Parasternal intercostal muscle remodeling in severe chronic obstructive pulmonary disease

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OVER THE PAST DECADE, OUR laboratory (21–23, 29, 30) and others (24) have demonstrated that, in patients with severe chronic obstructive pulmonary diseases (COPDs), the costal diaphragm exhibits a fast-to-slow transformation of fiber types and myosin heavy chain (MHC) isoform expression. However, the cellular and molecular adaptation of the rib cage muscles to severe COPD has not been fully elucidated. In the present study, we focus on the remodeling of the parasternal intercostal muscles exhibited by patients with severe COPD.

Previous workers have demonstrated that, in healthy humans, parasternal EMG activity invariably occurs synchronously with costal diaphragm activity. Moreover, healthy humans cannot inspire with the diaphragm alone without activating the parasternals (3, 9). Additionally, using both cross-innervation (2) and direct stimulation of muscle (see review in Ref. 32), investigators have demonstrated that increases in neural drive to a muscle elicit a fast-to-slow transformation of fiber types, MHC isoform expression, and contractile properties. Because previous workers (6, 11) have demonstrated that, in severe COPD, neural drive is increased to both the diaphragm and parasternals, we carried out the present study to test the hypothesis that the parasternal intercostals in patients with severe COPD exhibit fast-to-slow transformations in both fiber types and MHC isoform expression.

METHODS

Subjects

Our study cohort consisted of seven control subjects who were undergoing surgery for resection of a solitary pulmonary nodule and seven patients with severe COPD (with heterogeneous distribution of emphysema) who were undergoing lung volume reduction surgery. Full thickness parasternal intercostal muscle biopsies were carried out intraoperatively in all subjects at the level of the third interspace within 1 cm of the lateral sternal border.

Additionally, five of the control subjects and all of the COPD patients underwent full thickness costal diaphragm biopsies using previously described methodology (21–23, 29, 30). Informed consent for both parasternal and diaphragm biopsies was obtained from each of the subjects, and our protocol was approved by the Institutional Review Boards of the Philadelphia Veterans Affairs Medical Center and the University of Pennsylvania.

Pulmonary Function Tests

Before surgery, subjects underwent spirometry and measurement of lung volumes by plethysmography, and values were compared with predicted normal values (14, 16). Prior workers (33) have described artificually high values for lung volumes in patients with hyperinflation, and we used strategies for avoiding these errors described by others (34).

Biopsies

Parasternal and diaphragm biopsies were prepared for immunohistochemistry by the method of Larsson et al. (18, 19) and for biochemistry by the method of Larsson et al. (18, 19). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Immunohistochemistry

Methodology. Immunohistochemistry was carried out as previously described (29, 30) with some modifications. Briefly, we obtained 10-μm-thick serial transverse sections from each of the parasternal and diaphragm specimens. Two adjacent sections were initially incubated in block buffer that consisted of 2% bovine serum albumin and 1% normal goat serum (IgG concentration of 10 mg/ml) in PBS, pH 7.2. Subsequently, the blocked sections were stained with either mouse anti-slow MHC [clone NOQ7.5.4D (27), 1:50 dilution] or anti-fast MHC [clone MY-32 (28), 1:200 dilution] prepared in block buffer. Additionally, rabbit anti-laminin antibody (36) was included in each of these stains (Sigma no. L9393, 1:25 dilution) for visualization of the basement membrane. For these sections, we reacted with a mixture of Alexa Fluor 546 conjugated goat anti-mouse and Alexa Fluor 488 conjugated goat anti-rabbit secondary antibodies (Invitrogen, Carlsbad, CA; 1:200 dilution). Blocking and antibody staining steps were carried out in a humidified chamber for 1 h at room temperature. Unbound antibodies were removed by washing the sections three times (10 min each) with PBS. With the use of these stains, the MHCs that reacted with our antibody on each section appeared orange-red in color, whereas the laminin in the plasmalemma appeared green.

For each section, we sequentially imaged the laminin and myosin fluorescent signals. The laminin images were used to number and outline fiber cross-sectional area (CSA); subsequently, each of the myosin-stained images was superimposed on the corresponding laminin image. We then used this composite image to determine fiber type.

Fiber-type area fraction (%CSA). First, we outlined the perimeter of individual fibers using Scion Image (Scion, Frederick, MD) software and an Intuus graphics tablet (Wacom Technology, Vancouver, WA). We then tracked each of these fibers through the serial sections that were stained with our MHC antibodies. Based on immunoreactivity pattern to these antibodies, each of the fibers was categorized as slow (i.e., fibers expressing only the slow MHC isoform), fast (i.e., fibers expressing only fast MHC isoforms), or hybrid (i.e., fibers containing both slow and fast MHC isoforms). We analyzed a minimum of 400 fibers for each of the 14 subjects to obtain mean fiber-type proportions, as previously described (29, 30).

Vital Statistics and Pulmonary Function Measurements

The COPD patients did not differ significantly from the control subjects with respect to age, height, weight, or body mass index (Table 1). The COPD group consisted of five men and two women, whereas our control group consisted of three men and four women.

Table 1. Comparison of COPD and control subjects with respect to vital statistics and pulmonary function measurements

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>COPD (n = 7)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vital statistics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>62.8 ± 8</td>
<td>59.4 ± 4</td>
<td>0.73</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169.2 ± 2</td>
<td>167.4 ± 2</td>
<td>0.76</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.2 ± 2</td>
<td>66.5 ± 2</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 ± 2</td>
<td>24.1 ± 2</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Spirometry‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV 1.0, Liter</td>
<td>2.51 ± 0.14</td>
<td>0.83 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>%Predicted</td>
<td>106.2 ± 6</td>
<td>29.2 ± 2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>106.2 ± 10</td>
<td>67.7 ± 2</td>
<td>0.004</td>
</tr>
<tr>
<td>FEV 1.0/FVC * 100</td>
<td>85.2 ± 5</td>
<td>35.4 ± 2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Lung volumes (%predicted)‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>126.4 ± 4</td>
<td>236.2 ± 2</td>
<td>0.007</td>
</tr>
<tr>
<td>FRC</td>
<td>115.2 ± 17</td>
<td>181.1 ± 18</td>
<td>0.04</td>
</tr>
<tr>
<td>TLC</td>
<td>109.9 ± 2</td>
<td>128.9 ± 2</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE; n, no. of subjects. COPD, chronic obstructive pulmonary disease; BMI, body mass index; FEV 1.0, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; FRC, functional residual capacity; TLC, total lung capacity. †Group t-tests were used to compute the statistical significance of differences between control and COPD groups with respect to vital statistics and pulmonary function tests. Nominal P values for two-tail tests are reported. In this study, spirometry was measured in all control and COPD patients, whereas lung volumes and capacities were only measured in three of the seven controls and all of the COPD patients.
Table 1 indicates that the COPD patients had higher values for residual volume, functional residual capacity, and total lung capacity than the control subjects, whereas control subjects had higher values with respect to the forced expiratory volume in 1 s, forced vital capacity, and the ratio of the forced expired volume in 1 s to forced vital capacity than the control subjects. According to the GOLD criteria (31), one of our COPD patients had severe COPD, whereas six had very severe COPD.

**Comparison of COPD and Control Parasternals**

**Immunohistochemistry.** Figure 1 compares serial sections from a representative COPD and a representative control parasternal muscle. The figure shows that both control and COPD parasternals contained the following two types of fibers: 1) those expressing only MHC I (i.e., slow fibers); and 2) those expressing only MHC II isoforms (i.e., fast fibers). Additionally, the COPD parasternal shows a third type of fiber that contains both MHC I and MHC II isoforms (a hybrid fiber); this third type of fiber was also present in the control parasternals. Importantly, the figure shows that the COPD parasternal contained a higher proportion and a higher area fraction of slow fibers than the control parasternal. In contrast, the control parasternal exhibited a higher proportion and a higher area fraction of fast fibers than the COPD parasternal.

**Fig. 1.** Representative serial cross sections of parasternal muscle from chronic obstructive pulmonary disease (COPD) patients and control subjects. Sections in A and B were preincubated with NOQ7.5.4D antibody (27), which is specific for the slow myosin heavy chain (MHC), whereas sections in C and D were preincubated with the MY-32 antibody (28), which reacts with all fast MHCs. Additionally, in each section, all fibers are outlined by an antibody reactive to laminin (36). In each of the sections, representative slow and fast fibers are indicated by an open circle and square, respectively. Additionally, a hybrid fiber (containing both slow and fast MHCs) in the COPD parasternal section is indicated by an asterisk.

**Fig. 2.** Comparison of COPD patients (n = 7) and control subjects (n = 7) with respect to slow, fast, and hybrid fiber type proportions (A) and area fractions (B) in parasternal muscles. Open bars indicate means for control subjects, and solid bars represent means for COPD subjects. Error bars represent the SE.
regression equation and the line of identity. The square of the correlation coefficient (i.e., \( r^2 \)) represents the proportion of the increase in area fraction of slow fibers that can be attributed to variation in the proportion of slow fibers; therefore, the data shown in Fig. 3 indicate that 85% of the variation in area fraction of slow fibers can be accounted for by variations in fiber-type proportions.

**Morphometric ratio between slow and fast fiber types.** Table 2 indicates that the mean ratio between the CSAs of slow to fast fibers was the same in control and COPD groups. Additionally, the table indicates that COPD and control groups did not differ with respect to the ratio between least mean diameters of slow-to-fast fibers. These observations are consistent with our above-noted suggestion that the differences in area fractions of slow fibers between COPD and control parasternals are predominantly due to changes in fiber-type proportions and not fiber-type size.

**MHC isoform expression.** Figure 4 compares COPD and control parasternals with respect to MHC isoform proportions. Figure 4A shows that both the COPD and the control parasternals contained three types of MHC isoforms. Western blotting (not shown) indicated that the lower band was composed of MHC I, whereas the two upper bands were MHC IIa and IIx, respectively (see labels in A). These representative gel lanes strongly suggest that the proportion of slow MHC is greater in the COPD than in the control parasternal. Conversely, the proportion of fast MHC isoforms (i.e., the sum of the IIa and IIx bands) appears to be greater in the control than in the COPD parasternal.

Figure 4B summarizes our statistical comparisons of six COPD and six control parasternals with respect to MHC isoform proportions. In the COPD parasternals, 56 ± 4% of the MHC consisted of the slow isoform, whereas, in the control parasternals, only 46 ± 4% of the MHC consisted of the slow isoform. This difference between COPD and control parasternals was significant at the 0.04 level.

Figure 4B also shows that the control parasternals contained higher proportions of each of the fast MHC isoforms (i.e., IIa and IIx) than the COPD parasternals; however, each of these differences was not statistically significant (i.e., \( P = 0.10 \) for both the IIa and IIx comparisons). However, if one compares control and COPD parasternals with respect to the sum of the fast MHC isoforms, the difference between control and COPD parasternals is statistically significant (\( P = 0.04 \)).

**Within-Subject Comparison of Parasternals and Costal Diaphragms With Respect to Histochemical Features**

**Fiber-type proportions.** Figure 5 indicates that control subjects exhibited no statistically significant differences between the proportions of slow fibers in the parasternals and those in the costal diaphragm. However, in COPD subjects, the proportion of slow fibers in the parasternals was greater (\( P = 0.005 \)) than that noted in the costal diaphragms.

**Area fractions.** As with fiber-type proportions, control subjects exhibited no difference between parasternals and diaphragm. In contrast, the COPD subjects exhibited a 20% higher area fraction of slow fibers in the parasternals than in the diaphragm; this difference between group means approached but did not achieve statistical significance (i.e., \( P = 0.13 \)).

**DISCUSSION**

**Major Findings**

The major findings of the present study are that, compared with control subjects, the parasternal muscles of our severe COPD patients exhibited: 1) higher proportions and area fractions of slow fibers; and 2) higher proportions of the slow MHC isoform. These remodeling changes are similar to those that our laboratory has previously described to occur in the diaphragms of patients with severe COPD (21–23, 29, 30). To the best of our knowledge, this represents the first report of fiber-type and MHC isoform transformations noted in the human parasternal muscles.

**Critique of Methodology**

Previous workers have demonstrated that rostrocaudal as well as medial lateral differences in EMG activity occur in experimental animals (7, 20). However, De Troyer et al. (5, 8) showed (in the dog) that no medial-lateral gradient in fiber types was associated with these medial-lateral differences in EMG activity. In contrast, studies in the hamster by Kelsen et al. (15) showed that there was a rostrocaudal gradient in fiber-type proportions; i.e., the proportion of slow fibers decreased along the rostrocaudal axis of the parasternal muscles. We cannot exclude the possibility that a similar gradient exists in the human parasternal muscle. Nonetheless, even if both rostral-caudal and medial-lateral gradients in fiber-type proportions do exist in the human parasternal muscles, these gradients

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**Table 2. Comparison of control and COPD parasternal intercostal muscles with respect to fiber-type morphometric ratios**

<table>
<thead>
<tr>
<th>Morphometric Ratio</th>
<th>Control ((n = 7))</th>
<th>COPD ((n = 7))</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA I/CSA II</td>
<td>1.10 ± 0.10</td>
<td>1.10 ± 0.22</td>
<td>0.499</td>
</tr>
<tr>
<td>LMD I/LMD II</td>
<td>1.06 ± 0.07</td>
<td>1.03 ± 0.07</td>
<td>0.394</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), no. of subjects. CSA, cross-sectional area; LMD, least mean diameter.
cannot account for the fiber-type differences noted in this study, because all biopsies were obtained from the same medial portion of the parasternal intercostal in the third interspace.

Comparison of Increases in Proportion of Slow Fibers and Increases in Proportion of Slow MHC Isoform Expression

For each of our six COPD subjects (who underwent measurement of MHC expression and fiber-type proportions), Table 3 summarizes the percent increase above control mean for both of these measurements. The table indicates that the percent increase in slow-fiber-type proportions was over twice that noted in the increases in slow MHC expression. We hypothesize that this discrepancy is most likely accounted for by the fact that the slow fibers in the parasternals of patients with severe COPD have a lower concentration of MHC than those of control subjects.

Within-Subject Comparison of Slow Fiber-Type Proportions Between Parasternals and Diaphragm

As noted in the results, the parasternal muscles of our COPD subjects showed greater increases in the proportion of slow fibers than that noted in the costal diaphragm. The activity of a muscle can be increased either by 1) increases in the discharge frequency of single motor units that are already activated (i.e., rate coding) or 2) by activating additional motor units (i.e., recruitment). The observations of Gandevia et al. (10) suggest that, in the parasternals, virtually all increases in activity are due to recruitment, whereas increases in diaphragm activity are affected by both rate coding and recruitment. Therefore, compared with the diaphragm, the parasternals should have a greater proportion of their fibers continuously activated throughout the day, and we hypothesize that this difference accounts for the increased proportion of slow fibers manifest by the parasternals of our severe COPD subjects.

Comparison of Our Human Parasternal Findings with Data in the Literature

The histological literature on the human parasternals is limited to the autopsy study of Mizuno and Secher (25), who obtained postmortem open biopsies of the parasternals (in the right third interspace) of eight previously healthy men ranging in age from 39 to 51 yr. Their data show no statistically significant differences from the control subject data presented in the results with respect to both the proportion and area fraction of slow fibers. Their paper contains no data on MHC isoform expression.

Discrepancy Between COPD-Associated Remodeling of Parasternals and External Intercostals

Our parasternal observations are contrary to those noted by Gea et al. (12) and Gea (13), who reported that severe COPD elicits slow-to-fast adaptations in both fiber types and MHC isoforms in regions of the external intercostal (EI) muscles. Some comment is warranted regarding these latter observations. First, De Troyer et al. (4, 5) have demonstrated that the firing frequency of EI motor units (in healthy humans during resting breathing) exhibits both dorsoventral and rostrocaudal

Table 3. Comparison of increases in proportion of slow fibers and increases in slow MHC isoform expression in each of the COPD parasternal intercostal muscles

<table>
<thead>
<tr>
<th>Subject</th>
<th>Proportion of slow-fiber type</th>
<th>Proportion of slow MHC isoform</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD 1</td>
<td>52</td>
<td>18</td>
</tr>
<tr>
<td>COPD 2</td>
<td>84</td>
<td>65</td>
</tr>
<tr>
<td>COPD 3</td>
<td>60</td>
<td>15</td>
</tr>
<tr>
<td>COPD 4</td>
<td>47</td>
<td>24</td>
</tr>
<tr>
<td>COPD 5</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>COPD 6</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>50 ± 8.6*</td>
<td>23 ± 8.9*</td>
</tr>
</tbody>
</table>

*A paired t-test was used to compare increases above control mean values in proportion of slow fiber type and proportion of slow myosin heavy chain (MHC) isoform. The group means were statistically different (P = 0.0001).
gradients; i.e., maximum EMG activity is recorded in dorsal locations of the most rostral interspaces. Since we presume that the Gea group obtained their biopsies from the same location on the chest wall in both severe COPD and control subjects, the gradients in EMG activity reported by De Troyer et al. cannot account for the slow-to-fast transformation noted by Gea et al. (12) and Gea (13) in their patients with severe COPD.

Summary Statements

Our data indicate that the remodeling of the parasternal intercostal muscle in severe COPD is characterized by a fast-to-slow transformation in both fiber types and MHC isoforms. This remodeling is similar to that noted in the diaphragms of patients with severe COPD. In contrast, the literature indicates that severe COPD elicits remodeling of the EIs, another rib cage inspiratory muscle, characterized by a slow-to-fast transformation in both fiber types and MHC isoforms. This remodeling is similar to that noted in the diaphragms of patients with severe COPD. In contrast, the literature indicates that severe COPD elicits remodeling of the EIs, another rib cage inspiratory muscle, characterized by a slow-to-fast transformation in both fiber types and MHC isoforms. The physiological significance of this difference in remodeling between EIs and parasternal intercostals remains to be elucidated.

ACKNOWLEDGMENTS

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REFERENCES