Myosin heavy chain gene expression changes in the diaphragm of patients with chronic lung hyperinflation

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2INSERM Unité 153, Groupe Hospitalier Pitié-Salpêtrière, 75013 Paris, France; and 3Dipartimento di Scienze Biomediche Sperimentali, Centro Consiglio Nazionale delle Ricerche per la Biologia, Fisiopatologia Medica, Università di Padova, 35121 Padua, Italy

Mercadier, Jean-Jacques, Ketty Schwartz, Stefano Schiaffino, Claudine Wisnewsky, Simonetta Ausoni, Michele Heimburger, Rolana Marrash, René Pariente, and Michel Aubier. Myosin heavy chain gene expression changes in the diaphragm of patients with chronic lung hyperinflation. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L527–L534, 1998.—In striated muscle, chronic increases in workload result in changes in myosin phenotype. The aim of this study was to determine whether such changes occur in the diaphragm of patients with severe chronic obstructive pulmonary disease, a situation characterized by a chronic increase in respiratory load and lung volume. Diaphragm biopsies were obtained from 22 patients who underwent thoracic surgery. Myosin was characterized with electrophoresis in non-denaturing conditions, SDS-glycerol PAGE, and Western blotting with monoclonal antibodies specific for slow and fast myosin heavy chain (MHC) isoforms. Flow volume curves, total lung capacity, and functional residual capacity were measured before surgery in 20 patients. We found that the human diaphragm is composed of at least four myosin isoforms, one slow and three fast, resulting from the combination of three MHC species. Chronic overload was associated with an increase in the slow β-MHC species at the expense of the fast species (β–MHC, 78.2 ± 4.6 and 50.0 ± 6.5% in emphysematous and control patients, respectively; P < 0.005). Linear correlations were found between β-MHC percentage and forced expiratory volume in 1 s (r = −0.52; P < 0.02), total lung capacity (r = 0.44; P < 0.05), and functional residual capacity (r = 0.65; P < 0.003). The human adult diaphragm is composed of a balanced proportion of slow and fast myosin isoforms. In patients with chronic obstructive pulmonary disease, the proportion of fast myosins decreases, whereas that of slow myosins increases. This increase appears to be closely related to lung hyperinflation and may reflect an adaptation of the diaphragm to the new functional requirements.

human diaphragm; myosin electrophoresis; chronic obstructive pulmonary disease; lung distension

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is characterized by increased resistance to airflow, air trapping, and hyperinflation of the lungs. The increased resistance to airflow increases the work and energy required for breathing. Furthermore, lung hyperinflation places the inspiratory muscles, and particularly the diaphragm, at a mechanical disadvantage. Because most of the ventilatory burden in these patients is borne by the inspiratory muscles, the muscles, and especially the diaphragm, can fatigue and fail as pressure generators, leading to respiratory failure. Indeed, there is now ample evidence that the inspiratory muscles may fail if chronically overloaded (16). Inspiratory muscle fatigue has been documented in acute ventilatory failure and in chronic hypercapnic COPD when ventilation is voluntarily increased (19). However, in patients with stable severe COPD, the inspiratory muscles are capable of coping chronically with very high loads. Moreover, it has been shown recently in well-nourished patients with lung hyperinflation and stable COPD that the strength of diaphragm contraction and, to a greater extent, its inspiratory action are well preserved (27). This suggests that, in such patients, changes occur in the structure and function of the muscle that allow it to adapt to the altered functional demands.

Such adaptive alterations in the structure and function of striated muscles, including the heart, have been widely documented in a number of physiological and pathological conditions (for a review, see Ref. 30). With respect to skeletal muscle, it has been shown that chronic stimulation of a fast-twitch muscle at a slow rate results in a number of structural and functional changes that affect not only contractile protein phenotype but also capillary bed density, function of the sarcoplasmic reticulum, and the enzymes of both aerobic and anaerobic metabolism, leading to a fatigue-resistant, slowly contracting muscle (30). Among the changes in contractile protein phenotype, those of myosin heavy chain (MHC) are of special physiological importance because MHC is mainly responsible for the level of ATPase activity of the whole myosin molecule, which itself correlates with the maximum shortening velocity of the muscle fiber (3, 25, 30). During the last two decades, our knowledge of changes in the myosin phenotype of striated muscles during a variety of physiological and pathological conditions has improved greatly through the use of discriminatory electrophoretic techniques such as electrophoresis in non-denaturing conditions, which allows myosin forms to be separated according to charge differences of the whole hexameric molecule (10), and original SDS-PAGE, which can separate the various MHC isoforms (2, 5). Together with histochemistry, these techniques have shown that, after a chronic increase in muscle load, a shift occurs in myosin isoforms, from those with high ATPase activity (composed of “fast” MHCs) to those responsible for low
and lung hyperinflation. MHC phenotype with indexes of respiratory function, COPD. We also investigated possible correlations of the MHC phenotype of the human diaphragm and to chronic increases in respiratory load and lung volume. The aim of this study was therefore to establish the MHC phenotype of the human diaphragm and to identify any changes in patients with severe stable COPD. We also investigated possible correlations of the MHC phenotype with indexes of respiratory function, which account for the severity of airway obstruction and lung hyperinflation.

**Methods**

Patients, measurement of ventilatory function, and muscle sampling. We studied 22 patients (18 men and 4 women with a mean age of 54.4 ± 8.7 yr) due to thoracic surgery for lung cancer or resection of a large bulla (Table 1). None of them was receiving chronic glucocorticoid administration, and all had normal nutritional status. Ventilatory function was thoroughly documented before surgery (Table 2) except in two patients with lung metastases whose respiratory function was normal on a flow-volume curve. All measurements were performed in the sitting position. Several flow-volume curves were obtained with a Hewlett-Packard spirometer (Hewlett-Packard, Waltham, MA) to determine the forced expiratory volume in 1 s (FEV1) and forced vital capacity. Total lung capacity (TLC) and functional residual capacity (FRC) were measured with a constant-volume body plethysmograph (Gould System 2800; Gould Instruments, Cleveland, OH).

Muscle biopsy specimens of ~50–100 mg were obtained during surgery, in keeping with our institutional guidelines for human research, in the same costal region of the diaphragm. In addition, a sample of the pectoralis major was obtained from a patient undergoing a radical mastectomy. All muscle specimens were placed in liquid nitrogen immediately after sampling and stored at −80°C until use.

Gel electrophoresis in nondissociating conditions. About 20 mg of the muscle sample were crushed in liquid nitrogen and extracted at 4°C for 20 min with 4 volumes (wt/vol) of a slightly modified Guba's solution (0.3 M KCl, 0.1 M H2KPO4, 0.05 M HK2PO4, 0.001 M MgCl2, 0.01 M Na2P2O7, 1% (wt/vol) Na azide, and 1% (vol/vol) 2-mercaptoethanol) as previously described (10, 18). The homogenate was centrifuged at 4°C and 30,000 g for 20 min, and the supernatant was stored at −20°C in 50% (vol/vol) glycerol. PAGE in nondissociating conditions was performed with a Pharmacia apparatus (GE-411) capable of thermostating and circulating the buffer between the anodic and cathodic reservoirs (10, 18). Briefly, cylindrical polyacrylamide gels (60 × 5 mm) were prepared with 3.88% (wt/vol) acrylamide-0.12% (wt/vol) N,N'-methylene-bis-acrylamide. Each gel was loaded with 30 µl of a 40-fold dilution of the crude muscle extract (1–2 mg of myosin) with 0.01 M Na2P2O7, 50% (vol/vol) glycerol, and traces of bromphenol blue at pH 8.5. The buffer was kept at between 1 and 3°C, and electrophoresis was run at a constant voltage of 14 V/cm for 20–24 h. Staining and destaining of the

**Table 1. Patient profiles**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>55</td>
<td>M</td>
<td>Cancer</td>
</tr>
<tr>
<td>C2</td>
<td>61</td>
<td>M</td>
<td>COPD</td>
</tr>
<tr>
<td>C3</td>
<td>48</td>
<td>F</td>
<td>Cancer</td>
</tr>
<tr>
<td>C4</td>
<td>54</td>
<td>F</td>
<td>Cancer</td>
</tr>
<tr>
<td>C5</td>
<td>62</td>
<td>M</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>C6</td>
<td>48</td>
<td>F</td>
<td>Cancer</td>
</tr>
<tr>
<td>C7</td>
<td>56</td>
<td>F</td>
<td>Cancer</td>
</tr>
<tr>
<td>C8</td>
<td>40</td>
<td>M</td>
<td>Cancer</td>
</tr>
<tr>
<td>C9</td>
<td>61</td>
<td>M</td>
<td>COPD</td>
</tr>
<tr>
<td>E1</td>
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<td>M</td>
<td>COPD</td>
</tr>
<tr>
<td>E2</td>
<td>56</td>
<td>M</td>
<td>COPD</td>
</tr>
<tr>
<td>E3</td>
<td>54</td>
<td>M</td>
<td>COPD</td>
</tr>
<tr>
<td>E4</td>
<td>57</td>
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<td>COPD</td>
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</tr>
<tr>
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<td>M</td>
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<tr>
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<td>30</td>
<td>M</td>
<td>COPD</td>
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<tr>
<td>E11</td>
<td>57</td>
<td>M</td>
<td>COPD + Cancer</td>
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<tr>
<td>E12</td>
<td>45</td>
<td>M</td>
<td>COPD + Cancer</td>
</tr>
<tr>
<td>E13</td>
<td>58</td>
<td>M</td>
<td>COPD</td>
</tr>
</tbody>
</table>

C, control; E, emphysematous; M, male; F, female; COPD, chronic obstructive pulmonary disease.

**Table 2. Ventilatory function and slow myosin percentage**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>TLC, %predicted</th>
<th>FEV1, %predicted</th>
<th>FRC, %predicted</th>
<th>Slow Myosin, %total</th>
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<tbody>
<tr>
<td>C1</td>
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<td>56</td>
<td>101</td>
<td>60</td>
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<tr>
<td>C2</td>
<td>89</td>
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<td>C3</td>
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<td>96</td>
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<td>50</td>
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<td>C5</td>
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<td>63</td>
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<tr>
<td>C6</td>
<td>71</td>
<td>65</td>
<td>68</td>
<td>39</td>
</tr>
<tr>
<td>C7</td>
<td>108</td>
<td>93</td>
<td>104</td>
<td>21</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>92.9 ± 7.5</td>
<td>67.7 ± 6.5</td>
<td>109.6 ± 9.6</td>
<td>50.0 ± 6.5</td>
</tr>
</tbody>
</table>

**E1** 121 15 208 65
**E2** 86 11 134 52
**E3** 105 50 124 75
**E4** 111 19 184 100
**E5** 136 13 236 71
**E6** 148 21 242 100
**E7** 147 16 253 100
**E8** 115 19 169 82
**E9** 119 25 150 77
**E10** 130 63 157 72
**E11** 163 39 229 92
**E12** 130 43 160 79
**E13** 150 115 181 51

Mean ± SE 127.8 ± 5.9* 34.5 ± 8.1* 186.7 ± 11.9* 78.2 ± 4.6*

TLC, total lung capacity; FEV1, forced expiratory volume in 1 s; FRC, functional residual capacity. *p < 0.005 vs. controls.
gels were carried out in a Hoefer Scientific Instruments apparatus. Densitometric tracings of the gels were obtained with a Gilford model 240 spectrophotometer equipped with a Hewlett-Packard 70-44A multirecorder tracing table. The relative amounts of slow and fast myosin isoforms were calculated from the height of each peak. The reproducibility of the method has been shown to be 9% (18).

SDS-PAGE and Western blotting. Myosin was extracted by using a rapid procedure described by Schiaffino et al. (23). Briefly, muscle fragments were homogenized in 10 volumes of 20 mM KCl-2 mM K2HPO4-1 mM EGTA, pH 6.8, and centrifuged at 12,000 g for 2 min. After a wash with the same buffer, the pellets were resuspended in 40 mM Na2P2O7-1 mM MgCl2-1 mM EGTA, pH 9.5, for 15 min to extract myosin. The samples were then centrifuged at 12,000 g for 15 min, and the protein concentration in the supernatant was determined according to Bradford’s method (Bio-Rad). Myosin electrophoresis was performed using 6% polyacrylamide gels in the presence of 37.5% glycerol (24). Similar amounts of MHC, as determined by densitometry, were loaded on each lane.

Three monoclonal antibodies were used for the immunochemical characterization of MHCs. The first, BA-D5, was directed against β/slow MHC; the second, SC-75, against type II MHCs; and the third, BF-32, against both β/slow and type II a MHCs. We call type II a the fast MHC isoform recognized by BF-32 because only type I and II a fibers in human muscle sections were labeled by this antibody, type II b fibers being unreactive (data not shown). The procedure described by Towbin et al. (32) was used for Western blotting experiments. Briefly, proteins separated on SDS-polyacrylamide gels were transferred onto nitrocellulose paper at 250 mA overnight in 25 mM Tris·HCl-192 mM glycine buffer, pH 8.0. The paper sheets were incubated with appropriate dilutions of monoclonal antibodies, and reactivity was revealed by immunoperoxidase staining with rabbit anti-mouse IgG conjugated with horseradish peroxidase (Dako, Milan, Italy); diaminobenzidine (Serva, Milan, Italy) was used as the substrate in the presence of imidazole (22). The gels stained with Coomassie blue were scanned with a Shimadzu CS-930 dual-wavelength thin-layer chromatography scanner to estimate the relative amounts of the corresponding forms among total myosin (slow myosin percentage) was estimated only from the height of the major peak.

MHC SDS-PAGE. Diaphragmatic myosin from 8 of the 22 patients was submitted to SDS-PAGE and MHC immunochemical characterization. As with native myosin electrophoresis, SDS-PAGE revealed both the multiplicity of MHC isoforms and marked differences in the electrophoretic pattern among patients. Figure 2A shows examples of the different electrophoretic patterns in four patients. In contrast to pyrophosphate gel electrophoresis, the fast-migrating band corresponds, in both human and rat diaphragms, to myosin composed of type I (β/slow) MHCs, whereas the slow-migrating bands correspond to myosins composed of

**RESULTS**

Myosin electrophoresis in nondenaturing conditions. Figure 1 shows typical electrophoretic patterns of native diaphragmatic myosin from three patients undergoing bulla resection (Emphysematous patients (patients E1–3)) or lung cancer surgery (control patients (patients C1–3)). In these electrophoretic conditions, myosin from type I fatigue-resistant muscle fibers (myosin composed of β-MHC) is also the one that exhibits the slowest electrophoretical mobility. Like diaphragmatic myosin in other mammalian species, diaphragmatic myosin in the two categories of patients separated into fast- and slow-migrating bands corresponding to fast and slow myosin isoforms, respectively. However, the relative amounts of fast and slow myosin forms differed markedly from patient to patient in both groups. Slow myosin was detected in all samples as a single dark band or a major dark band associated with a faint band migrating slightly ahead. In sharp contrast, the abundance of fast myosin isoforms differed greatly among the patients. Three fast myosin bands were clearly detected in some patients (patients E1 and C1), whereas fast myosins were at the detection limit in others (patients E3 and C3). An intermediate pattern was observed in patients E2 and C2. Table 2 shows the proportions of slow myosin relative to total myosin. Because the faint band migrating slightly ahead of the major slow myosin band appeared as a small shoulder of the major peak on densitometric tracings, the proportion of slow myosin among total myosin (slow myosin percentage) was estimated only from the height of the major peak.

**Figure 1.** Electrophoresis of diaphragm myosin in nondenaturing conditions. E1–3, emphysematous patients with chronic obstructive pulmonary disease (COPD); C1–3, control patients (see Table 1 for details). S, slow myosin; F, fast myosin.

**Figure 2A.** SDS-glycerol PAGE of diaphragm myosin. I, type I myosin heavy chain; II, type II myosin heavy chain; C4 and C5, control patients; E3 and E4: emphysematous patients with COPD (see Table 1 for details). B: myosin electrophoresis in nondenaturing conditions of same muscle samples as in A.
fast MHCs. One fast- and two slow-migrating bands were detected in two of the four samples (patients C4 and C5), probably corresponding, by analogy with other muscles and mammalian species, to slow (type I) and fast (type II) MHCs, respectively. Type I and II MHCs were present in approximately equal proportions, and among the two type II MHC bands, the faster migrating species predominated. Type I myosin was consistently observed in all the samples studied, whereas the proportion of type II MHCs varied markedly, being equivalent to that of type I MHC in some samples (patients C4 and C5), almost undetectable in another (patient E4), and intermediate in the remainder (patient E3). In the latter case, the faster migrating type II MHC species always predominated and was sometimes the only visible MHC form. Figure 2B shows the electrophoretic patterns of myosin in nondenaturing conditions. In the eight muscle samples subjected to both denaturing and nondenaturing electrophoresis, there was a strong link between the proportion of slow myosin (Fig. 2B, S) and type I MHC (Fig. 2A, I).

Characterization of MHCs by Western blot analysis. Figure 3A shows the electrophoretic pattern (Coomassie blue staining) of myosin purified from three patients (patients C5, C4, and E4) and from a human adult pectoral muscle (P). Three MHC bands were separated in the pectoral muscle and in two diaphragmatic samples (patients C5 and C4) and corresponded to type I, IIA, and IIB MHCs, respectively, based on the nomenclature used by Klitgaard et al. (12). The electrophoretic pattern of myosin from the diaphragm of patients C5 and C4 was identical to that of myosin from the pectoral muscle. The first monoclonal antibody, BA-D5, reacted against the fast-migrating band in all four samples, confirming that this band was composed of type I (β/slow) MHC (Fig. 3B). The second monoclonal antibody, SC-75, reacted with the two slow-migrating MHC bands in patients C5 and C4 and the pectoral muscle and with a band in patient E4, migrating the same distance as the faster component of the two slow-migrating bands in patients C5 and C4 and the pectoral muscle, confirming that these slow-migrating bands were composed of type I MHCs (Fig. 3C). The third monoclonal antibody, BF-32, which labeled type I and IIA but not type IIB fibers in human muscle sections, reacted with both the fast-migrating band and the fast component of the slow-migrating bands, indicating that these bands were composed of type I (β/slow) and type IIA MHCs, respectively (Fig. 3D). Finally, the sample from patient E4 contained an increased proportion of type I (β/slow) MHC and a decreased proportion of type IIA MHC relative to patients C4 and C5, whereas type IIB MHC was undetectable.

Slow myosin in the diaphragm of patients with and without severe COPD and correlations with ventilatory function. Analysis of native myosin phenotype in the 20 patients in whom ventilatory function was assessed allowed us to establish that the proportion of slow myosin among total myosin was increased by 56% in those patients with severe stable COPD compared with control patients (78.2 ± 4.6 vs. 50.0 ± 6.5%; P < 0.005; Table 2). Grouping patients according to their functional capacity rather than their diagnosis would lead to a very similar result as seen from the values in Table 2. In addition, we could identify correlations between the proportion of slow myosin and parameters reflecting a chronic increase in airway resistance (FEV₁) or lung distension (TLC and FRC) in all study patients except two (patients C3 and C4) with lung metastases whose respiratory function was normal on a flow-volume curve. The raw view of the data scattergram was highly suggestive for linear correlations between the above-mentioned variables, which was confirmed by linear regression analysis. Indeed, we found a negative linear correlation between FEV₁ and the slow myosin percentage (r = −0.52; P < 0.02) and positive linear correlations between the slow myosin percentage and both TLC and FRC (r = 0.44; P < 0.05 and r = 0.65; P < 0.003, respectively; Fig. 4). Because these results confirmed our initial assumption that myosin phenotypic conversion toward type I (β/slow) MHC is proportional to lung distension and airway obstruction, we did not test whether the variables were related in a nonlin-

Fig. 3. A: SDS-glycerol PAGE of diaphragm myosin. See Table 1 for details about patients. P, human pectoralis muscle; I, type I (β/slow) myosin heavy chain; IIA and IIB, type IIA and IIB myosin heavy chains, respectively, based on nomenclature used by Klitgaard et al. (12). B–D, Western blots of myosin specimen shown in A. In B, myosin was revealed with BA-D5, a monoclonal antibody directed against β/slow myosin heavy chain. In C, myosin was revealed with SC-75, a monoclonal antibody specific for type I myosin heavy chains. In D, myosin was revealed with BF-32, a monoclonal antibody that reacts with both type I and IIA muscle fibers. See text for details.
The best correlation coefficient \((r = 0.65; P < 0.003)\) was observed between slow myosin percentage and FRC, suggesting that the position of the diaphragm at the end of spontaneous expiration may be an important determinant of MHC phenotypic change in patients with COPD.

DISCUSSION

These results show that the human diaphragm is composed of at least four myosin species resulting from the combination of three MHC isoforms and that chronic mechanical overload of the human diaphragm results in the accumulation of slow \(\beta\)-MHC at the expense of the fast isoforms. In addition, our results suggest that this MHC transition appears to increase with the deterioration of respiratory function (i.e., airway obstruction and especially lung hyperinflation).

Pyrophosphate gel analysis separates myosin isoforms according to charge differences of the whole hexameric myosin molecule. This technique allowed us to identify two band groups in crude protein extracts from the human diaphragm: the first one was a fast-migrating group composed of three bands and the second was a slow-migrating group composed of, in most cases, a dark major band associated with a faint band migrating slightly ahead (some patients had a single dark band). By analogy with a variety of muscles from various mammalian species, these two band groups probably corresponded to fast and slow myosin isoforms, respectively (13, 14, 34). Analysis of necropsy samples from various areas of the costal diaphragm from a patient free of bronchopulmonary disease (data not shown) indicated that this band did not arise from postmortem myosin proteolysis and showed no significant difference in the myosin pattern throughout the normal human costal diaphragm.

The proportion of slow myosin is smaller in the diaphragm of the rat (13, 14) than in the patients studied here whose respiratory function was normal or close to normal. Because the ventilatory rate in the former species is higher than in humans, the myosin phenotype of diaphragm muscle may be related to it. This is reminiscent of the differences in cardiac myosin phenotype among mammalian species and of the relationships among the proportion of a given myosin species, myosin ATPase activity, and the maximum contraction velocity of the muscle, which have to be compatible with heart rate (6, 15). In the ventricles of small mammals, fast myosin, high myosin ATPase activity, and contraction velocity seem to be adapted to a high heart rate but at the expense of the efficiency of muscle contraction (1, 26). Conversely, in the ventricles of big mammalian species, the almost exclusive expression of slow myosin (9, 15) appears adapted to low heart rates and may be regarded as an adaptive evolutionary mechanism. In this respect, the larger proportion of slow myosin in the human diaphragm than in that of small rodents could be also interpreted as an evolutionary adaptation of the human diaphragm to a lower ventilatory rate.

PAGE in the presence of SDS and high glycerol concentrations can be used to separate MHC isoforms. In contrast to pyrophosphate gel electrophoresis, the fast-migrating band corresponds, in both human and rat diaphragms, to myosin composed of type I (\(\beta\)/slow) MHCs, whereas the slow-migrating bands correspond to myosins composed of fast MHCs. The fact that the
fast-migrating MHC isoform accounted for approximately one-half of the total MHC in samples from patients with normal or close-to-normal respiratory function is in keeping with the proportion of the slow-migrating band in pyrophosphate gel analysis of the corresponding muscle samples and confirms that the normal human diaphragm contains a balanced proportion of fast and slow MHCs in total myosin. Among the 20 patients whose diaphragmatic samples were subjected to the two electrophoretic procedures, we observed a strong positive linear correlation between the proportion of slow myosin in pyrophosphate gel electrophoresis and the proportion of type I (β/slow) MHC in SDS-glycerol PAGE, confirming that the slow-migrating band observed with the former technique corresponds to myosin composed of β-MHCs. This was further borne out by Western blot analysis because the fast-migrating MHC band reacted with monoclonal antibodies (BA-DS and BF-32) directed against β/slow MHCs and type I and IIa fibers in human muscle sections, respectively.

In contrast to β/slow MHC (fast-migrating band), SDS-glycerol PAGE and immunochemical analysis revealed marked differences in the pattern of fast MHCs (slow-migrating bands) between human and rat dia-

phragms. First, only two bands were detected in the human diaphragm compared with three in the rat diaphragm (14). This is due to the presence in the latter species of an additional band corresponding to an additional MHC, type Ix, which migrates between the two type II (a and b) MHCs (14). The two bands detected in the human diaphragm were type II MHCs because they both reacted with monoclonal antibody SC-75. In addition, monoclonal antibody BF-32 showed that the fastest migrating MHC component in the human diaphragm was type IIa, whereas the fastest migrating band in the rat diaphragm was type I (14). The low-mobility MHC band in human muscle was initially identified as type IIb (4, 12), but in situ hybridization combined with ATPase histochemistry and anti-MHC immunocytochemistry on serial sections (28) later indicated that human muscle fibers identified as type IIb (based on ATPase histochemistry) contained MHC transcripts homologous to rat type IIx MHC transcripts (7). On the other hand, type I and type IIa human muscle fibers contain transcripts homologous to rat β/slow and type IIa MHCs, respectively (28). The presence of type IIx-like mRNAs in human type IIb fibers has been confirmed by RT-PCR analyses on single muscle fibers (8). It is thus likely that the low-mobility MHC band in human muscle in fact corresponds to a type IIx MHC species rather than to a type IIb MHC species. The existence of a type IIb MHC in human skeletal muscle remains to be established.

The other important finding in this study was that the proportion of type I (β/slow) myosin increased in patients with severe COPD at the expense of type II (fast) isoforms. Although the role of decreased thyroid hormone plasma concentrations in this increased proportion of slow myosin cannot be totally ruled out, it seems most unlikely in stable well-nourished COPD patients in whom such a decrease has not been reported to date. Furthermore, the correlations we found between the proportion of slow myosin and the parameters of respiratory function do not support this hypothesis. Indeed, to get insights into the mechanisms responsible for the increased proportion of slow myosin, we looked for correlations between this proportion and the parameters of respiratory function. In this respect, it should be noted that our aim was not to assess diaphragm muscle contractile properties but rather to measure the parameters of respiratory function that may testify for the severity and duration of the increase in respiratory load and lung volume. By doing so, our goal was just to bring together, in patients with various degrees of airway obstruction and lung distension, a biochemical parameter involved in muscle adaptation to load and the parameters testifying for chronic increase in muscle load. Our hypothesis was that the latter alterations, which have been shown to chronically increase the load imposed on the diaphragm, will result in the need for a change in myosin phenotype as observed in the heart submitted to chronic hemodynamic overload (9, 17, 18). Although control and COPD patients alone did not allow us to observe significant correlations, the pooling of the two subject categories, by including a whole range of values of slow myosin percentages and functional parameters, allowed us to observe positive correlations between the proportion of slow myosin and both TLC and FRC and a negative correlation with FEV1. Although no absolute cause-to-effect relationship can be ascertained from our studies, these correlations suggested that the degree of the fast-to-slow myosin transition increases with lung dis-

tension and airway obstruction. In this respect, it is interesting to note that the strongest correlation was observed with FRC, which determines the length of the diaphragm before inspiration. As FRC increases, the diaphragm shortens, which diminishes its capacity to generate the driving pressure of the respiratory system (29).

In the present study, the fact that myosin subtype correlated with the reported functional parameters does not mean that the MHC phenotype determines these physiological parameters but rather that the higher the airway obstruction and lung distension, the higher the proportion of slow myosin. This myosin phenotype conversion is reminiscent of the changes in myosin phenotype in fast skeletal muscle facing unusual functional requirements (30) and those occurring in the ventricles of small rodents (18) and in human atria (9, 17) subjected to a chronic increase in the hemodynamic load. However, the precise nature of the triggering factor(s) involved in myosin phenotypic alteration in the flattened and/or overloaded human dia-

phragm remains to be elucidated. The shortening of diaphragm operating length, which results from its flattening in patients with COPD, and the resulting detrimental mechanical and energetical conditions under which the muscle has to cope with an increased respiratory load could be one of the triggers. However, we cannot exclude that other triggers are also responsible. For instance, changes in the frequency of stimula-
tion are also an important trigger for myosin phenotypic alteration in skeletal muscle (30). Culture of cardiac myocytes and isolated perfused hearts has been very fruitful in determining a number of triggers and neurohumoral factors involved in myocyte phenotypic changes during mechanical overload of the heart, pointing to the important role of myocyte stretch acting or not acting through an autocrine production of angiotensin II, depending on the developmental stage considered (11, 31). The use of differentiated skeletal muscle in tissue culture has also allowed the demonstration of the role of mechanical stimulation and the involvement of various growth factors and signal transduction pathways in skeletal muscle remodeling (20, 33). Further studies will be needed to determine which among these various mechanisms and pathways are operating in the chronically overloaded human diaphragm.

In conclusion, this study extends the concept of myosin plasticity to the diaphragm, the main muscle involved in the control of ventilatory function. Like other striated muscles, gene expression in the diaphragm can thus adapt to new functional requirements. Other possible changes such as hypertrophy of a specific fiber type (type I7) or modifications of diaphragm blood supply or capillary bed density in the overloaded diaphragm remain to be identified. However, the present data provide new information concerning the relationship between the functional properties of the human diaphragm and its biochemical and molecular composition. The changes observed at the molecular level in the human diaphragm of COPD patients are in-line with a previous physiological study (27) that found that diaphragmatic force is well preserved in severe COPD in a stable state. Taken together, our data and the physiological data previously described (27) raise the question of the usefulness of the therapeutic approach based on respiratory muscle rehabilitation currently used in COPD patients.

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