Diaphragm electromyogram root mean square response to hypercapnia and its intersubject and day-to-day variation

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Diaphragm electromyogram root mean square response to hypercapnia and its intersubject and day-to-day variation. J Appl Physiol 98: 274–281, 2005. First published September 10, 2004; doi:10.1152/japplphysiol.01380.2003.—Diaphragm activation can be quantified by measuring the root mean square of crural EMG (RMSdi) (Beck J, Sinderby C, Lindstrom L, and Grassino A, J Appl Physiol 85: 1123–1134, 1998). To examine intersubject and day-to-day variation in the RMSdi-PCO2 relationship, end-tidal PCO2, minute ventilation (Ve), respiratory frequency (fR), and RMSdi were measured in seven healthy subjects on two occasions during steady-state ventilation at seven levels of inspired O2 fraction (FiO2) from 0 to 0.08 in random order. RMSdi was measured with a multielectrode esophageal catheter and controlled for signal contamination and diaphragm position. RMSdi was normalized for values obtained during quiet breathing at functional residual capacity, at FiCO2 of 0.04, and during an inspiratory capacity maneuver (RMSdi%max) as well as ECG R-wave amplitude at functional residual capacity (RMSdi/ECG). fR and thickness of the costal diaphragm measured by ultrasound. RMSdi increased linearly with PCO2 (mean r2 = 0.83 ± 0.10); at the highest FiCO2, RMSdi%max was 40.2 ± 11.6%. Relative to the intersubject variation in the Ve-PCO2 relationship, intersubject variations in the slopes and intercepts of the RMSdi-PCO2 relationships were 1.7 and 1.8 times, respectively, and RMSdi%max-PCO2 relationships 0.9 and 1.3 times, respectively, and were unrelated to fR and diaphragm thickness. Relative to the day-to-day variation in the Ve-PCO2 relationship, day-to-day variation in the slopes and intercepts of the RMSdi-PCO2 relationships were 2.8 and 4.4 times, respectively, and RMSdi/ECG-PCO2 relationships 1.3 and 2.2 times, respectively. It was concluded that the RMSdi-PCO2 relationship measures chemosensitivity and is best compared between subjects via RMSdi%max and on separate occasions in the same subject via RMSdi/ECG.

IN HUMANS, THE VENTILATORY CONTROL SYSTEM IS CONVENTIONALLY STUDIED BY RELATING A MECHANICAL RESPONSE OF THE RESPIRATORY SYSTEM SUCH AS MINUTE VENTILATION (Ve), MEAN INSPIRATORY FLOW RATE (Vr/Ti), OR AIRWAY PRESSURE DURING THE FIRST 100 MS AFTER AIRWAY OCCLUSION (36) TO A SINGLE STIMULUS SUCH AS THE AORTIC PCO2. HOWEVER, THESE RESPONSES MAY UNDERESTIMATE NEURAL DRIVE WHEN RESPIRATORY MUSCLE FUNCTION IS IMPAIRED BY MUSCLE WEAKNESS OR CHANGES IN MUSCLE LENGTH BECAUSE OF HYPERINFLATION AND, IN THE CASE OF Ve AND Vr/Ti, WHEN RESPIRATORY SYSTEM RESISTANCE OR ELASTANCE IS INCREASED. DIAPHRAGM ELECTROMYOGRAPHY (EMGdi) IS A MORE DIRECT MEASURE OF RESPIRATORY MUSCLE ACTIVATION. IN ANIMALS, DIRECT RELATIONSHIPS HAVE BEEN FOUND BETWEEN RECTIFIED INTEGRATED EMGdi AND END-TIDAL PCO2 (PETO2) (1), THE PHRENIC NEUROGRAM (22), AND DIAPHRAGM O2 CONSUMPTION (28, 34). IN HUMANS, THE DIAPHRAGM ACCOUNTS FOR A SUBSTANTIAL FRACTION OF INSPIRATORY WORK (23, 24), AND THE AVERAGE RATE OF INSPIRATORY EMGdi MOVING TIME AVERAGE INCREASES LINEARLY WITH PETO2, TRANSDIAPHRAGMATIC PRESSURE (Pdi), Vr/Ti, AND OCCLUSION PRESSURE (25). HOWEVER, EMGdi IS RARELY USED TO ASSESS VENTILATORY CONTROL IN CLINICAL PRACTICE BECAUSE OF DIFFICULTIES IN DETECTION AND QUANTIFICATION. BECK AND SINDERBY AND COLLEAGUES (4, 5, 31, 33) HAVE RECENTLY DEVELOPED METHODS TO IMPROVE QUANTIFICATION OF EMGdi IN HUMANS BY DETECTING ACTIVITY USING A MULTIELECTRODE ESOPHAGEAL CATHETER, EVALUATING SIGNAL QUALITY USING SPECTRAL ANALYSIS, AND MEASURING ITS ROOT MEAN SQUARE TO QUANTIFY SIGNAL STRENGTH. THE ROOT MEAN SQUARE REFLECTS THE NUMBER AND FIRING RATE OF MOTOR UNITS RECRUITED (3) AND IS REGARDED AS A MORE VALID MEASURE OF EMG POWER THAN FULL-WAVE RECTIFICATION AND INTEGRATION (2). BECK ET AL. (3) FOUND THAT DIAPHRAGM ROOT MEAN SQUARE (RMSdi) WAS A RELIABLE INDEX OF GLOBAL DIAPHRAGM ACTIVATION UP TO BUT NOT EXCEEDING ~75% OF MAXIMAL ACTIVATION.

THE USE OF RMSdi AS A MEASURE OF DIAPHRAGM ACTIVATION HAS A NUMBER OF LIMITATIONS. FIRST, BECAUSE RMSdi VARIES WITH THE SQUARE ROOT OF ACTIVATION (3), THE RELATIONSHIP BETWEEN RMSdi AND A RESPIRATORY STIMULUS SUCH AS PCO2 MAY NOT BE LINEAR. SECOND, ABSOLUTE VALUES OF RMSdi MAY VarIE WIDELY BETWEEN SUBJECTS (3). TO ALLOW COMPARISONS BETWEEN SUBJECTS, RMSdi HAS BEEN NORMALIZED TO VALUES OBTAINED AT REST (RMSdi rest) (3) OR VOLUNTARY MAXIMAL INSPIRATION (RMSdi max) (30). HOWEVER, THESE APPROACHES MAY BE SUBOPTIMAL BECAUSE RMSdi rest IS LIKELY TO HAVE A LOW SIGNAL-TO-NOISE RATIO, RMSdi max UNDERESTIMATES ACTIVATION (3), AND BOTH ARE DEPENDENT ON EFFORT. FINALLY, THE VARIATION IN RMSdi IN A SUBJECT FROM ONE OCCASION OF MEASUREMENT TO ANOTHER HAS NOT BEEN DEFINED.

THERE ARE SEVERAL POTENTIAL SOURCES OF INTERSUBJECT VARIATION IN RMSdi. FIRST, ANATOMICAL DIFFERENCES IN THE RELATIONSHIP OF THE CRURAL DIAPHRAGM TO THE GASTROESOPHAGEAL JUNCTION COULD INFLUENCE THE POSITION OR ORIENTATION OF THE ELECTRODE RELATIVE TO DIAPHRAGM MUSCLE. SECOND, RMSdi MAY VARY BETWEEN SUBJECTS BECAUSE OF DIFFERENCES IN DIAPHRAGM ANATOMY SUCH AS MUSCLE FIBER DIAMETER, WHICH AFFECTS THE PROPAGATION VELOCITY AND THUS SHAPE AND SIZE OF THE MUSCLE FIBER ACTION POTENTIAL (13), AND THE NUMBER OF FIBERS IN A MOTOR UNIT (20). THESE DIFFERENCES MAY BE REFLECTED IN DIAPHRAGM THICKNESS AND, ALTHOUGH THERE ARE NO ESTABLISHED METHODS TO MEASURE THE THICKNESS OF THE CRURAL DIAPHRAGM IN SITU, THICKNESS OF THE COSTAL DIAPHRAGM CAN BE MEASURED BY ULTRASOUND (8). THIRD, THERE MAY BE DIFFERENCES...
between subjects in the filtering properties of the electrode or surrounding tissue. These factors are likely to also affect the EMG frequency spectrum. Finally, studies of diaphragm activation in chronic airflow limitation and after polio by Sinderby et al. (30, 32) compared RMSdi values per breath without considering differences in pattern of breathing between subjects. As the ventilatory response to a stimulus consists of an increase in both tidal volume and respiratory frequency (fR), it may be more appropriate to express RMSdi as a function of time rather than per breath (21).

Day-to-day variation in RMSdi may also result from alterations in the position or orientation of the electrode relative to muscle and/or filtering properties of the electrode or surrounding tissues. An equivalent variation may be seen in the amplitude of other electrical signals detected by the esophageal electrode. The electrocardiogram (ECG) unavoidably contaminates esophageal recordings of diaphragm EMG and could provide an effort-independent electrical signal to control for these day-to-day variations. Because the pericardium is adherent to the left hemidiaphragm, at a constant lung volume, the position and electrical axis of the heart may have a relatively constant relationship to the gastroesophageal junction and crural diaphragm.

The aims of this study were to define 1) the response of RMSdi to hypercapnia in healthy subjects, 2) the intersubject and day-to-day variations in the relationships between RMSdi and PCO2, and 3) the effect of normalization for RMSdi\textsubscript{rest}, root mean square at a submaximal effort (RMSdi\textsubscript{submax}), RMSdi\textsubscript{max}, diaphragm EMG center frequency (CFdi), diaphragm thickness, fR, and ECG R-wave amplitude (ECGR) on intersubject and day-to-day variations in the relationships between RMSdi and PCO2. It was hypothesized that the relationship between RMSdi and PCO2 was near linear at moderate diaphragm activation, intersubject and day-to-day variations in the relationship between RMSdi and PCO2 were substantial, and these variations could be minimized by normalization. It was found that the relationship between RMSdi and PCO2 was linear at moderate diaphragm activation, intersubject and day-to-day variations in the relationship between RMSdi and PCO2 were substantial, and these variations could be minimized by normalization. It was found that the relationship between RMSdi and PCO2 was near linear at moderate diaphragm activation, intersubject and day-to-day variations in the relationship between RMSdi and PCO2 were substantial, and these variations could be minimized by normalization.

Subjects. Seven healthy subjects, five men and two women, age (mean ± SD) 40.7 ± 11.1 yr, body mass index 25.2 ± 2.1 kg/m², and forced expiratory volume in 1 s of 3.59 ± 0.70 liters, participated in the study. Informed consent was obtained from each subject, and ethical approval was granted by the Committee for Human Rights, University of Western Australia.

Measurements. In each subject, the response to hypercapnia was examined at seven fractional concentrations of inspired CO2 (FICO2), i.e., 0, 0.02, 0.04, 0.05, 0.06, 0.07, and 0.08, on two occasions at an interval of between 6 and 98 days (mean 34 ± 34 days). The fractional concentration of CO2 in expired gas was measured with a rapid gas analyzer (Morgan Benchmark, model 503, P. R. Morgan, Kent, UK) and used to calculate the P\textsubscript{ET}CO2. A pneumotachograph was used to measure inspiratory flow and was integrated over time to obtain inspired volume.

EMGdi signals were recorded and processed by methods described by Beck and Sinderby and colleagues (4, 5, 31, 33). A purpose-built Silastic esophageal catheter, 2.5 mm in diameter with an array of eight electrode rings at 1-cm intervals at its distal end (Dentsleeve, Wayville, South Australia, Australia), was used. Each electrode ring was 2 mm wide and consisted of fine stainless steel wire wrapped around the catheter. Six bipolar electrode pairs were created by wiring the electrodes in an overlapping array, i.e., 1 vs. 3, 2 vs. 4, 3 vs. 5, 4 vs. 6, 5 vs. 7 and 6 vs. 8 (Fig. 1). EMG signals were collected at a rate of 2 kHz, amplified (Grass Wideband A.C. amplifier, model 17P3C, Quincy, MA), band-pass filtered between 10 and 1,000 Hz, digitized, and, via purpose-written software (Labview Version 6), displayed online on a personal computer and stored for subsequent analysis. The EMG signals were later reviewed and processed in the time and frequency domains as described by Sinderby et al. (31, 33) with purpose-written software in Labview (version 6) to calculate and display RMSdi, CFdi, and signal-contamination criteria of selected EMG segments from each electrode pair. The catheter was positioned across the diaphragm so that the electrical center of the diaphragm was in the center of the electrode span. The electrical center of the diaphragm was identified by a change in polarity of the EMG signal across the center of the diaphragm. For each breath, a single RMSdi value was obtained from a segment of diaphragm EMG activity during inspiration that was free of cardiac activity, i.e., between the T and P waves of successive ECG complexes. The RMSdi was measured by subtracting the signal below from the signal above the electrical center, to improve the signal-to-noise ratio (33). The duration of each such segment was between 300 and 400 ms, and RMSdi represents the mean deviation of the signal from zero over the selection. The electrical center of the diaphragm remained within the span of the electrode array in all tests. RMSdi and CFdi were only accepted if the frequency spectra fulfilled criteria for an uncontaminated signal recommended by Sinderby et al. (31).

Protocol. Subjects were seated and inspired from a 50-liter bag containing FICO2 of between 0 and 0.08 in random order, inspired O2 fraction 0.21, and the remainder nitrogen (Fig. 1). Subjects were blinded to the level of FICO2. To equilibrate with the gas mixture, the subject took two vital capacity inspirations and 20 tidal breaths from the bag before collection of 30 breaths for analysis. For each condition of measurement, the RMSdi value for the last 10 breaths was averaged, because the data showed that the coefficient of variation (CV) increased when fewer than 10 breaths were analyzed (Fig. 2). There was no change in P\textsubscript{ET}CO2 during the last 10 breaths of each collection. EMG data could not be retrieved for one condition of measurement of one study in two subjects, i.e., FICO2 0 in the first study in subject 5 and FICO2 0.08 in the second study in subject 4. In all subjects, the thickness of the costal diaphragm in the midaxillary line was measured in a seated posture during quiet breathing by an experienced radiologist using a two-dimensional ultrasound (Toshiba PowerVision 6000, model SSA-370A) and methods described by Cohn et al. (8).

Normalization. RMSdi\textsubscript{rest} and RMSdi\textsubscript{submax} were the RMSdi values at FICO2 0 and 0.04, respectively. RMSdi\textsubscript{max} was measured at the beginning and end of each study as the mean of three slow inspirations from functional residual capacity to total lung capacity. There was no difference between RMSdi\textsubscript{max} measured at the beginning and end of each study (0.347 ± 0.111 vs. 0.340 ± 0.093 arbitrary units), and RMSdi\textsubscript{max} at the beginning of each study was used in normalizations. For all studies, the mean RMSdi\textsubscript{max} was 90.9 ± 4.5% of the highest RMSdi\textsubscript{max}. The ECGR was the mean amplitude of five R waves of the ECG at end expiration at FICO2 = 0 detected by the electrode pair at the center of electrical activity of the diaphragm. The thickness of the costal diaphragm was the mean of five measurements at the midaxillary line at functional residual capacity.

Data analysis and statistics. Maximum voluntary ventilation was estimated as 35 times forced expiratory volume in 1 s. The relationships between responses (RMSdi, Ve, and V\textsubscript{T/T}i) and P\textsubscript{ET}CO2 were examined via linear and polynomial regressions. To determine the factors associated with intersubject variability in RMSdi, at each level of FICO2, the relationships between RMSdi and RMSdi\textsubscript{rest}.
RMSdisubmax, RMSdimax, ECGR, CfDi, fB, and diaphragm thickness were examined by linear regressions. Intersubject variability in the relationship between RMSdi and PETCO2 and the effect of normalization with RMSdi%rest (RMSdi%rest), RMSdi%submax, RMSdimax (RMSdi%max), ECGR (RMSdi/ECGR), fB (RMSdi/fB), and diaphragm thickness (RMSdi/Tdi) were examined by using the CV of the slopes and intercepts of the linear regressions of the first study in each subject. Day-to-day variability in the relationship between RMSdi and PETCO2 and the effect of various normalizations, as above, were examined by using the CV of the difference in slopes and intercepts of the linear regressions between the two studies in each subject. The variation in CFdi between subjects and at different levels of FICO2 was analyzed by using a two-way ANOVA. The difference between RMSdi%max at the beginning and end of each test was compared via a paired t-test. Mean slopes and intercepts are expressed as means ± SE, and all other data are expressed as means ± SD. Significance was defined as P < 0.05.

RESULTS

Response to hypercapnia. At FICO2 0.08, the subjects achieved 33.9 ± 9.0% of predicted maximum voluntary ventilation (range 20.0–47.8%), a mean inspiratory flow rate of 1.57 ± 0.42 l/s (range 0.86–2.37 l/s), and RMSdi 40.2 ± 11.6% of RMSdimax (range 19.2–62.3%). CFdi varied between subjects (P < 0.001) but was not influenced by FICO2 (P = 0.126). In all tests, RMSdi, V˙E, and VT/TI were linearly related to PETCO2 [mean coefficient of determination (r^2) = 0.83 ± 0.10, 0.96 ± 0.02, and 0.96 ± 0.02, respectively] (Table 1). A second-order polynomial did not significantly increase the r^2 value of the RMSdi-PETCO2 relationship. In most subjects, the r^2 value of the linear regression of RMSdi against PETCO2 relationship increased when fB was considered (RMSdi-fB-PETCO2, mean r^2 = 0.88 ± 0.07). The relationship between mean RMSdi%max and mean PETCO2 at each FICO2 for all subjects in the first study is shown in Fig. 3.

Intersubject variation. Intersubject differences in RMSdi at equivalent FICO2 were related to RMSdi%rest, RMSdi%submax, and RMSdi%max (Table 2) but not to CFdi, ECGR, fB, or diaphragm thickness. Diaphragm thickness at functional residual capacity ranged from 15 to 43 mm and was closely related to body mass index (r^2 = 0.94) and height (r^2 = 0.73). Intersubject differences in RMSdi%max at equivalent FICO2 were not related to fB.
Table 1. Linear regressions of RMSdi against PETCO2 (n = 7) at each study

<table>
<thead>
<tr>
<th>Subject</th>
<th>First Study</th>
<th>Second Study</th>
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<tbody>
<tr>
<td></td>
<td>Slope, AU/Torr × 10^{-3}</td>
<td>Intercept, AU × 10^{-1}</td>
</tr>
<tr>
<td>1</td>
<td>2.62</td>
<td>−0.71</td>
</tr>
<tr>
<td>2</td>
<td>6.39</td>
<td>−2.43</td>
</tr>
<tr>
<td>3</td>
<td>6.10</td>
<td>−2.06</td>
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<tr>
<td>4</td>
<td>8.30</td>
<td>−2.94</td>
</tr>
<tr>
<td>5</td>
<td>2.73</td>
<td>−0.65</td>
</tr>
<tr>
<td>6</td>
<td>2.15</td>
<td>−0.44</td>
</tr>
<tr>
<td>7</td>
<td>2.98</td>
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<tr>
<td>Mean</td>
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<td>−1.47</td>
</tr>
<tr>
<td>SE</td>
<td>0.91</td>
<td>0.38</td>
</tr>
<tr>
<td>SD</td>
<td></td>
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<tr>
<td>CV, %</td>
<td>20.5</td>
<td>25.6</td>
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Table 3 and Fig. 4 show the means and variations of the slopes and intercepts for the relationships between measured responses and PETCO2 at the first study. Variation in slopes and intercepts between subjects was least for the VT/Ti-PETCO2 relationship (Table 3 and Fig. 4). The slope of the VE-PETCO2 relationship varied from 0.57 to 2.65 l·min⁻¹·Torr⁻¹ (mean 1.68 ± 0.21 l·min⁻¹·Torr⁻¹). Relative to the VE-PETCO2 relationship, intersubject variation in slopes and intercepts for the RMSdi-PETCO2 relationship was 1.7 and 1.8 times greater, respectively (Table 3). Normalization for RMSdi and, to a lesser degree, RMSdi rest, RMSdi submax, and ECGR reduced the intersubject variation in the slopes and intercepts of the RMSdi-PETCO2 relationships (Table 3 and Fig. 4). Intersubject variation in slopes of the RMSdi%max-PETCO2 and VE-PETCO2 relationships were similar (Table 3 and Fig. 4).

**DISCUSSION**

This study found that diaphragm activation, quantified by measuring RMSdi, increased linearly in response to hypercarbia up to moderate levels of diaphragm activation, implying that the RMSdi response to hypercarbia measures chemosensitivity. At the highest FICO2 used, RMSdi was <65% of RMSdi max in all subjects. The relationships between VE and PETCO2 indicated substantial intersubject and day-to-day variation in chemosensitivity. Intersubject variation in the RMSdi-PETCO2 relationship was almost twice the variation in the VE-PETCO2 relationship in the same subjects and can be attributed, first, to individual differences in chemosensitivity and, second, to differences in anatomy and muscle-electrode relationship because the effect of the second source of intersubject variation was virtually removed by normalizing RMSdi for RMSdi rest, RMSdi submax, and RMSdi max at each FICO2 in the first study.

**Table 3. Coefficients of determination (r²) for linear regressions of RMSdi rest, RMSdi submax, and RMSdi max at each FICO2 in the first study.**

<table>
<thead>
<tr>
<th>FICO2</th>
<th>Mean PETCO2, Torr</th>
<th>n</th>
<th>RMSdi rest</th>
<th>RMSdi submax</th>
<th>RMSdi max</th>
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<tbody>
<tr>
<td>0.02</td>
<td>44.9 ± 2.3</td>
<td>7</td>
<td>0.57</td>
<td>0.69*</td>
<td>0.63*</td>
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<tr>
<td>0.04</td>
<td>48.0 ± 1.9</td>
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<td>0.69*</td>
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<td>0.63*</td>
</tr>
<tr>
<td>0.05</td>
<td>50.8 ± 1.8</td>
<td>7</td>
<td>0.58</td>
<td>0.82*</td>
<td>0.65*</td>
</tr>
<tr>
<td>0.06</td>
<td>53.3 ± 2.0</td>
<td>7</td>
<td>0.59</td>
<td>0.79*</td>
<td>0.66*</td>
</tr>
<tr>
<td>0.07</td>
<td>57.1 ± 1.7</td>
<td>7</td>
<td>0.82*</td>
<td>0.63*</td>
<td>0.83†</td>
</tr>
<tr>
<td>0.08</td>
<td>61.9 ± 1.6</td>
<td>7</td>
<td>0.87†</td>
<td>0.60*</td>
<td>0.86‡</td>
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**Table 2. Coefficients of determination (r²) for linear regressions of RMSdi against PETCO2 (n = 7) at each study.**

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**Fig. 3. Relationship between mean ± SD RMSdi values normalized with RMSdi values during an inspiratory capacity maneuver (RMSdi%max) and mean ± SD end-tidal PCO2 (PETCO2) (n = 7) at each FICO2 in the first study.**
RMSdimax. Day-to-day variation in the slope of the RMSdi-PETCO2 relationship was 2.8 times the day-to-day variation in the VE-PETCO2 relationship in the same subjects. The day-to-day variation in the RMSdi-PETCO2 relationship in a subject was also attributable in part to day-to-day variation in chemosensitivity; the residual variation in the RMSdi-PETCO2 relationship in a subject could be minimized by normalizing RMSdi for RMSdi at rest or ECGR, implying that the variation was mainly due to differences in the muscle-electrode relationship. Before discussing these findings and conclusions, we consider the extent to which the methods used allow accurate estimates of diaphragm activation in response to hypercapnia and influence interpretation of the results.

**Limitations.** The conclusions of this study are based on estimates of diaphragm EMG signal strength measured using methods proposed by Beck and Sinderby and colleagues (4, 5, 31, 33). To maintain constant muscle-to-electrode distance, EMG activity was continuously recorded from six bipolar electrode pairs spanning 7 cm (Fig. 1). To improve signal-to-noise ratios, the signals above and below the electrical center of the diaphragm were subtracted; this required the electrical center of the diaphragm to remain within the middle 5 cm of the array. Although diaphragm excursion during the inspiratory capacity maneuver used to measure RMSdimax is likely to have exceeded 5 cm (7, 35), the electrical center remained within the span of electrode pairs at all times, supporting the notion that the electrodes move with the diaphragm. The progressive increase in RMSdi with hypercapnia supports the findings of Beck et al. (5) that this method enables the electrode to remain in a relatively constant spatial relationship with the crural diaphragm during respiration.

Diaphragm EMG was only sampled between the T and P waves of successive ECG complexes to avoid ECG artifact. In most studies, this consisted of between 0.3 and 0.4 s of EMG activity. Inability to sample from an identical part of each inspiration led to some variability in RMSdi from breath to breath; this variability was reduced by using the mean RMSdi over 10 breaths during steady-state ventilation at each condition of measurement. The data in this study showed that, by using 10 breaths, there was a 95% likelihood that the CV in each subject was <10% at FICO2 0.08 (Fig. 2). RMSdi was not corrected for alterations in action potential propagation velocity because CFDi did not change at different levels of FICO2.

Esophageal electrodes detect EMG activity arising from the crural diaphragm. The costal and crural parts of the diaphragm differ in embryological origin (16), innervation (17), proportions of muscle-fiber types (27), and actions on the lower rib cage (11). There is conflicting evidence on the homogeneity of activation and shortening of the costal and crural diaphragms. Several studies report that the costal and crural diaphragms contract simultaneously (6, 29) and that their electrical activity increases similarly when an inspiratory load is added (22). In intact awake lambs, Cooke et al. (9) found identical EMG responses of the costal and crural diaphragms to various hypoxic and hypercapnic gas mixtures, indicating that these components of the diaphragm comprise a single functional unit. Other studies show regional differences in activation of the recruitment of the costal and crural diaphragms (12). Studies in dogs show that regional EMG activity within intercostal muscles is directly related to regional mechanical advantage (10, 18, 19). In the diaphragm of dogs, regional mechanical advantage and blood flow are heterogeneous (37, 15) but matched and similar between adjacent costal and crural regions (15). The distribution of regional blood flow does not change with exercise (15). Assuming that the distribution of activation within the diaphragm is proportional to the distribution of mechanical advantage, these findings suggest a direct relationship between crural and costal EMG that does not change with exercise. Consistent with this, in humans, Beck et al. (3) found that activation of the crural diaphragm, assessed by the EMG technique we studied, was linearly related to a measure of global activation of the diaphragm (ratio of Pdi at maximal Pdi) independent of diaphragm length up to 75% of maximal Pdi. Thus available evidence suggests that activation of the crural diaphragm is representative of global activation of the diaphragm.

The mean slopes and intercepts of the relationship between VE and hypercapnia in this study are lower than those reported...
by Read and Leigh (26) and Irsigler (14) and can be attributed primarily to differences in chemosensitivity. In 126 healthy subjects, Irsigler (14) found that the mean slope of the relationship between V˙E and P CO2 was 2.60 \( \pm \) 0.11 l\( \cdot \)min\(^{-1} \)\( \cdot \)Torr\(^{-1} \), but there was considerable variation so that the range in slopes was 1.5 to 5.0 l\( \cdot \)min\(^{-1} \)\( \cdot \)Torr\(^{-1} \) in 80% of the subjects. Most of the remaining subjects in that study had slopes <1.5 l\( \cdot \)min\(^{-1} \)\( \cdot \)Torr\(^{-1} \), and women were less responsive than men (14). In the present study, one female subject (subject 6) had a slope below 1.5 l\( \cdot \)min\(^{-1} \)\( \cdot \)Torr\(^{-1} \). The possibility that the difference in methods, i.e., steady-state ventilation at several levels of PCO2 in this study compared with progressive hypercapnia induced by rebreathing used by Read and Leigh (26) and Irsigler (14), also contributed to the observed differences cannot be excluded.

At the second study, there was less intersubject variation in the slopes and intercepts of the RMSdi-PetCO2 relationship, there was no relationship between RMSdi and RMSdi\(_{\text{max}}\), and intersubject variation in the RMSdi-PetCO2 relationship was not reduced by normalization. These findings may represent a type II statistical error due to the relatively small sample size.

The levels of diaphragm activation achieved in this study were moderate, so that the results do not establish the limits of linearity of the relationship between RMSdi and PetCO2 during hypercapnia. The range of FICO2 used was adequate to establish the ventilatory and diaphragmatic EMG responses to hypercapnia in healthy subjects; in practice, higher levels of response are unnecessary and unlikely to be tolerated in healthy subjects because of the large increase in ventilation and the dyspnea experienced. In chronic airflow limitation, the level of diaphragm activation during quiet breathing (30) approximates that found in this study at the highest PCO2 breathed. In such subjects, the RMSdi responses to progressive hypercapnia could approach the value during maximal inspiration and the
relationship between RMSdi and PETCO₂ could become nonlinear.

Implications. The finding of a linear relationship between PCO₂ and RMSdi, up to moderate levels of diaphragm activation (Table 1 and Fig. 3), is consistent with the findings of Beck et al. (3), who found a linear relationship between RMSdi and the ratio of Pdi to maximal Pdi up to 75% of maximal Pdi. This confirms that RMSdi accurately reflects moderate levels of activation of the diaphragm, although it could underestimate higher levels of activation because RMSdi varies with the square root of activation (3). In most subjects, the \( r^2 \) value for the relationship between RMSdi and PCO₂ increased when \( f_B \) was considered, implying that most subjects responded to different levels of PCO₂ by varying the frequency and amount of diaphragm activation in slightly different proportions. The linear relationship between RMSdi and PETCO₂, up to 60% RMSdi max suggests that RMSdi accurately reflects neural drive to the diaphragm. As a measure of chemosensitivity, it provides an output that is independent of diaphragm length, diaphragm weakness and threshold, resistive and elastic loads.

Intersubject variation in the relationship between RMSdi and PETCO₂ was partly attributable to intersubject variation in chemosensitivity, evidenced by the variability in the relationship between \( \dot{V}E \) and PETCO₂ (Table 3). The variability in the RMSdi-PETO₂ relationship exceeded that of the \( \dot{V}E \)-PCO₂ relationship (Table 3 and Fig. 4). This additional variability could be due to differences in diaphragm anatomy, spatial relationship of electrode to muscle, possibly because of anatomical differences at the gastroesophageal junction, and/or filtering properties of the electrode or surrounding tissue. These factors are likely also to influence RMSdi rest, RMSdi submax and RMSdi max, which could be used to reduce these intersubject differences, so that the main source of intersubject variation in RMSdi%PCO₂ relationship was chemosensitivity. These differences are also likely to influence CFdi, which increases with action potential propagation velocity and decreases as the muscle-to-electrode distance increases (5). However, intersubject differences in CFdi in the present study did not account for intersubject variation in RMSdi. This could be due to cancellation by the different sources of variation or the wide range in CFdi obtained with esophageal electrodes. The \( f_B \) and thickness of the costal diaphragm did not account for intersubject differences in the relationship between RMSdi and hypercapnia. These findings validate the approach of Sinderby et al. (30), who used the ratio of RMSdi to RMSdi max to show increased diaphragm activation in subjects with severe chronic airflow limitation and restriction due to previous poliomyelitis compared with healthy subjects.

In keeping with the findings of Irsigler (14), considerable day-to-day variability in the \( \dot{V}E \) and \( \dot{V}T / Ti \) responses to hypercapnia was found, and this accounted for part of the day-to-day variation in RMSdi. Additional day-to-day variation in the RMSdi response to hypercapnia is likely to be due to variations in the muscle-to-electrode distance and orientation and perhaps changes to the filtering properties of the electrode. Such differences are likely to be best corrected by normalization with an effort-independent electrical signal of constant amplitude that is detected by the same esophageal electrode used to detect EMG activity. The data show that the \( R \) wave of the ECG measured from the electrode closest to the electrical center of the diaphragm at end expiration at rest can be used to reduce day-to-day variation in RMSdi and has the advantage over RMSdi rest or RMSdi max in being independent of effort.

These findings suggest that RMSdi measured via a multi-electrode esophageal catheter and methods described by Beck and Sinderby and colleagues (4, 5, 31, 33) can be used to quantify moderate levels of diaphragm activation and the neural drive to the diaphragm during progressive hypercapnia. It is recommended that RMSdi is normalized for RMSdi max for comparisons between subjects and normalized for the amplitude of the ECG R wave for comparisons of repeat measurements in the same subject.

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