafter until twPdi amplitude showed < 10% variability to the preceding two stimulations.

In order to avoid twitch potentiation, stimuli were separated from each other by a pause of ≥40 s. For stimulation at FRC, abdominal movements were visually observed after instructing the patient to expire and hold the breath until the magnetic stimulus was delivered (which was delivered within 2 s after FRC had been reached). Both twPes and twPgas were obtained using balloon catheters (Cooper Surgical, Trumbull, CT, USA) transnasally inserted into the stomach and distal oesophagus. The oesophageal and gastric balloon were filled with 1.0 mL and 2.5 mL of air, respectively. Balloon catheters were connected to a differential blood pressure transducer (DPT-100™, Utah Medical Products, Athlone, Ireland), which was interfaced to a fully-isolated blood pressure amplifier and the PowerLab™ data acquisition device (both ADInstruments, Oxford, UK), and pressure data for twPgas, twPes and twPdi (twPes – twPgas) were continuously displayed using LabChart™ software (ADInstruments, Oxford, UK, sampling rate of 1000 Hz) on a personal computer and saved for later analysis.

Maximum rate of contraction (MRC), maximum rate of relaxation (MRR), half relaxation time (t½) and area under the curve (AUC) were derived from the twPdi curve following CEMS. MRC is defined as the positive peak of the pressure derivative as a function of time (i.e. the steepest slope of the inclining twPdi curve) (Similowski et al., 1991).

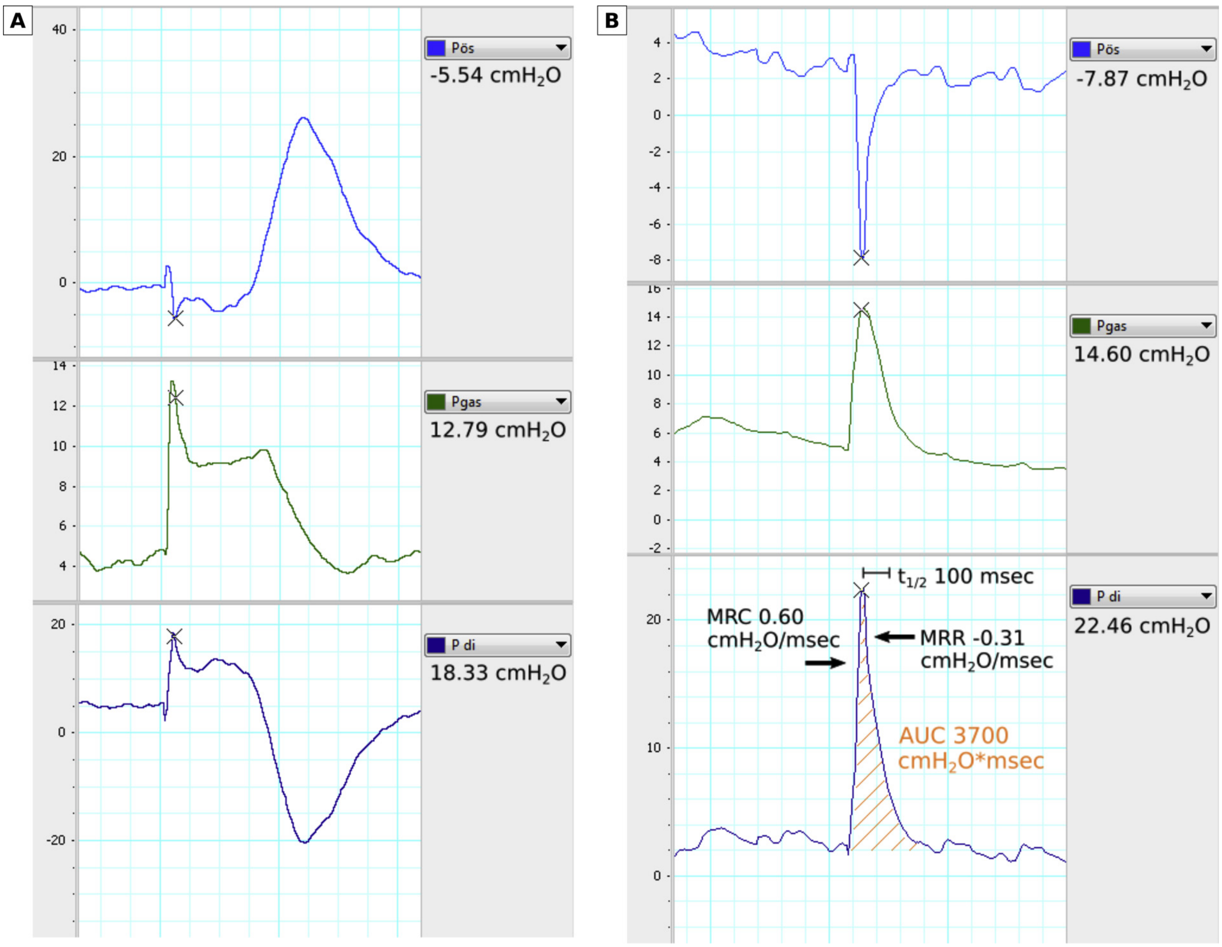

Fig. 1. Representative trace showing transdiaphraghmatic pressure following cortical magnetic stimulation **A** and following cervical magnetic stimulation **B** (at functional residual capacity). AUC, area under the curve; $t_{1/2}$, half time of recovery. Note: pressure values were converted from mmHg to cmH_2O for the final analysis.

MRR is defined as the negative peak of the pressure derivative as a function of time and measures the initial part of the pressure decay (Coirault et al., 1995). MRC and MRR vary with twPdi amplitude and were adjusted for peak twPdi (Similowski et al., 1991). Finally, t½ was defined as the time taken for peak tension to decrease by 50%. Fig. 1 shows representative tracings of twPdi following supramaximal COMS (Fig. 1A) and CEMS (Fig. 1B) at FRC.

As twPdi following MS can be variable even in healthy volunteers, (Zifko et al., 1996) a fully automated inspiratory pressure trigger was used to reliably perform MS at a defined time point during inspiration (Kabitz et al., 2007b). Subjects breathed through a spirometry device that continuously measured and displayed mouth-pressure on a personal computer running the LabChart™ software. Inspiration was detected when inspiratory volume exceeded 10 mL. When inspiratory pressure reached –5.1 cmH_2O, a mechanical shutter automatically closed the spirometry flow head for 100 ms. The magnetic stimulus was automatically delivered 80 ms after the pressure threshold was reached meaning that the shutter was definitely closed by the time of stimulus release. Triggered MS was performed directly after the corresponding measurement at FRC to ensure identical placement of the coil. COMS was performed first and CEMS thereafter.

2.5. Statistical analysis

All analyses were performed using Sigma Plot™ software (Version 13.0, Systat, Erkrath, Germany). Results are expressed as mean and standard deviation for continuous variables or percentage for categorical variables unless otherwise specified. For normal values, the 95% confidence interval (CI) was computed for the statistical mean and the lower limit (LLN) of the 95% CI of the mean was calculated and reported. Normal distribution was tested using the Kolmogorov-Smirnov test. Differences between groups were compared using Student's t-test. Subjects were stratified into two groups by sex and two groups based on age (dichotomized by the median [30 years]). Side-to-side differences were tested using a paired *t*-test. Pearson's product moment correlation was used for correlation analyses. For graphical presentation of the transdiaphragmatic pressure gradients obtained after magnetic stimulation, box plots were used where the mid horizontal line shows the mean, boxes show the upper and lower quartiles and whiskers show the 5th and 95th percentiles (outliers are displayed as dots). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Subjects

Sixty-three healthy volunteers were included in the study. Average age was 34 ± 13 years; 24 (38%) subjects were male (36 ± 16 years) and 39 (62%) were female (33 ± 12 years). Table 1 summarizes anthropometric, demographic, lung function and pressure data for all participants and stratified by gender.

All participants showed normal spirometric lung function tests (according to existing normal values) (Evans and Whitelaw, 2009; Dean, 1999). Mean PImax was 94 ± 27 cmH_2O and PEmax was

Table 1
Demographic data, lung function and pressure recordings, overall and stratified by gender.

	Healthy volunteers			LLN		P-value
	Overall (n = 63)	Males (n = 24)	Females (n = 39)	Males	Females	
Clinical characteristics						
Age (years, range)	34 (18-78)	36 (18-78)	33(20-59)			n.s.
Female (%)	62					
Height (cm, range)	175 (160-192)	184 (174-192)	170 (160-178)			< **0.05**
BMI (kg/m^2)	25 ± 2	24 ± 2	25 ± 2			n.s.
Weight (kg)	76 ± 9	83 ± 8	72 ± 7			< **0.05**
Lung function data						
FVC, L (% predicted)	4.6 ± 1.1 (107 ± 15)					
FEF25-75, L/sec (% predicted)	3.5 ± 1.1 (83 ± 21)					
FEF50, L/sec (% predicted)	4.2 ± 1.2 (86 ± 21)					
FEV_1/FVC (%)	81 ± 8 5					
PEF, L/sec (% predicted)	8.5 ± 2.1 (103 ± 18)					
PImax (cmH_2O)	94 ± 27	109 ± 29	85 ± 22	97	78	< **0.05**
PEmax (cmH_2O)	130 ± 31	149 ± 29	118 ± 27	137	110	< **0.05**
Pressure recordings after MS						
Transcortical MS at FRC						
twPdi (cmH_2O)	13.6 ± 12.3	12.3 ± 12.2	15.0 ± 12.5	9.7	11.3	n.s.
twPes (cmH_2O)	−6.8 ± 8.2	−4.1 ± 10.9	−8.2 ± 5.4	−1.4	−2.7	n.s.
twPgas (cmH_2O)	6.8 ± 9.5	8.2 ± 10.9	6.8 ± 9.5	4.1	4.1	n.s.
Cervical MS at FRC						
twPdi (cmH_2O)	21.8 ± 9.5	21.8 ± 9.5	21.7 ± 8.2	19.0	19.0	n.s.
twPes (cmH_2O)	−15.0 ± 6.8	−15.0 ± 8.2	−16.3 ± 6.8	−12.2	−12.2	n.s.
twPgas (cmH_2O)	6.8 ± 5.4	6.8 ± 6.8	5.4 ± 4.1	4.1	4.1	n.s.
MRR twPdi (cmH_2O/ms)	−11.2 ± 9.4	−11.0 ± 9.5	−11.3 ± 9.5	−7.2	−8.3	n.s.
MRC twPdi (cmH_2O/ms)	32.1 ± 17.0	34.9 ± 17.7	30.4 ± 16.6	27.8	25.2	n.s.
t½ twPdi (ms)	200 ± 270	260 ± 410	160 ± 120	430	200	n.s.
AUC twPdi (cmH_2O x ms)	4978 ± 5086	6093 ± 7616	4298 ± 2434	3046	3536	n.s.
Transcortical MS at -5.1 cmH_2O						
twPdi (cmH_2O)	17.7 ± 13.6	19.0 ± 16.3	16.3 ± 12.1	12.2	13.6	n.s.
twPes (cmH_2O)	−6.8 ± 9.5	−6.8 ± 8.2	−5.4 ± 10.9	−4.1	−2.7	n.s.
twPgas (cmH_2O)	10.9 ± 16.3	12.2 ± 19.0	10.9 ± 15.0	4.1	6.8	n.s.
Cervical MS at -5.1 cmH_2O						
twPdi (cmH_2O)	15.0 ± 7.0	16.8 ± 7.1	14.6 ± 6.8	15.0	12.2	n.s.
twPes (cmH_2O)	−10.9 ± 6.8	−12.2 ± 8.2	−10.9 ± 5.4	7.8	7.3	n.s.
twPgas (cmH_2O)	4.1 ± 5.4	4.6 ± 5.4	3.7 ± 5.4	1.7	1.4	n.s.
MRR twPdi (cmH_2O/ms)	−23.6 ± 29.5	−19.1 ± 18.3	−26.4 ± 34.6	−11.5	−15.1	n.s.
MRC twPdi (cmH_2O/ms)	42.7 ± 25.7	39.8 ± 22.5	44.4 ± 27.6	30.4	35.4	n.s.
t½ twPdi (ms)	140 ± 150	150 ± 170	140 ± 140	220	180	n.s.
AUC (cmH_2O x ms)	2679 ± 3033	3618 ± 4311	2094 ± 1673	1809	1537	n.s.

Data are presented as mean ± standard deviation or %, unless otherwise stated. LLN, lower limit of the 95% confidence interval computed around the mean value; AUC, area under the curve; FEF, forced expiratory flow; FEV_1, forced expiratory volume after 1 s; PEmax, maximal expiratory pressure; PImax, maximal inspiratory pressure; MRC, maximal rate of contraction (in relation to twitch amplitude); MRR, maximal rate of relaxation (in relation to twitch amplitude); MS, magnetic stimulation; n. s. not statistically significant; PCF, peak cough flow; PEF, peak expiratory flow; twPdi, twitch transdiaphragmatic pressure (gastric pressure – oesophageal pressure); twPes, twitch oesophageal pressure; twPgas, twitch gastric pressure; t½, half time of recovery (the upper limit of normal is given); VC, vital capacity.

130 ± 31 cmH_2O, with significantly higher values in male than in female participants for both parameters (Table 1).

Subjects were stratified by gender and age in order to test whether age and sex impact non-volitional diaphragm strength (Tables 1 and 2). Full data sets were obtained in all volunteers. Fig. 1 displays representative twPdi curves following supramaximal COMS (Fig. 1A) and CEMS (Fig. 1B) at FRC.

3.2. COMS and CEMS at FRC

After COMS at FRC, reproducible twPdi amplitudes (< 10% variability) were obtained in 50/63 volunteers (13 subjects showed maximum 20% variability). LLN was 10.4 cmH_2O, independent of age (Table 1) or gender (Table 2). TwPdi amplitudes were poorly reproducible, both inter-individually (reflected by a coefficient of variation of 0.90) and intra-individually (as reflected by as much as 13 subjects in whom maximum twPdi amplitude showed 20% variability) (Fig. 2A).

After CEMS at FRC, stable twPdi amplitudes were obtained in all subjects with the inter-individual coefficient of variation being 0.43. The LLN was 19.0 cmH_2O, again unaffected by age (Table 1) or gender (Table 2). Changes in twPes contributed more to twPdi than variations in twPgas. Mean twPes and twPgas were significantly higher in subjects aged 18–30 years (LLN 23.1 cmH_2O) versus ≥30 years (LLN 16.3 cmH_2O). twiPdi amplitudes were reproducible within and between subjects (Fig. 2B). The spread of twPdi following CEMS in relation to age is displayed in Supplemental Fig. S2.

TwPgas, twPes, and twPdi were all significantly higher after CEMS versus COMS of the phrenic nerves (Fig. 2B).

3.3. COMS and CEMS with inspiratory pressure trigger

TwPdi following COMS and CEMS of the phrenic nerves using an inspiratory pressure trigger are depicted in Fig. 2 and in Tables 1 and 2.

Following COMS, reproducible twPdi amplitudes were obtained in all 63 subjects with sufficient intra-individual variability and a coefficient of variation of 0.77 with regard to inter-individual variability. The LLN for twPdi amplitude was 13.0 cmH_2O independent of gender and age (Tables 1 and 2). TwPdi amplitudes were easier to obtain, slightly (but not significantly) higher, and more reproducible both inter- and

Table 2
Normal values of transdiaphragmatic pressure gradients and lung function by age.

	Age 18-30 years (n = 32)	Age ≥30 years (n = 31)	P-value
Clinical characteristics			
Age (years)	24 ± 3	45 ± 12	**< 0.05**
Female (%)	51	50	n.s.
Height (cm)	176 ± 8	174 ± 9	n.s.
BMI (kg/m^2)	25 ± 2	25 ± 2	n.s.
Weight (kg)	77 ± 8	74 ± 10	n.s.
Lung function data			
FVC, L (% predicted)	5.0 ± 1.0 (108 ± 12)	4.3 ± 1.1 (106 ± 18)	**< 0.05**
FEF25-75, L/sec (% predicted)	4.0 ± 1.0 (87 ± 18)	3.0 ± 1.0 (79 ± 23)	**< 0.05**
FEF50, L/sec (% predicted)	4.6 ± 1.2 (88 ± 19)	3.8 ± 1.2 (83 ± 23)	**< 0.05**
FEV$_1$/FVC (%)	82 ± 8	79 ± 7	n.s.
PEF, L/sec (% predicted)	8.8 ± 2.2 (101 ± 15)	8.1 ± 1.9 (105 ± 21)	n.s.
PImax (cmH$_2$O)	98 ± 28	89 ± 26	n.s.
PEmax (cmH$_2$O)	132 ± 33	128 ± 30	n.s.
Transdiaphragmatic pressure data following MS			
Transcortical MS at FRC			
twPdi (cmH$_2$O)	15.0 ± 13.6	15.0 ± 9.5	n.s.
twPes (cmH$_2$O)	−8.2 ± 8.2	−6.8 ± 8.2	n.s.
twPgas (cmH$_2$O)	6.8 ± 9.5	8.2 ± 9.5	n.s.
Cervical MS at FRC			
twPdi (cmH$_2$O)	24.5 ± 9.5	19.0 ± 6.8	**< 0.05**
twPes (cmH$_2$O)	−17.7 ± 8.2	−13.6 ± 6.8	**< 0.05**
twPgas (cmH$_2$O)	6.8 ± 6.8	5.4 ± 2.7	n.s.
MRR twPdi (cmH$_2$O/sec)	−9.5 ± 6.7	−12.9 ± 11.4	n.s.
MRC twPdi (cmH$_2$O/sec)	33.0 ± 20.1	31.2 ± 13.4	**n.s.**
t ½ twPdi (ms)	230 ± 360	170 ± 140	n.s.
AUC twPdi (cmH$_2$O x ms)	6079 ± 6501	3835 ± 2679	n.s.
Transcortical MS at -5.1 cmH$_2$O			
twPdi (cmH$_2$O)	16.4 ± 13.6	19.1 ± 15.0	n.s.
twPes (cmH$_2$O)	−8.2 ± 9.5	−4.1 ± 9.5	n.s.
twPgas (cmH$_2$O)	8.2 ± 13.6	15.0 ± 17.7	n.s.
Cervical MS at -5.1 cmH$_2$O			
twPdi (cmH$_2$O)	16.3 ± 6.8	13.6 ± 5.4	n.s.
twPes (cmH$_2$O)	−10.9 ± 5.4	−10.9 ± 8.2	n.s.
twPgas (cmH$_2$O)	5.4 ± 6.8	2.7 ± 4.1	n.s.
MRR twPdi (cmH$_2$O/sec)	−18.9 ± 20.5	−28.4 ± 36.2	n.s.
MRC twPdi (cmH$_2$O/sec)	40.9 ± 27.7	44.4 ± 23.9	n.s.
t½ twPdi (ms)	150 ± 170	130 ± 140	n.s.
AUC twPdi (cmH$_2$O x ms)	5304 ± 5086	5032 ± 5440	n.s.

Data are presented as mean ± standard deviation or %, unless otherwise stated. AUC, area under the curve; FEF, forced expiratory flow; FEV$_1$, forced expiratory volume after 1 s; PEmax, maximal expiratory pressure; PImax, maximal inspiratory pressure; MRC, maximal rate of contraction (in relation to twitch amplitude); MRR, maximal rate of relaxation (in relation to twitch amplitude); MS, magnetic stimulation; n. s. not statistically significant; PCF, peak cough flow; PEF, peak expiratory flow; twPdi, twitch transdiaphragmatic pressure (gastric pressure – oesophageal pressure); twPes, twitch oesophageal pressure; twPgas, twitch gastric pressure; t½, half time of recovery (the upper limit of normal is given); VC, vital capacity.

intra-individually compared with stimulation at FRC (Fig. 2A). Following CEMS, reproducible twPdi amplitudes could be obtained in all subjects with inter-individual variability being sufficient (coefficient of variation 0.46). The LLN for twPdi amplitude was 13.3 cmH$_2$O with no difference between genders (Table 1). TwPes accounted for changes in twPdi more than twPgas. Compared to COMS and CEMS of the phrenic nerves at FRC, pressure amplitudes were slightly smaller when the inspiratory pressure trigger was used for CEMS.

3.4. Maximum rates of contraction and relaxation

Mean MRC following supramaximal CEMS at FRC adjusted for twPdi amplitude was 32.1 ± 17.0 cmH$_2$O/msec/cmH$_2$O. Its LLN was 27.3 cmH$_2$O/msec/cmH$_2$O with no impact of gender. MRC was not significantly different in the younger age group (33.0 ± 20.1 cmH$_2$O/msec/cmH$_2$O vs. 31.2 ± 13.4 cmH$_2$O/msec/cmH$_2$O; $p < 0.05$). Mean MRR was –11.2 ± 9.4 cmH$_2$O/msec/cmH$_2$O with no impact of both gender and age, and LLN was –8.0 cmH$_2$O/msec/cmH$_2$O). Half time of recovery (t½) was 200 ± 270 msec with an upper limit of normal of 270 msec, and was unaffected by age or gender. Following CEMS using an inspiratory pressure trigger, the respective values for both MRC and MRR were consistently higher, but differences were not significant (Tables 1, 2).

4. Discussion

Our study provides extensive normal values for twPes, twPgas and twPdi following both COMS and CEMS of the diaphragm in healthy adults. We used a standardised protocol for MS at FRC but also performed MS combined with an inspiratory pressure trigger (Windisch et al., 2005; Kabitz et al., 2007b). So far, normal values for twPdi after phrenic nerve stimulation in healthy subjects have been collected in rather small samples ranging from 5 to 23 subjects and using different stimulation techniques (Similowski et al., 1989; Wragg et al., 1994a; Luo et al., 2002; Similowski et al., 1996; Hamnegard et al., 1996; Mier et al., 1990; Polkey et al., 1997; Mier et al., 1989b). In total, 95 healthy subjects were examined in 10 studies. In order to facilitate comparison of results, Table 3 summarises the findings previously reported. In all studies, magnetic stimuli were delivered at FRC. An inspiratory pressure trigger was used in only one study, which did not apply twPdi recordings.16] COMS was used in one study, but no twPdi response could be elicited at FRC (Mier et al., 1990). In-depth analysis of the human diaphragm contractile properties including t½, MRC and MRR was provided by only one study (Similowski et al., 1991).

With regard to twPdi following CEMS, published results show substantial variation that may be attributed to either stimulation technique or age. In contrast to previous reports, our study is the first large enough to show that age affects twPdi. Half of the previous studies reported twPdi at FRC to be about 25 cmH$_2$O which is close to our results (Esau et al., 1983; Similowski et al., 1996; Gandevia and McKenzie, 1985; Laghi et al., 1996).

Our study confirms that the contractile properties of the diaphragm change with age. Although volunteers were rather young in our study cohort, stratification for age revealed that twPdi andtwPes were significantly lower in the higher age group. This finding is in line with one previous study specifically focusing on age-dependency of the twPdi amplitude (Polkey et al., 1997).

Mean values for MRR and MRC were –11 cmH$_2$O/msec/cmH$_2$O and 32 cmH$_2$O/msec/cmH$_2$O, similar to the only previous study reporting these values in five healthy men (–11 and 35 cmH$_2$O/msec/cmH$_2$O, respectively) (Similowski et al., 1991).

Utilisation of an inspiratory pressure trigger in combination with CEMS has been shown to allow for prediction of twPdi by twitch mouth pressure (Kabitz et al., 2007b). In our study, standardisation of the exact timing of the magnetic stimulus was associated with less intraindividual variability in twPdi amplitudes after COMS compared with MS at FRC, and Pdi amplitudes were still significantly higher in younger subjects. Interestingly, mean twitch Pdi amplitude was significantly lower when the inspiratory pressure trigger was used compared to CEMS at FRC. While the concept of facilitation could suggest even higher values when an inspiratory pressure trigger is used, the impact of lung volume itself may account for this finding. Hamnegard and coworkers elegantly showed that twPdi decreases as lung volume increases (Wragg et al., 1994b). On the other hand, in-depth analysis of twPdi revealed consistently higher values for MCR and MRR using an

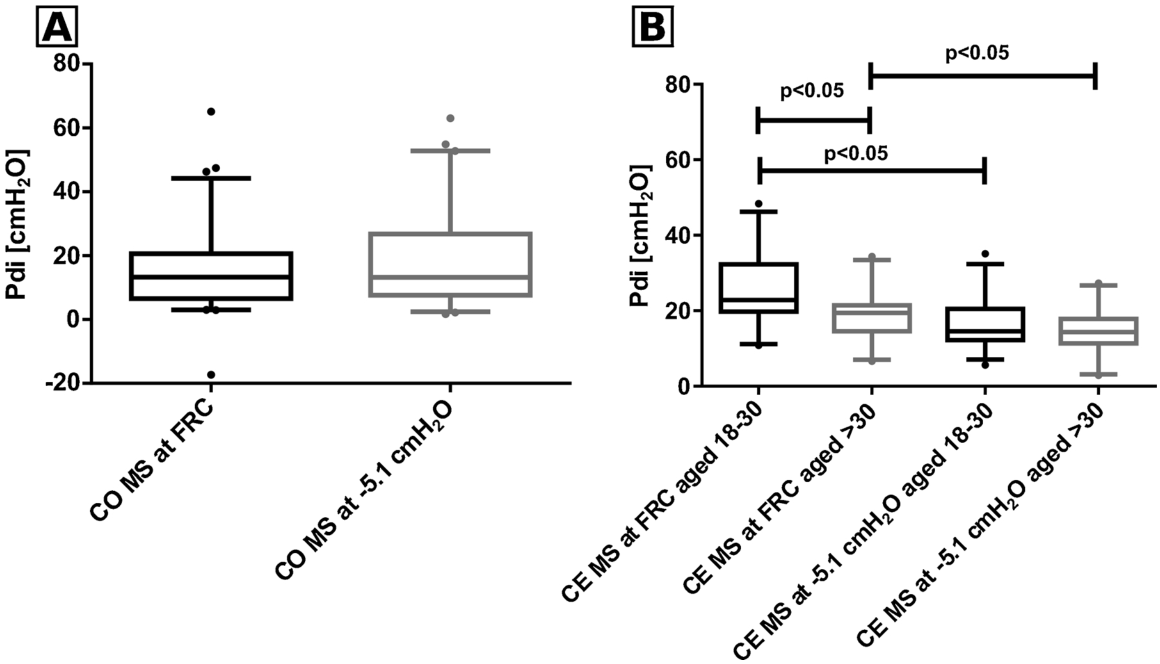

Fig. 2. Normal values of transdiaphragmatic pressure gradients following cortical **A** and cervical **B** magnetic stimulation at functional residual capacity and using an inspiratory pressure trigger. The mid horizontal line shows the mean, boxes show the upper and lowe quartiles and whiskers show the 5th and 95th percentiles. Outliers are displayed as dots.

inspiratory pressure trigger that would speak out in favour of facilitation.

With regard to COMS of the phrenic nerves, one previous study used a similar protocol to ours (Similowski et al., 1996). In this study, deflection of the twPdi curve did not occur in response to stimulation of the relaxed diaphragm at FRC, and twPdi only increased at higher lung volumes. In contrast, we could elicit a pressure response already at FRC. This finding corresponds with the observation that COMS of the phrenic nerves at FRC leads to reproducible diaphragm MEP (Similowski et al., 1997; Khedr and Trakhan, 2001; Lissens, 1994). In the previous study, supramaximality may not have been reliably achieved which we tried to achieve by performing at least five consecutive stimulations (see below).

In addition, evaluation of a larger cohort revealed that twPdi responses to COMS were rather small (15.0 ± 12.2 cmH_2O) and highly variable: 20/63 healthy volunteers showed a twPdi amplitude below 7 cmH_2O (data not shown).

Twitch Pdi following COMS using an inspiratory pressure trigger was slightly higher compared with stimulation at FRC. Differences were not significant but can possibly be explained by central facilitation which has been described for motor evoked potentials recorded from other muscles than the diaphragm. (Zifko et al., 1996). In addition, COMS may also have activated accessory inspiratory muscles (Hamnegard et al., 1995).

4.1. Study limitations

Despite its comprehensive approach our study has several limitations. Firstly, inter- and intra-observer variability may have affected the study results. We aimed to address this by extensive training and by performing up to five tests per patient until variability was < 10%. Secondly, threshold testing for magnetic output was not performed. Instead, the same magnetic field output was used in every subject to achieve reproducible twPdi amplitudes. Thirdly, our normal values may not be applicable to other populations. This is particularly true for older individuals because mean age was relatively low in our cohort. Fourthly, twitch mouth pressure was not measured but has been shown to reliably predict twPdi when an inspiratory pressure trigger is used. (Kabitz et al., 2007b)

Finally, we cannot exclude that in some subjects, supramaximal

Table 3
Summary of published values for twitch transdiaphragmatic pressure after magnetic stimulation of the phrenic nerves in healthy subjects.

First author, year	N	Age, years	% Male	Stimulation site	twPdi at FRC (cmH_2O)	
					CEMS	COMS
Similowski, 1989 (Similowski et al., 1989)	6	28 ± 5 (n.a.)	83	C7 anterior, bilateral	33 ± 10 (n.a.)	n.a.
Similowski, 1991 (Similowski et al., 1991)	5	62 ± 9 (n.a.)	100	C5–C7 anterior, bilateral	26 ± 4 (n.a.)	n.a.
Wragg, 1994 (Wragg et al., 1994a)	9	n. a. (30–50)	67	C5–C7 anterior, bilateral	37 (27–48)	n.a.
Wragg, 1994 (Wragg et al., 1994b)	6	n. a. (31–45)	100	C5–C7 anterior, bilateral	30 (19.4–38.4)	n.a.
Laghi, 1995 (Laghi et al., 1995)	12	31 (23–36)	92	C5–C7 anterior, bilateral	39 ± 1 (SE) (33–42)	n.a.
Hamnegard, 1995 (Hamnegard et al., 1995)	6	38 ± 8 (n.a.)	100	C5–C7 anterior, bilateral	40 (n.a.)	n.a.
Similowski, 1996 (Similowski et al., 1996)	7	28 ± 4 (n.a.)	57	C5–C7 anterior, bilateral	28 ± 2 (SEM) (n.a.)	Available, but absence of response at FRC
Polkey, 1996 (Polkey et al., 1996)	7	30 (n.a.)	57	C5–C7 anterior, bilateral	25 ± 2 (SEM) (n.a.)	n.a.
Kabitz, 2007 (Kabitz et al., 2007b)	21	25 ± 2 (n.a.)	100	C5–C7 anterior, bilateral	23 ± 6 (n.a.)	n.a.

Values are mean ± standard deviation (range), unless otherwise stated.
CEMS, cervical magnetic stimulation; COMS, cortical magnetic stimulation, n.a., not available; SE, standard error; SEM, standard error of the mean; twPdi, twitch transdiaphragmatic pressure (gastric pressure – oesophageal pressure).

magnetic stimulation was not achieved even by using the maximum magnetic output of the coil. This problem has been adressed before (Mills et al., 1996; Man et al., 2004; Mador et al., 2002), depends on both the magnetic stimulator and coil and may be technically difficult to overcome. In a subset of volunteers, titration of the magnetic output from 90% to 100% was accompanied by an increase in CMAP amplitude and/or twPdi amplitude greater than 10% leaving the possibility that higher magnetic output than 2 T might have triggered an even higher response. However, for clinical research purposes, this aspect may be considered negligible as long as a case-control study design is chosen, and strict adherence to a standardized methodology is warranted.

5. Conclusion

Inspiratory muscle strength testing using MS with recording of twPdi is feasible in healthy subjects. We recommend further utilisation of this diagnostic method in patients with impending or manifest respiratory muscle weakness.

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Competing interests

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resp.2019.04.011.

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