**Task specific differences in respiration-related activation of the deep and superficial pelvic floor muscles**

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**Running Head:**

Deep and superficial pelvic floor muscles and respiration

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**Abstract**

The female pelvic floor muscles (PFM) are arranged in distinct superficial and deep layers that function to support the pelvic/abdominal organs and maintain continence, but with some potential differences in function. Although general recordings of PFM activity show amplitude modulation in conjunction with fluctuation in intra-abdominal pressure such as that associated with respiration, it is unclear whether the activity of the two PFM layers modulate in a similar manner. This study aimed to investigate the activation of the deep and superficial PFM during a range of respiratory tasks in different postures. Twelve females without pelvic floor dysfunction participated. A custom-built surface electromyography (EMG) electrode was used to record the activation of the superficial and deep PFM during quiet breathing, breathing with increased dead-space, coughing, and maximal and submaximal inspiratory and expiratory efforts. As breathing demand increased, deep PFM layer EMG had greater coherence with respiratory airflow at the frequency of respiration than the superficial PFM ($P=0.038$). During cough, the superficial PFM activated earlier than the deep PFM in sitting ($P=0.043$). In contrast, during maximal and submaximal inspiratory and expiratory efforts, the superficial PFM EMG was greater than that for the deep PFM ($P=0.011$). These data show that both layers of PFM are activated during both inspiration and expiration, but with a bias to greater activation in expiratory tasks/phases. Activation of the deep and superficial PFM layer differed in most of the respiratory tasks, but there was no consistent bias to one muscle layer.

Key words: pelvic floor muscles, electromyography, levator ani, bulbocavernosus, respiration

**New and Noteworthy:** Although pelvic floor muscles are generally considered as a single entity, deep and superficial layers have different anatomy and biomechanics. Here we show

task-specific differences in recruitment between layers during respiratory tasks in women. The deep layer was more tightly modulated with respiration than superficial, but activation of the superficial layer was greater during maximal/submaximal occluded respiratory efforts and earlier during cough. These data highlight tightly coordinated recruitment of discrete pelvic floor muscles for respiration.

## Introduction

The female pelvic floor includes multiple striated muscles that contribute to control of urinary continence, modulation of intra-abdominal pressure (IAP), and support of the organs of the pelvic and abdominal cavities. Support of the floor of the abdominal cavity is important for multiple reasons. First, increase of IAP requires support of the abdominal cavity in all directions, which includes contraction of the abdominal muscles, the diaphragm and the pelvic floor muscles (PFM)(39). This is necessary for functions such as lifting (18), postural control (19), and respiratory functions including breathing (6) and coughing (37). Reduced capacity of the PFM, which often accompanies conditions such as urinary incontinence, might compromise control of IAP and prevent optimal performance of these functions. Second, without adequate support, the pelvic organs can descend outside the abdominal cavity (e.g. pelvic organ prolapse) resulting in pain (12, 43) and reduced quality of life (13-15). Despite the importance of PFM for control of IAP, understanding of their role in this function is incomplete.

The PFMs are arranged in deep and superficial layers that have different attachments and biomechanical actions (3, 35). Although this implies potential differences in their role in control of IAP, few studies have considered the activation of the deep and superficial PFM layers separately. The deep layer of PFM includes the levator ani group and the coccygeus muscle (1, 25) with attachments to the inner surface of the pubic bone, fascia of the obturator internus muscle and the sacrum/coccyx (41). The superficial layer is formed by the bulbocavernosus, ischiocavernosus, and deep and superficial transverse perineal muscles (35) that lie below the perineal membrane, and located lateral to the vaginal opening and perineal body (38). Although some data show activation of the superficial PFMs before the deep PFM during voluntary contraction (9), this has not been studied in automatic functions such as respiratory tasks.

PFM activity has been studied during a range of respiratory tasks, but generally only quantified using methods that are either unable to discriminate between PFM layers (e.g. surface electromyography [EMG] (21, 23, 27) and vaginal pressure (24, 28)) or only measure activity from one muscle (e.g. intra-muscular EMG (5, 36), surface EMG (23), and real-time ultrasound imaging (7, 26, 29, 31)). Using these methods, PFM activation has been shown to increase prior to and during a cough (27) and when IAP is increased with a Valsalva manoeuvre (36). During breathing, PFM are tonically active throughout the respiratory cycle, but also phasically modulate their activity such that activation is greater during expiration (21). In view of the potential differences in biomechanics and evidence of differential activation of muscle layers with voluntary efforts (9), it is plausible that activation of deep and superficial PFM may differ during breathing-related tasks. Preliminary evidence to support this hypothesis comes from one study that made qualitative judgement of presence of displacement of the superficial perineum (clitoral motion - assumed to indicate superficial PFM activation) and reduction of midsagittal hiatal diameter (which reflects deep PFM activation) in women attending a gynaecology clinic (10). In that study, Dietz and colleagues reported that the two layers of muscle did not always displace together during a cough - midsagittal hiatal diameter reduced in 79% of women, whereas 91% displayed clitoral motion. Temporal differences were also observed between muscle layers - hiatal diameter reduced in advance of bladder neck movement in 31% of women but clitoral motion preceded bladder neck motion in just 5% of women. Similar qualitative measures were reported, but with some differences in outcome, in two other studies (44, 45). Although these observations provide some initial indication of differential activation of the two muscle regions, no studies have reported quantitative measures or investigated women without dysfunction.

The overall objective of this study was to compare the activation of the deep and superficial PFM layers during a range of respiratory tasks in women without pelvic floor

dysfunction. The specific aims were to: (i) compare the activation of the deep and superficial PFM during quiet breathing and breathing with increased demand; (ii) compare the amplitude and timing of activation of the deep and superficial PFM during coughing in sitting and standing; and (iii) compare the amplitude of activation of deep and superficial PFM during maximal and submaximal inspiratory and expiratory efforts.

## Methods

### *Participants*

Twelve English-speaking female participants with mean(SD) age of 34(10) years volunteered for this study. Ten participants were nulliparous and two were multiparous (each with two vaginal deliveries). Participants were excluded if they were younger than 18 years, had a history of pelvic floor dysfunction or any major respiratory, neurological or orthopaedic condition. The Institutional Medical Research Ethics Committee approved the study and all participants provided informed written consent.

### *Electromyography*

An EMG electrode was custom-made using an Educator device (Neen, Patterson Medical, UK). The modification of the probe included addition of multiple strands of silver wire to record EMG from the left and right sides of the deep and superficial layers of the PFM. A single-use probe was manufactured for each participant by passing the wires through drilled holes, such that 2 pairs of wires (exposed length 15 mm) were exposed on each side, one pair was located at 45 mm and the other at 5 mm from the flange of the device, which is design to maintain the position of the electrode at the introitus. These wires were located such that they were aligned with the deep and superficial muscles and approximately along the direction of muscle fibres when the electrode was inserted vaginally. The connection cable exited the distal end of the electrode and was secured with tape to the inner thigh. EMG data

were bandpass filtered (20–1000 Hz), amplified 2000 times (Neurolog, Digitimer, UK), and sampled at 4000 samples/s with a Power1401 acquisition system using Spike2 software (CED, UK).

*Respiratory measures*

During breathing and coughing, airflow was recorded using a pneuomotachometer (Model 3813, Hans Rudolf Inc., USA) connected to a low differential pressure transducer (Model DP45-16, Validyne Engineering, USA) via a disposable mouthpiece (SureGard Filter, Bird Healthcare, Australia). For the maximal and submaximal inspiratory and expiratory pressure trials, mouth pressure was recorded using a modified disposable mouthpiece (SureGard Filter, Bird Healthcare, Australia) with a blocked exit and a tube that entered a side-port for connection to a variable reluctance pressure sensor (Model DP15-34, Validyne Engineering, USA).

A nose clip (Model 9014, Hans Rudolf Inc., USA) was used during respiratory recordings. Data were low pass filtered at 50 Hz and amplified 50 times with a dual output carrier demodulator (Model CD19A, Validyne Engineering, USA) and recorded with the EMG data.

*Procedure*

Participants were positioned in long sitting with a back rest reclined to 45° from vertical. Participants performed four tasks;

(i) Maximum voluntary contraction (MVC): participants were asked to squeeze and lift the PFM around the pelvic openings as hard as possible and hold the contraction for 5s for two repetitions (See Figure 1 for a representative example).

(ii) Dead space breathing - participants breathed for up to 120s through the filter/pneumotachometer held in the mouth which was connected to a tube (2L volume) to

induce hypercapnia. Participants could cease the trial if they became distressed by the increased respiration.

(iii) Cough - participants performed a single voluntary cough to an effort of 8/10 (10 = maximum voluntary effort) for three repetitions. Coughing was performed in sitting and in standing with nose clip and lips sealed on the mouthpiece attached to the pneumotachometer which was attached to a one-way valve that enabled recording of exhalation.

(iv) Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) – in separate trials participants were instructed to take a breath out to end tidal volume or inhale a moderate volume, prior to performance of a maximal inhalation or exhalation, respectively, against an occluded airway (blocked respiratory mouthpiece), as hard as possible for 3-5 s for three repetitions.

(v) Sub-maximal inspiratory pressure (SIP) and sub-maximal expiratory pressure (SEP) – participants were provided with feedback of mouth pressure and were instructed to match 25% of the pressure achieved in the MIP/MEP tasks in separate trials. Pressures were maintained for 3-5 s for three repetitions.

*Data Analysis*

Data from two subjects for the maximal and submaximal inspiratory and expiratory tasks were not included due to technical failure of the equipment. EMG and respiratory data were analysed with MatLab (R2013b, Mathworks Inc, USA) using custom written programs. Left and right EMG data were averaged for each muscle layer and repetition, and all values were normalised to MVC. The MVC normalization value was determined by selecting the largest peak EMG amplitude recorded from the deep/right channel across the two repetitions of MVC.

*Dead space breathing:* Data were analysed in two phases. Initial analysis of the data for 10 participants showed that dead space breathing did not induce a significant increase in

155 respiratory flow until at least the 9$^{th}$ breath. On this basis, data for the initial seven breaths
156 were considered to represent quiet breathing and are referred to as “early dead space
157 breathing”. This is consistent with the observations of Campbell and Green (6). The final
158 seven breaths were analysed to represent the period of hypercapnia or increased respiratory
159 demand and are referred to as “late dead space breathing”.
160 Spectral measures assessed the relation between EMG modulation of each PFM layer
161 and respiratory airflow during the early and late dead space breathing. This analysis was
162 conducted on the basis that if PFM EMG was modulated at frequency of breathing, then
163 airflow and PFM EMG would have a high coherence value at this frequency (19). Coherence,
164 which relates to correlation in the frequency domain, is bounded between 0 reflecting no
165 relation, and 1 reflecting perfect relation between modulation of airflow and PFM EMG. For
166 spectral analyses, EMG data were bandpass filtered between 20-500 Hz using a fourth order
167 dual pass Butterworth filter. EMG data were then rectified, and low pass filtered (5 Hz) using
168 a fourth order dual pass Butterworth filter. This filtering creates linear envelopes which
169 emphasize the slow signal components and remove high frequency peaks. The
170 pneumotachometer signal was bandpass filtered between 0.1 and 2 Hz using a dual pass
171 Butterworth filter. An adapted Fourier approach of Rosenberg et al (33) was used to
172 transform the signals from the time domain to the frequency domain. Briefly, data were
173 divided into five sections of 8.192 s with 50% overlap using a Hanning window. To increase
174 the resolution of frequencies of the Fourier transform to 0.0305 Hz, each section was zero-
175 padded to a length of $2^{17}$. Spectral estimates were then constructed with algebraic
176 combinations from the 5 sections with a fast Fourier transform algorithm (16). The main
177 frequency of respiration was determined as the frequency with the maximum spectral power
178 of airflow. Coherence between airflow and each of the two PFM EMG signals was calculated
179 as the squared ratio between the cross-spectrum divided by the square-root of the product of

180 the airflow and EMG spectra (16). Coherence between airflow and EMG was considered
181 significant if the value exceeded 0.5271 which was determined as follows; $1-(0.05)^{1/(\#\ sections-1)}$
182 (16, 33).

183 To investigate the temporal relationship between modulation of PFM EMG (at the
184 main frequency of respiration) and airflow, the phase angle between EMG and airflow was
185 calculated. The amplitude of temporal shift of EMG and breathing modulations is reflected
186 by the size of the phase angle, with zero degrees reflecting concurrent modulation (i.e. in-
187 phase) and 180 degrees reflecting modulation in opposite directions (i.e. out-of-phase). A
188 negative phase angle indicates modulation of EMG before airflow. Phase angles were only
189 analysed when coherence between airflow and EMG exceeded the significance threshold.

190 To assess the effect of breathing effort on the proportion of PFM EMG power that
191 was modulated with breathing, a ratio was calculated by dividing the EMG power at the main
192 breathing frequency (which is generally ~0.2Hz) in the power spectrum by the sum of EMG
193 power of the frequencies between 0-2Hz.

194 *Cough:* For analysis of EMG amplitude in relation to cough, five time points were manually
195 selected from the airflow or EMG signals: (i) a point located within 5s before the cough
196 where PFM EMG was observed to be silent, (ii) onset of expulsion from airflow, (iii) peak of
197 expulsion from airflow, (iv) initial trough or inflection point in airflow following peak
198 expulsion, and (v) end of expulsion from airflow (Figure 2). These points were used to
199 calculate root mean square (RMS) EMG during four epochs: (a) 1s about the point selected
200 for EMG at rest, (b) 100ms prior to the onset of expulsion, (c) between peak expulsion and
201 the initial airflow trough that followed peak expulsion, and (d) 100ms after the end of
202 expulsion.

203 To determine the onset of deep and superficial PFM EMG during cough, RMS EMG
204 was calculated for twenty 10-ms epochs calculated over the 200ms that preceded the onset of

205 expulsion. This is the period during which onset of PFM EMG is expected (37). The time of
206 EMG onset was determined by the following criteria: (1) the first epoch that was equal to or
207 larger than a threshold defined as three standard deviations greater than mean activity at rest
208 (calculated as mean of five consecutive 10-ms epochs between 500-400 ms prior to onset of
209 expiration with smallest value), and (2) the selected epoch had to be followed by at least one
210 epoch equal to or larger than the threshold.

211 *MIP/MEP and SIP/SEP:* RMS EMG amplitude was calculated for a 1-s epoch starting 500
212 ms before the time of peak positive or negative mouth pressure achieved during the task. For
213 the submaximal tasks, the RMS EMG was calculated for a 1-s period closest to the target
214 25% of maximum mouth pressure.

215 *Statistical analysis*

216 A linear mixed model analysis was used to compare the coherence, proportion of total
217 power and phase angle between the two respiratory Demands (early vs. late dead space
218 breathing; repeated measure) and Muscle layers (deep vs. superficial PFM averaged for left
219 and right sides for each layer; within participant) in the dead space breathing trials. For
220 analysis of the cough, linear mixed model analysis was used to compare the EMG amplitudes
221 between participants Position (sitting vs. standing; repeated measure), Muscle layers (deep
222 vs. superficial PFM averaged for left and right sides for each layer; within participant), and
223 Cough phase (rest vs. pre-onset of expulsion vs. peak expulsion vs. post-end of expulsion). A
224 linear mixed model analysis was used to compare EMG amplitudes between maximal and
225 submaximal respiratory pressure tasks between Tasks (inspiration vs. expiration; repeated
226 measure), Effort levels (maximal vs. submaximal), and Muscle layers (deep vs. superficial
227 PFM averaged for left and right sides for each layer; within participant). Descriptive statistics
228 and t-tests were used to compare the averaged onsets of the left and right channels for each
229 layer during cough within and between participant position (i.e. during sitting and standing).

Separate t-tests for sitting and standing was because onset were unavailable for some participants in the standing position (EMG amplitude change failed to reach threshold for detection of onset) where background EMG was higher.

## Results

*Activation of PFM layers during respiration*

Raw EMG during early and late dead-space breathing for a representative participant is shown in Figure 3. The proportion of EMG power at the frequency of respiration of both PFM layers was significantly higher during late than early dead space breathing (Main effect - Demand; P=0.0093; Figure 4) providing evidence that as respiratory demand increased, activation of both PFM layers for breathing also increased. There was no difference in EMG power between the PFM layers (Main effect - Muscle layer; P= 0.11; Figure 4), which suggests that during dead space breathing both muscles layers respond with similar amplitude increase.

Based on the *a priori* defined threshold for significant coherence (0.5271), 7/12 participants had significant coherence (i.e. coordinated modulation of airflow and PFM EMG) for one or both muscle layers during early dead space breathing, and 9/12 participants during late dead space breathing (Figure 5). This concurs with statistical analysis that showed significantly greater coherence between respiratory airflow and muscle activity during late than early dead space breathing (Main effect - Demand; P=0.006), which indicates the relationship between modulation of EMG of both PFM layers with respiratory airflow increased as respiratory demand increased. Comparison between layers showed significantly greater coherence was between airflow and deep than superficial PFM EMG (Main effect – Muscle layer; P= 0.038) during both early and late dead space breathing, which suggests a bias to activation of the deep PFM for respiration.

Of data with significant coherence, analysis of phase angle showed in-phase modulation of PFM EMG and airflow, that is PFM EMG was greater during expiration. There was a small negative phase angle for both PFM layers indicating that EMG modulation precedes that of airflow (phase angle: early dead space breathing - deep -23 ±30, superficial -31±51; late dead space breathing; deep -7±32, superficial -10±27).

*Amplitude and timing of PFM activation during cough in sitting and standing*

Figure 6 shows raw EMG during a cough in sitting and standing for a representative participant. Analysis of EMG amplitude during cough showed a significant interaction between cough Phases, Muscle layer and participant body Position ($P=0.0044$; Figure 7). Post hoc analysis showed that the deep PFM EMG was greater than that of the superficial layer during standing during the rest phase ($P=0.002$; Figure 7), but not the other phases ($P>0.12$; Figure 7). Both muscles were more active at rest in standing than sitting, but similar between positions for the other phases (all $P>0.12$; Figure 7). Both muscles were more active in pre-expulsion, peak expulsion and post peak expulsion than rest phase in both positions (all: $P<0.003$; Figure 7).

When times of EMG onset were compared between PFM layers, there was a significantly earlier onset of superficial than deep PFM EMG in sitting (superficial - 11.3 (4.6) 10-ms epochs before onset of expulsion vs. deep - 8.4 (4.8) epochs; $P=0.043$) but not standing (superficial - 13.1 (14.7) epochs vs. deep - 9.7 (5.7) epochs; $P=0.062$). There were no differences in EMG onset of deep ($P=0.57$) or superficial ($P= 0.94$) PFM between positions.

*Amplitude of deep and superficial PFM EMG during maximal and submaximal inspiratory and expiratory efforts*

Examination of the amplitudes of EMG normalised to MVC during maximal and submaximal inspiratory and expiratory efforts showed significantly greater normalised

superficial PFM EMG than deep PFM EMG across conditions (Main effect - Layer: P=0.011); Table 1; Figure 8 shows raw data for a representative participant). Both muscle layers were more active during maximal than submaximal inspiratory and expiratory efforts (Main effect - Effort: P<0.001). Both PFM layers were more active during expiration than inspiration (Main effect - Phase: P=0.025).

## Discussion

This study aimed to compare the activation of the deep and superficial PFM during a range of respiratory tasks in women without pelvic floor dysfunction. The data show differences between superficial and deep PFM layers during the respiratory tasks, but these were task specific. Although, the deep PFM were more tightly modulated with respiration than the superficial PFM, the superficial PFM had greater normalised EMG during maximal and submaximal respiratory efforts and were activated earlier during the cough in sitting. These observations have relevance for the interpretation of the role of the PFM in respiration.

### *PFM layers work together during respiration*

The present data show that EMG of both PFM layers modulate with breathing (with greater activation during expiration) and demonstrate greater coherence with airflow as respiratory demand increases. This provides evidence for a role of activation of both PFM layers in associated with airflow during respiration. These data are consistent with earlier investigations of global PFM activation (21, 40). Using electrodes that could not separately record from the two PFM layers, Hodges et al (21) showed modulation of PFM EMG with breathing that increased with tidal volume in women without pelvic floor dysfunction. Talasz et al. (40) reported for a similar group that movement of the PFM was phase-locked with that of the diaphragm during quiet and forceful breathing, and cough. Other work has shown incremental increases in PFM activation (inferred from digital palpation of PFM contraction) during with different intensities of expiratory flow (25%, 50% and 75%) (39).

There are three inter-related interpretations of the activation of female PFM during respiration. First, activation of the PFM is required to maintain continence as IAP increases during both the inspiratory and expiratory phases of breathing (6, 20) as a consequence of alternating activation and motion of the abdominal wall and diaphragm (21). Second, PFM activation is necessary to support the pelvic/abdominal organs (i.e. prevent organ descent) when challenged by gravity and increased IAP. Third, to contribute to the IAP increase required to generate respiratory movements (i.e. increased IAP elevates the relaxed diaphragm during expiration in upright positions (40)).

The three separate roles of PFM are generally congruent (i.e. all require elevated PFM activation as IAP increases) and the relative contribution of the PFM to each role is not possible to disentangle. One issue requires additional consideration. In the current study, although both PFM layers were active throughout the respiratory cycle, EMG was higher during expiration than inspiration. This is similar to previous reports (21, 34). Those data show greater elevation of both PFM and abdominal EMG during expiration, despite the increase in IAP in both respiratory phases (21). Although this appears counterintuitive considering the role of PFM to control IAP, the observation can be explained by the well-known force-velocity relationship of EMG. The downward motion of the pelvic floor with inspiration that is phase-locked with diaphragm descent (40) implies lengthening of the PFM. Lower PFM EMG during this phase probably reflects the fact that a similar muscle force can be generated by less EMG as the muscle is contracting eccentrically. Thus, it cannot be inferred that PFM generate greater force and have a greater role during expiration. The PFM maintain a role throughout the respiratory cycle.

Higher PFM EMG during MEP than MIP tasks also cannot be interpreted to imply greater role of PFM during expiration. Activation of PFM during both tasks confirm a role of PFM during inspiratory and expiratory efforts. In this case greater PFM EMG with expiration

cannot be explained by PFM lengthening during expiration as airway occlusion would have limited respiratory motions. Instead it is likely explained by higher IAP in this task. Greater changes in mouth pressures can be generated during MEP than MIP (8), and this concurs with higher IAP. Taken together with the new data, these observations imply that activity of both PFM layers contribute to respiration during both respiratory phases, and that differences between phases depends on muscle length change and IAP.

*Task dependent difference in respiratory activation of deep and superficial PFM*

A new observation from the present study was that the bias of respiratory activation to the deep or superficial PFM was task dependent; deep PFM had greater coherence with airflow, whereas superficial PFM EMG was greater with isometric inspiratory/expiratory efforts and increased earlier with coughing in sitting. This task-dependence has several possible explanations. First, during late dead space breathing, PFM must maintain tension at an elevated level for a prolonged period. The bias to deep PFM might be explained by a superior mechanical advantage for the deep PFM to generate urethral pressure and support the pelvic organs. Although not directly compared, greater advantage might be expected from the deep PFM which loop around behind the urethra, vagina and rectum and compress these structures against the pubic symphysis, than the superficial PFM which lie lateral to the vaginal and urethra. This requires further investigation. The deep PFM also have greater bulk. Greater capacity of the deep PFM to maintain tension is also suggested by the observation of the greater activation of the deep than superficial PFM at rest in standing, prior to the cough. This activation would be interpreted to support abdominal contents against gravity in standing, independent of a role in respiration (21, 34).

Second, features of the superficial PFM may explain the bias to activation of these muscles in cough and maximal/submaximal pressure tasks. In sitting, onset of superficial PFM EMG occurred earlier during a cough than that of deep PFM. Earlier onset of the

superficial than deep PFM has also been reported during voluntary PFM contraction in response to verbal instruction (9). This bias differed from the observation of simultaneous activation of deep and superficial PFM in 81% of women by Yang et al (44). However, that interpretation was based on qualitative judgement from two-dimensional real-time ultrasound, which is unable to match the temporal resolution or accuracy of our EMG and algorithm-based analysis. One possible reason for the faster activation of the superficial PFM (including bulbocavernosus and ischiocavernosus muscles) is the higher proportion of fast twitch muscle fibres than the deep muscles (32). This may enable the superficial muscles to produce a faster increase in force to counteract the rapid increase in IAP with expulsion (30, 42). Unlike standing, the two muscle layers activated together during coughing in standing. There are two possible explanations. First, standing places greater demand on the PFM to support the pelvic contents and induces greater activation of deep PFM, as mentioned above. As activation is already present, there may be less requirement for an additional ballistic increase in PFM EMG. Second, the explanation may be methodological - onset determination using our method based on a change from mean baseline activity is potentially less sensitive to detect onset when tonic rest activation in greater in standing.

With respect to the activation of superficial muscles to a greater percentage of MVC during the MIP/MEP and SIP/SEP, this could be inferred to suggest greater role for activation of these muscles. Alternatively, it might simply reflect that these muscles have smaller size and lesser mechanical advantage (see above), and thus need to contract to a greater proportion of their maximum to generate sufficient tension to contribute to the task. Previous work has highlighted that greater neural drive does not necessarily infer relative capacity to perform a task(22).

*Methodological considerations*

There is likely to be some cross-talk between recordings of deep and superficial PFM in the present study. The surface EMG electrode was designed to use separate electrode pairs to bias recording separately for the deep and superficial PFM layers. This electrode and the alternative design of electrode described by Devreese et al. (9) have successfully identified differences in activation between the muscle layers, which provides evidence that at least part of the activity they record reflects the separate muscles. No commercially available EMG electrodes allows separate recording from the PFM layers and most previous studies have used invasive methods, such as intramuscular EMG (5, 36). Although real-time ultrasound provides another alternative (10, 44, 45), further investigation and validation of this method is required to established methods to quantify parameters and to test their relationship to muscle activation.

*Clinical implications*

The new insights provided by the current study have potential clinical implications for assessment and management of PFM. Most notably, the data provide a foundation to investigate respiration-related activation of the PFM in women with pelvic floor dysfunction and/or respiratory disorders. Although coughing is routinely considered in assessment of stress urinary incontinence (17), assessment of respiration more broadly may provide further insight into PFM activation and the coordination between muscle layers. This may be particularly relevant for women with comorbid conditions affecting PFM function and breathing. For instance, urinary and faecal incontinence are a commonly reported by women with cystic fibrosis (25.8% and 22.6%, respectively)(2) and bronchiectasis (48% of females) (11). Further work to evaluate respiration-related activation of the PFM in women with urogenital conditions is required to refine and then validate assessments.

*Conclusion*

In summary, the current study provides further evidence for the importance of PFM for respiration and provides new insight into task dependent differences in respiratory activation of the superficial and deep PFM layers. These observations confirm that deep and superficial PFM must be considered individually to understand PFM function.

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## Figure legends

**Figure 1** Raw EMG data of superficial and deep pelvic floor muscles from a representative participant during maximal voluntary contraction.

**Figure 2** Points selected for interpretation of the cough EMG. Airflow data are shown for a representative participant. The selected points are highlighted with an "x".

**Figure 3** Raw EMG data of superficial and deep pelvic floor muscles from a representative participant during respiration. Three breaths are shown during early (left) and late dead space breathing (right). Power spectra for each muscle and coherence between airflow (PNTX – pneumotachometer) and EMG for the deep and superficial muscles are shown for each condition. Vertical dashed lines indicate the frequency of respiration identified from the power spectrum of flow. EMG calibration – 10 μV; PNTX calibration – 0.5 L/s.

**Figure 4** Group data for the EMG power at frequency of respiration expressed as proportion of the sum of power spectrum (between 0 and 2 Hz). Data are shown for deep and superficial PFM layers separately for early and late dead space breathing. Mean and standard deviation are shown. *=P<0.001. Prop. – proportion. EMG – electromyography.

**Figure 5** Coherence between EMG and airflow at frequency of respiration for individual participants (circles) and group (rectangle, mean and standard deviation). Data are

shown separately for deep and superficial PFM during early and late dead space breathing. Lines connect data points for the sperate muscles for each participant. The *a priori* defined threshold for significant coherence is shown with the dashed line. Note the greater proportion of data points above the threshold in late dead space breathing. EMG – electromyography.

**Figure 6** Raw EMG data of superficial and deep pelvic floor muscles from a representative participant during cough in the sitting (left) and standing (right) positions. PNTX – pneumotachometer. **a** - onset of expulsion from airflow, **b** - peak of expulsion from airflow, **c** - initial trough or inflection point in airflow following peak expulsion, and **d** - end of expulsion from airflow.

**Figure 7** Group data for amplitude of pelvic floor muscle (PFM) electromyography (EMG) during the four phases of cough for the deep and superficial PFM layers in sitting and standing positions. Difference between deep and superficial PFM at rest is shown. EMG was also greater for both muscles in standing than sitting, and less at rest that the other phases. MVC – maximal voluntary contraction.

**Figure 8** Raw EMG data of superficial and deep pelvic floor muscles from a representative participant during maximal expiratory pressure (top left), sub-maximal expiratory pressure (top right), maximal inspiratory pressure (bottom left) and sub-maximal inspiratory pressure.

458

459 **Table 1** Root mean square electromyography amplitude during maximal and

460 submaximal pressure tasks

| | **Deep** | **Sup** | **Difference** | **Deep** | **Sup** | **Difference** |
|---|---|---|---|---|---|---|
| | **MIP** | | | **MEP** | | |
| Mean | 0.28 | 0.32 | 0.04* | 0.36 | 0.40 | 0.04* |
| SD | 0.23 | 0.19 | | 0.24 | 0.13 | |
| | SIP | | | SEP | | |
| Mean | 0.16 | 0.21 | 0.05* | 0.17 | 0.22 | 0.05* |
| SD | 0.08 | 0.07 | | 0.10 | 0.07 | |

461

462 Data are shown as percent MVC. Sup – superficial; MEP – Maximal expiratory pressure;

463 MIP – Maximal inspiratory pressure; SEP – sub-maximal expiratory pressure; SIP – Sub-

464 maximal inspiratory pressure, SD – standard deviation.

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588

**Table 1** Root mean square electromyography amplitude during maximal and submaximal pressure tasks

| | **Deep** | **Sup** | **Difference** | **Deep** | **Sup** | **Difference** |
|---|---|---|---|---|---|---|
| | **MIP** | | | **MEP** | | |
| Mean | 0.28 | 0.32 | 0.04* | 0.36 | 0.40 | 0.04* |
| SD | 0.23 | 0.19 | | 0.24 | 0.13 | |
| | SIP | | | SEP | | |
| Mean | 0.16 | 0.21 | 0.05* | 0.17 | 0.22 | 0.05* |
| SD | 0.08 | 0.07 | | 0.10 | 0.07 | |

Data are shown as percent MVC. Sup – superficial; MEP – Maximal expiratory pressure; MIP – Maximal inspiratory pressure; SEP – sub-maximal expiratory pressure; SIP – Sub-maximal inspiratory pressure, SD – standard deviation.

Figure 1

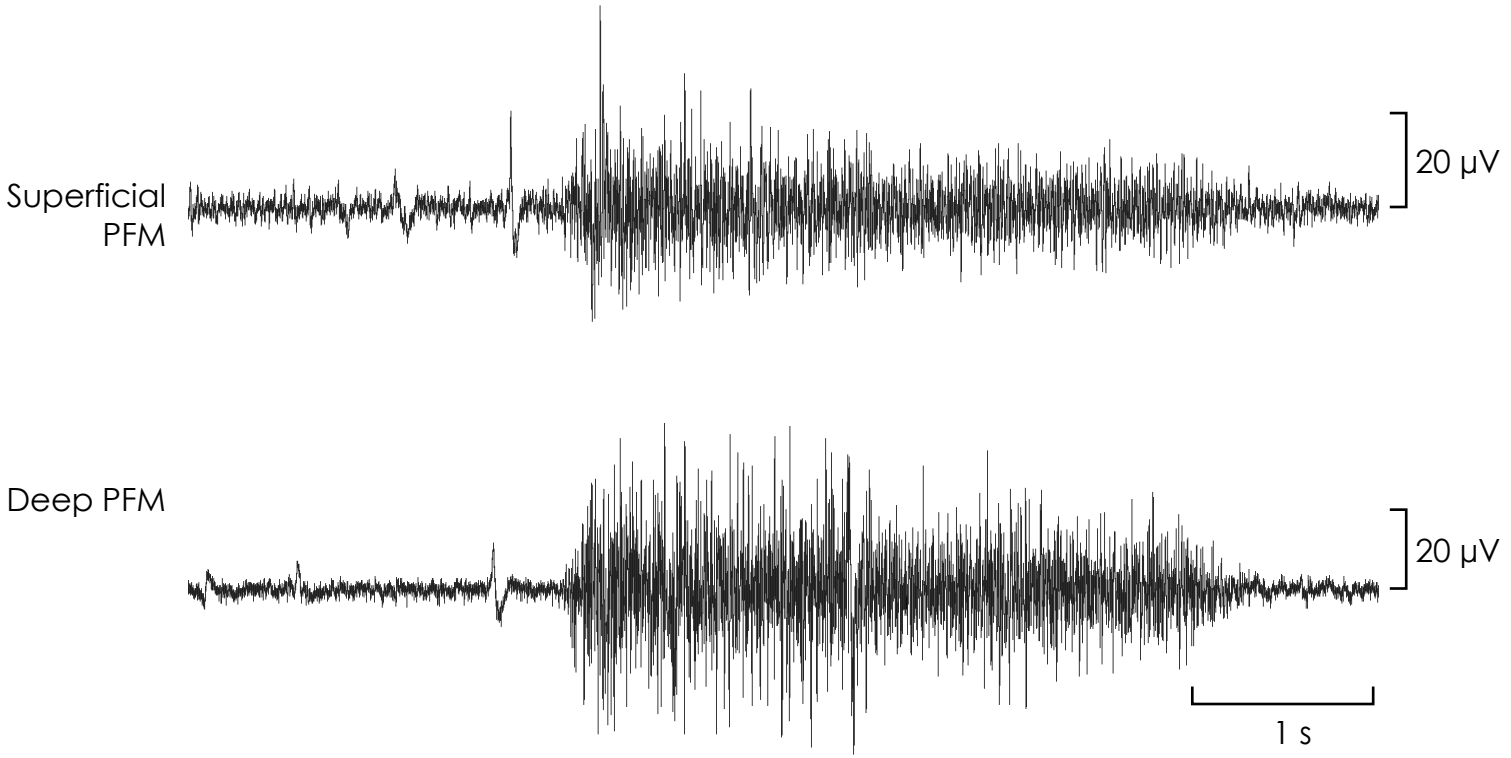
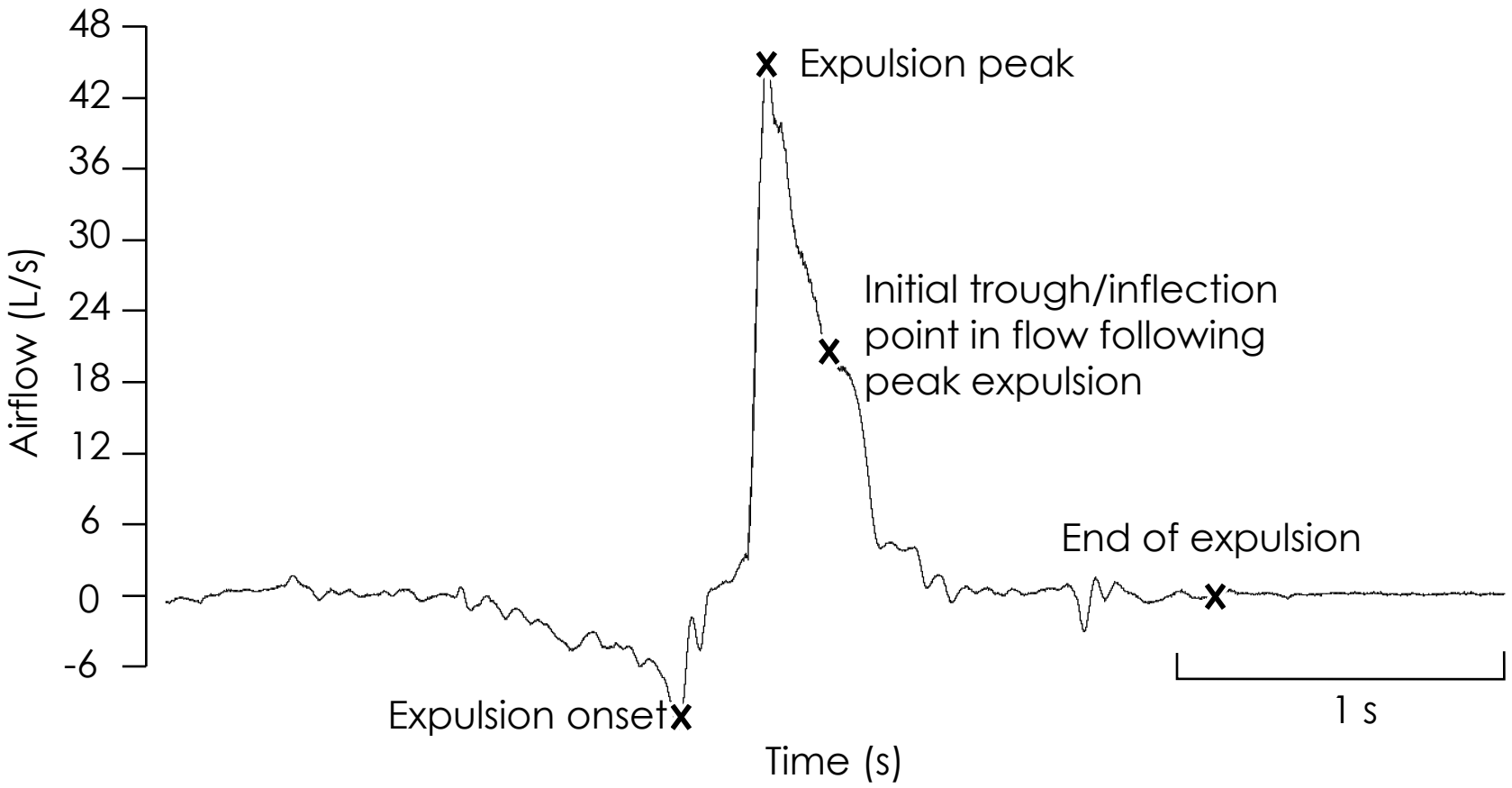
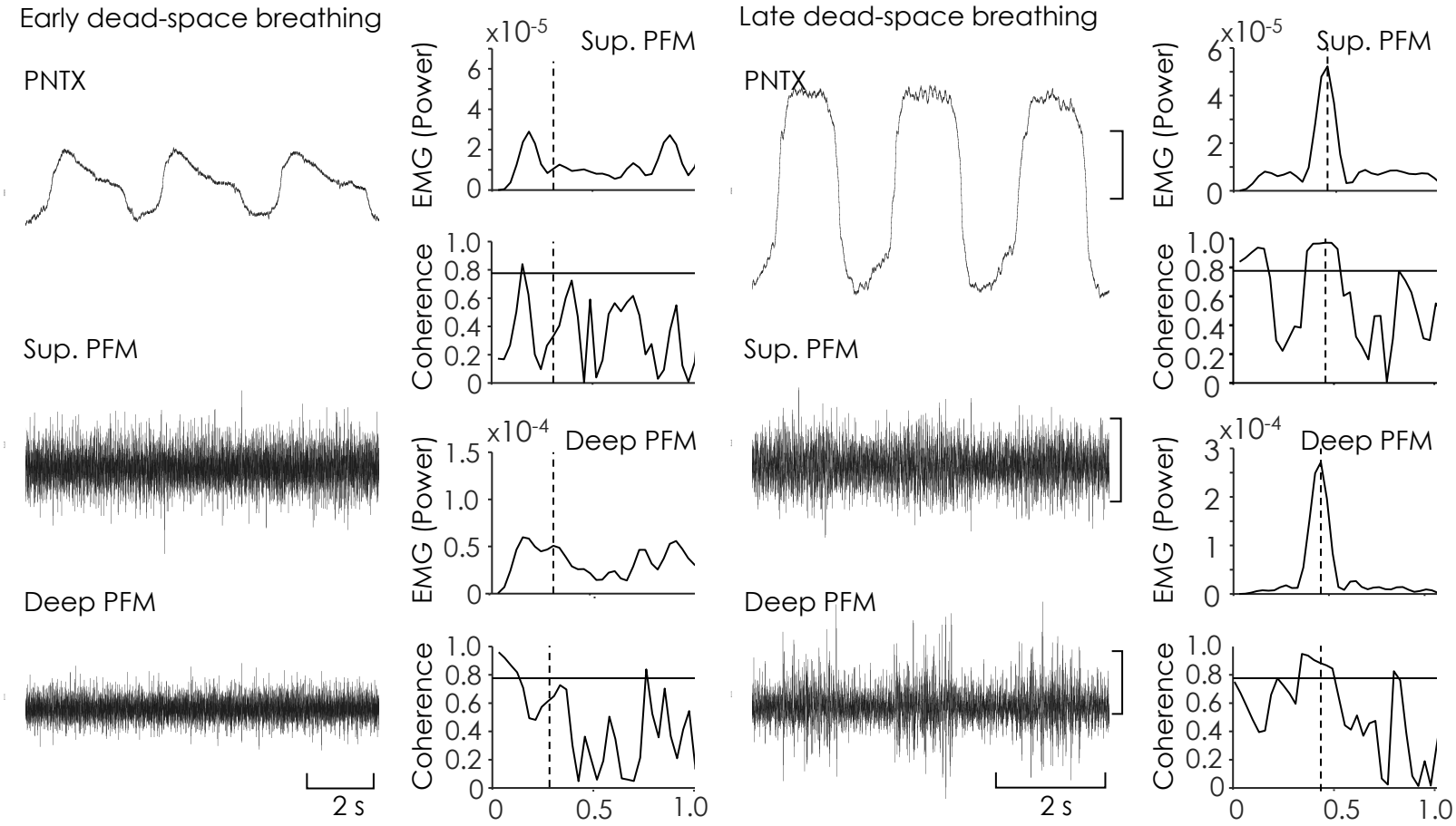
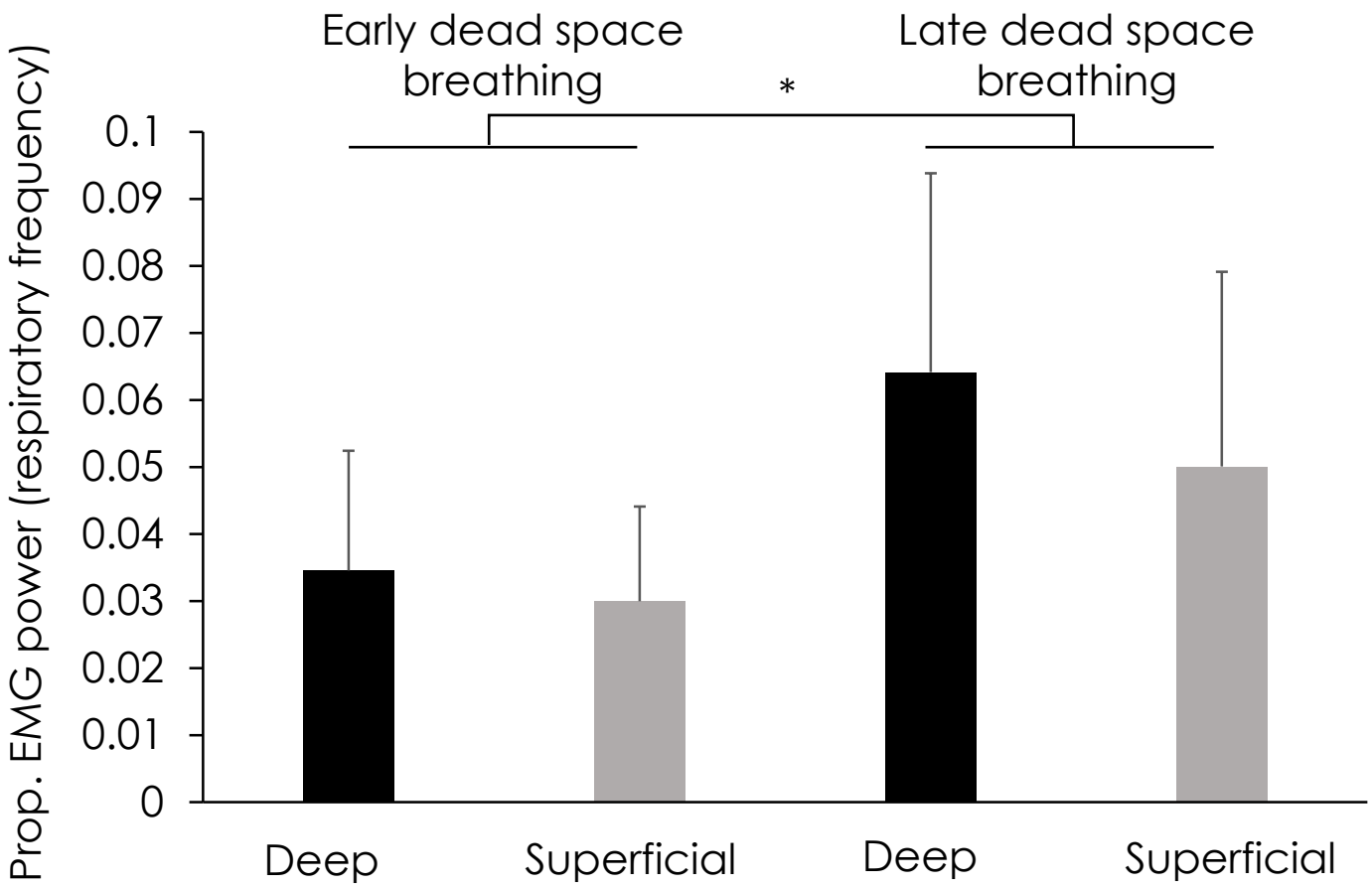


Figure 2







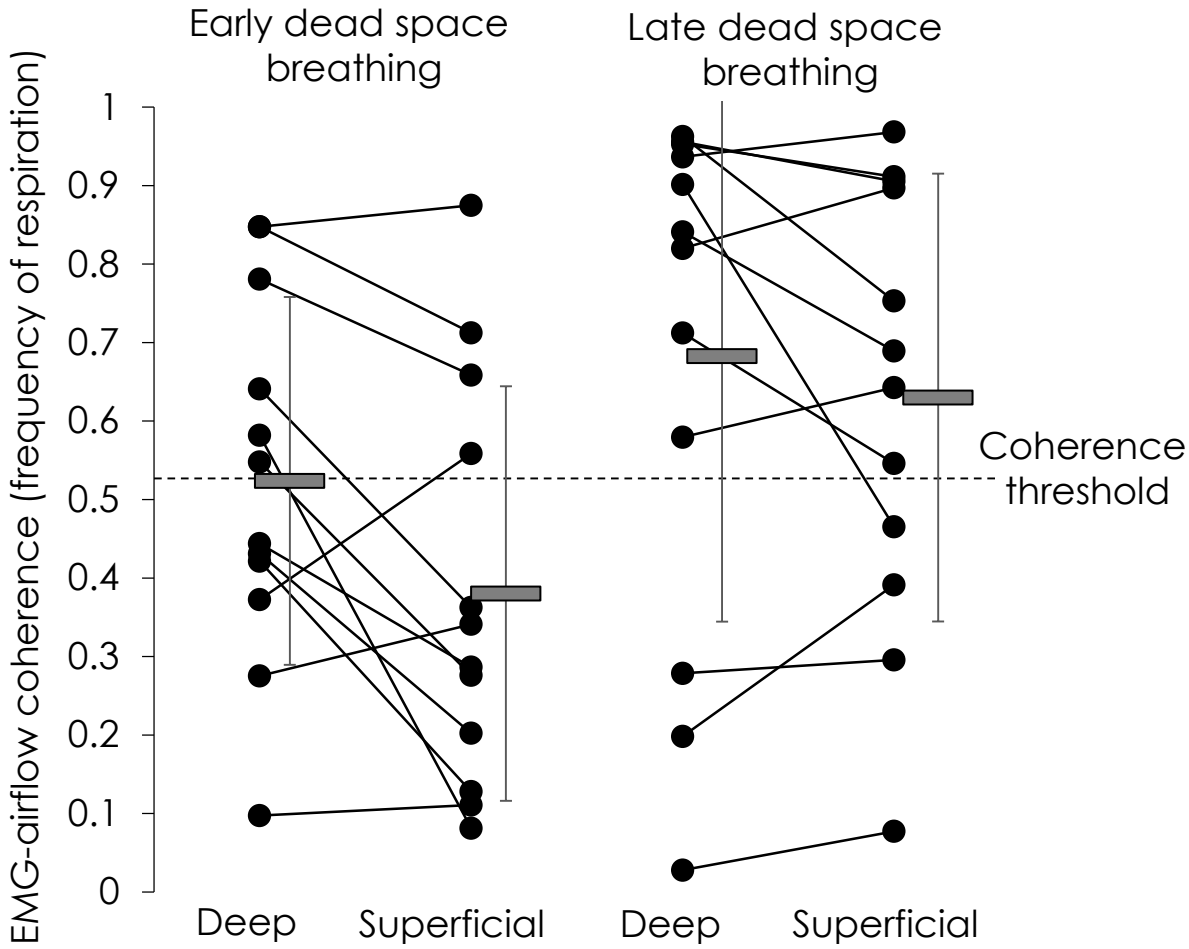
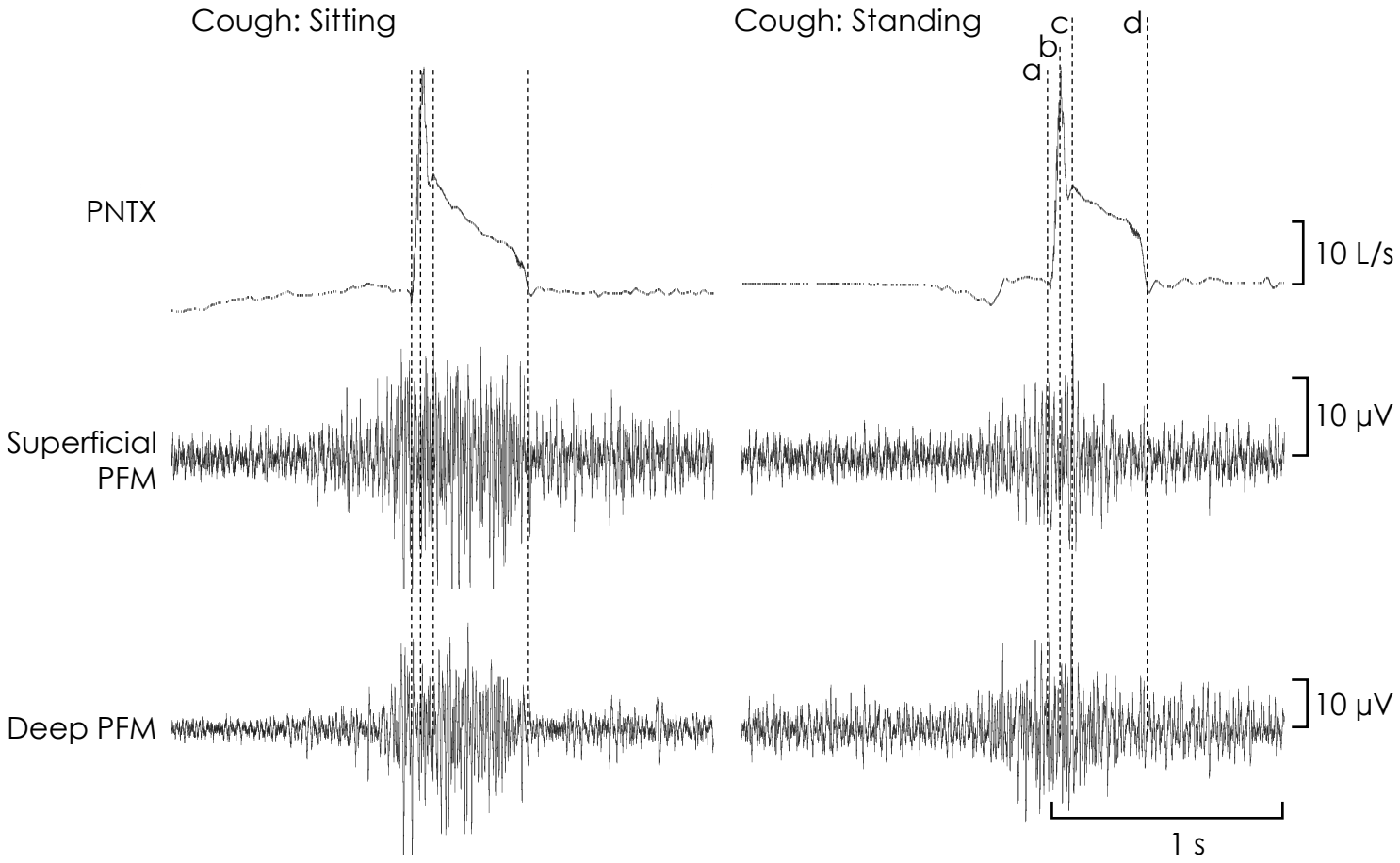


Figure 6



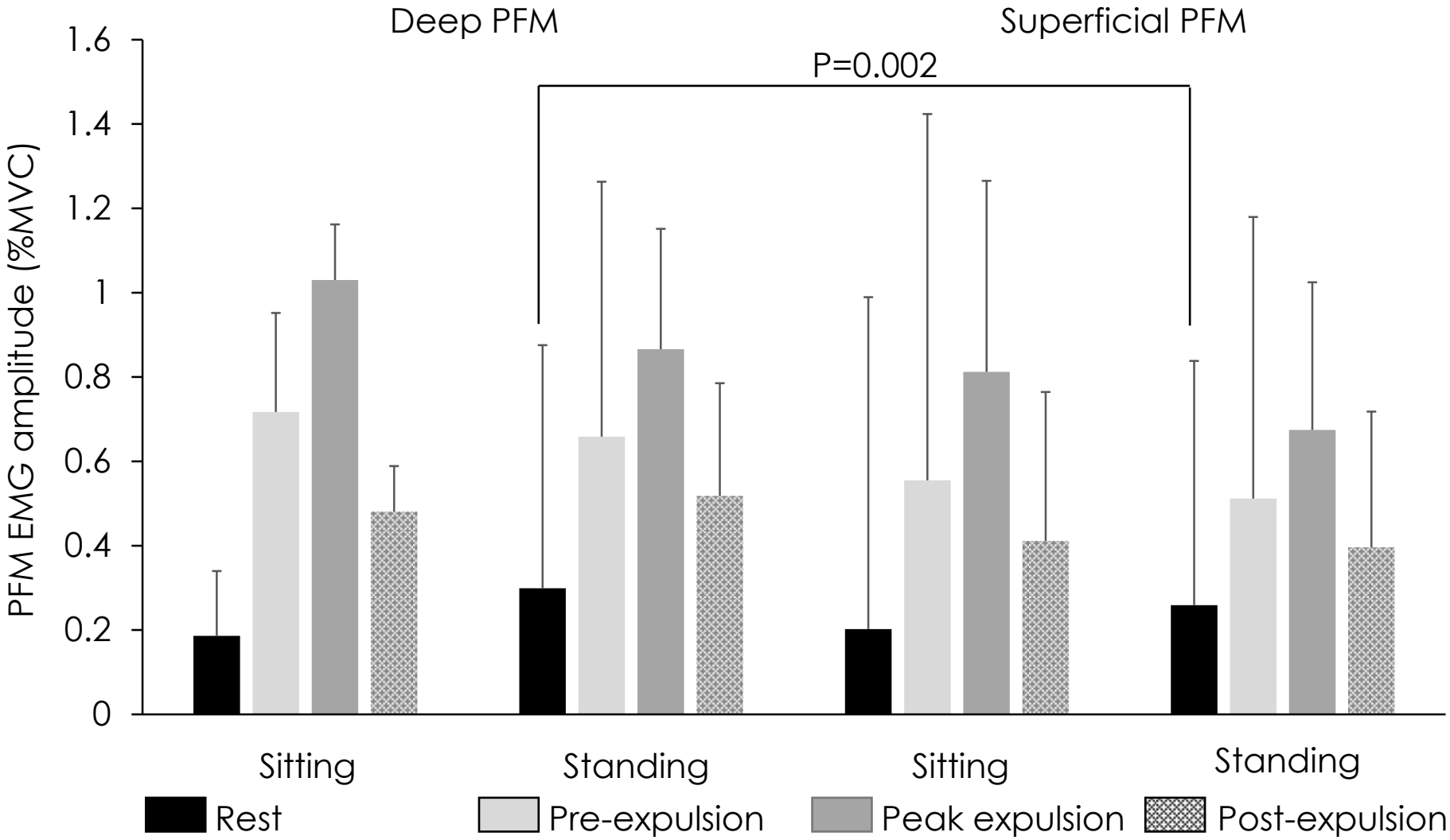


Figure 8