

## CELLULAR ADAPTATIONS IN THE DIAPHRAGM IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

SANFORD LEVINE, M.D., LARRY KAISER, M.D., JOHN LEFEROVICH, M.S., AND BORIS TIKUNOV, PH.D.

**ABSTRACT**

**Background** In patients with severe chronic obstructive pulmonary disease, the diaphragm undergoes physiologic adaptations characterized by an increase in energy expenditure and relative resistance to fatigue. We hypothesized that these physiologic characteristics would be associated with structural adaptations consisting of an increased proportion of less-fatigable slow-twitch muscle fibers and slow isoforms of myofibrillar proteins.

**Methods** We obtained biopsy specimens of the diaphragm from 6 patients with severe chronic obstructive pulmonary disease (mean  $\pm$ SE forced expiratory volume in one second,  $33\pm 4$  percent of the predicted value; residual volume,  $259\pm 25$  percent of the predicted value) and 10 control subjects. The proportions of the various isoforms of myosin heavy chains, myosin light chains, troponin, and tropomyosin were determined by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. We also used immunocytochemical techniques to determine the proportions of the various types of muscle fibers.

**Results** The diaphragm-biopsy specimens from the patients had higher percentages of slow myosin heavy chain I ( $64\pm 3$  vs.  $45\pm 2$  percent,  $P<0.001$ ), and lower percentages of fast myosin heavy chains IIa ( $29\pm 3$  vs.  $39\pm 2$  percent,  $P=0.01$ ) and IIb ( $8\pm 1$  vs.  $17\pm 1$  percent,  $P<0.001$ ) than the diaphragms of the controls. Similar differences were noted when immunohistochemical techniques were used to compare the percentages of these fiber types in the two groups. In addition, the patients had higher percentages of the slow isoforms of myosin light chains, troponins, and tropomyosin, whereas the controls had higher percentages of the fast isoforms of these proteins.

**Conclusions** Severe chronic obstructive pulmonary disease increases the slow-twitch characteristics of the muscle fibers in the diaphragm, an adaptation that increases resistance to fatigue. (N Engl J Med 1997;337:1799-806.)

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**T**WO decades ago, Roussos and Macklem<sup>1</sup> demonstrated that the diaphragm, the major inspiratory muscle, can become fatigued. Although the site of muscular fatigue can occur anywhere in the motor pathway between the cerebral cortex and the muscle fibers themselves, previous investigators have demonstrated that a particular type of fatigue — i.e., low-frequency fatigue — occurs at the level of the muscle cell itself.<sup>2,3</sup>

Bellemare and Bigland-Ritchie<sup>4</sup> and others<sup>5-9</sup> have demonstrated that various types of exercise can elicit low-frequency diaphragmatic fatigue in normal subjects. Surprisingly, there has been little mention of exercise-induced low-frequency diaphragmatic fatigue in chronic obstructive pulmonary disease (COPD). Indeed, Polkey et al.<sup>10</sup> recently reported that low-frequency diaphragmatic fatigue did not develop in patients with severe COPD during treadmill exercise to “exhaustion.”

Bellemare and Grassino<sup>11</sup> have shown that the diaphragms of patients with severe COPD expended more energy — as evaluated by the diaphragmatic time-tension index — than the diaphragms of normal subjects. We hypothesized that this combination of a chronic increase in energy expenditure and relative resistance to low-frequency fatigue (in the diaphragms of patients with severe COPD) would be associated with profound structural adaptations characterized by an increase in slow-twitch muscle characteristics (i.e., increased proportions of less-fatigable slow-twitch fibers and slow isoforms of the myofibrillar proteins).

**METHODS****Patients and Control Subjects**

We obtained biopsy specimens from the costal diaphragm of 6 patients with severe COPD (3 men and 3 women) who were undergoing lung-volume-reduction surgery and 10 control subjects (5 men and 5 women). We used two subgroups of controls. The first consisted of four subjects with a mild impairment in pulmonary function who were undergoing resection of solitary pulmonary nodules; in those subjects, biopsies were performed at the time of surgery. The second type consisted of six brain-dead organ donors in whom biopsies were performed at the time of organ harvest before circulatory arrest.

Since the brain-dead control subjects were nonsmokers who had no history of symptoms or signs of cardiopulmonary or neuromuscular disease before their neurologic catastrophes, we presumed that these subjects would have had normal pulmonary-function tests before the onset of their neurologic events. Moreover, since the interval between the onset of the neurologic events (in these subjects) and biopsy of the diaphragm was less than 24 hours, the myofibrillar protein composition of these diaphragms should not have changed during this period.<sup>12,13</sup>

From the Pulmonary and Critical Care Divisions, Philadelphia Veterans Affairs Medical Center, Allegheny University of the Health Sciences, and the University of Pennsylvania (S.L.); and the Division of Thoracic Surgery (L.K.) and the Pennsylvania Muscle Institute (S.L., J.L., B.T.), University of Pennsylvania — all in Philadelphia. Address reprint requests to Dr. Levine at the Pulmonary and Critical Care Division (111P), Veterans Affairs Medical Center, University and Woodland Aves., Philadelphia, PA 19104.

Informed consent for biopsies in both the patients and the controls who underwent surgery was obtained from each of these participants, and our protocol was approved by the institutional review boards of the Philadelphia Veterans Affairs Medical Center and the Hospital of the University of Pennsylvania. In contrast, for the brain-dead organ donors, informed consent was obtained from the family of each subject, and this portion of the protocol was approved by the human-studies committee at Columbia-Presbyterian Hospital, New York.

### Biopsies

Full-thickness biopsy specimens (approximately 20 to 25 mm by 6 to 8 mm; weight, 4 g) were obtained from the same region of the right anterior costal diaphragm lateral to the insertion of the phrenic nerve, frozen in isopentane, and then transferred to liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until being used.

### Overview of Biochemical Determinations

Myosin light chains were analyzed from preparations of purified myosin, whereas purified myofibrils were used for the measurement of all other myofibrillar proteins (myosin heavy chains, troponin, and tropomyosin).

### Preparation of Myofibrils and Purification of Myosin

Myofibrils were prepared according to the method of Solaro et al.<sup>14</sup> with the addition of a protease-inhibitor set (2 mM sodium azide, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM of sodium pyrophosphate, and 1  $\mu\text{g}$  each of leupeptin and pepstatin A per milliliter) in the homogenization buffer. To purify myosin, part of the myofibrillar suspension was dialyzed for five to six hours against 200 volumes of 20 mM TRIS-hydrochloric acid (pH 7.5), 0.5 M potassium chloride, 1 mM magnesium chloride, and 0.5 mM  $\beta$ -mercaptoethanol, and then subjected to a "bath-flow" procedure with a resin of phalloidin-stabilized, actin-coated Sepharose B prepared according to the method of Grandmont-Leblanc and Gruda.<sup>15</sup> This resin was first equilibrated against the dialysis buffer and then incubated for 30 minutes at  $25^{\circ}\text{C}$  with the myofibrillar solution in a ratio of 1 mg of myofibril per gram of resin. The resin was washed twice with 10 volumes of dialysis buffer, and the myosin was then diluted by 2 M potassium chloride. Some trace amounts of actomyosin were then precipitated with 35 percent ammonium sulfate, and the myosin was subsequently precipitated from the supernatant by increasing concentrations of ammonium sulfate (up to 50 percent). Protein concentrations were determined by Bradford's reaction.

### Sodium Dodecyl Sulfate-Polyacrylamide-Gel Electrophoresis

Myofibrils or purified myosin preparations were dialyzed overnight against 200 volumes of 100 mM TRIS-hydrochloric acid (pH 8.0) and 1 mM  $\beta$ -mercaptoethanol. The samples for sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE) were then prepared by diluting the proteins with Laemmli's buffer by a factor of 4 to 6 and then incubating them at  $95^{\circ}\text{C}$  for five minutes. SDS-PAGE for myosin heavy chains was performed according to the method of Talmadge and Roy,<sup>16</sup> which we adapted for use with human tissue by increasing the total running time to 32 hours and decreasing the voltage (i.e., 50 V for 1 hour, 100 V for 2 hours, and 220 V for 29 hours at  $8^{\circ}\text{C}$ ). The amount of protein loaded per lane was 0.15  $\mu\text{g}$  for silver-stained gels and 1.5  $\mu\text{g}$  for Coomassie-stained gels.

SDS-PAGE for myosin light chains, troponins, and tropomyosin was performed according to the method of Laemmli<sup>17</sup> on 15 percent polyacrylamide gels supplemented with 10 percent glycerol; 1.5  $\mu\text{g}$  of protein was loaded per lane. Coomassie-stained gels were scanned on a Pharmacia Ultrascan densitometer (LKB). The densitometric signal (i.e., peak absorption) for our 0.75-mm-thick gels was linear up to concentrations of 5  $\mu\text{g}$  of protein per lane.

### Two-Dimensional SDS-PAGE

Two-dimensional SDS-PAGE was performed as described by O'Farrell.<sup>18</sup> The sample preparation and running conditions were identical to those described previously.<sup>19</sup>

### Western Blot Analysis

Immunoblotting for myosin heavy chains was performed according to the method of Hughes et al.<sup>20</sup> The supernatants of all monoclonal antibodies against myosin heavy chains were obtained from the Developmental Studies Hybridoma Bank, with the exception of antibody BF-F3, which was kindly provided by Dr. Stefano Schiaffino.<sup>21</sup> We used F(ab)<sub>2</sub> anti-IgG and anti-IgM, peroxidase-conjugated goat antimouse secondary antibodies (Cap-pel). The bands were visualized with ECL peroxidase substrate (Amersham) and exposed to Kodak XAR-2 x-ray film.

### Immunocytochemical Analysis

The types of fiber were identified by indirect immunofluorescence with monoclonal antibodies specific for the following myosin heavy chains: NOQ7.54D for type I,<sup>22</sup> SC71 for type IIa,<sup>22</sup> and BF-F3 for type IIb.<sup>21</sup> Our staining protocols have been previously described.<sup>22,23</sup> We determined the proportions of the various types of fiber by counting the fibers on the serial 10- $\mu\text{m}$  sections, each stained with a specific antimyosin antibody. Approximately 200 fibers were counted per cross-section. We classified the fibers as type I, IIa, or IIb on the basis of the antibody that yielded maximal fluorescence on fluorescence antibody staining; therefore, our classification method did not assess the proportions of fibers that may have expressed more than one myosin heavy chain. The cross-sectional areas of the fibers were determined according to previously described methods.<sup>24</sup>

### Statistical Analysis

Quantitative data are described as means  $\pm$  SE. Group t-tests were used to evaluate the statistical significance of differences between groups.<sup>25</sup> Differences were attributed to chance unless they were significant at the 0.05 level.

## RESULTS

### Characteristics of the Study Subjects

The patients did not differ significantly from the control subjects with respect to age, height, or weight (Table 1). Pulmonary-function measurements were carried out only in the patients and the four control subjects who underwent surgery. The patients had greater residual volume, functional residual capacity, and total lung capacity than the control subjects, and the controls had higher values with respect to the forced expiratory volume in one second and the ratio of the forced expiratory volume in one second to forced vital capacity.

### Analyses of Myosin Heavy Chains

#### SDS-PAGE and Immunoblotting

As shown in Figure 1, SDS-PAGE revealed that isoform IIb is either absent or markedly diminished in the patients. In contrast, isoform I is more prominent in the patients than in the controls. When immunoblotting was performed with the monoclonal antibody A4.1025 (Fig. 2), the distribution of myosin heavy chains in both controls and patients was the same as that shown in Figure 1, since this mon-

**TABLE 1.** CHARACTERISTICS OF THE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND THE CONTROL SUBJECTS.

CHARACTERISTIC	CONTROLS (N=10)	PATIENTS (N=6)	P VALUE
	mean ±SE		
Age (yr)	49±7	59±4	0.30
Height (cm)	167±2	164±5	0.58
Weight (kg)	67±3	68±4	0.78
Spirometry*			
Forced expiratory volume in 1 sec			
Liters	2.00±0.29	0.95±0.19	0.01
Percent of predicted	69±3	33±4	<0.001
Forced expiratory volume in 1 sec:			
forced vital capacity (%)	70±4	38±3	<0.001
Lung volume (% of predicted)*			
Residual volume	111±14	259±25	0.002
Functional residual volume	100±8	194±19	0.006
Total lung capacity	89±8	145±7	0.001

\*Lung function was measured only in the patients and the four control subjects who underwent surgery. Spirometry and plethysmographic lung volumes were assessed with conventional techniques, and the values were compared with predicted values.<sup>26,27</sup>

oclonal antibody recognizes all myosin-heavy-chain isoforms. When monoclonal antibodies A4.951 and A4.974, which react specifically with myosin heavy chains I and IIa, respectively, were used, reactions were positive for both controls and patients. Immunoblotting with monoclonal antibody BF-F3, which is specific for isoform IIb, was strongly positive only in controls and was either absent or weakly positive in patients.

**Quantitation of Myosin-Heavy-Chain Isoforms**

We used Coomassie-stained densitograms to quantitate the proportions of the myosin-heavy-chain isoforms present in diaphragm-biopsy specimens from control subjects and patients with COPD. Control subjects had higher percentages of myosin heavy

chains IIa and IIb, whereas patients had a higher percentage of myosin heavy chain I (Table 2).

**Immunocytochemical Analysis**

**Qualitative Observations**

A comparison of cross-sections of diaphragm from a representative patient with COPD and a control subject (Fig. 3) showed that the patient had a higher proportion of type I fibers, a lower proportion of type IIa fibers, and no evidence of type IIb fibers. In addition, the cross-sectional areas of the fibers from the patient were smaller.

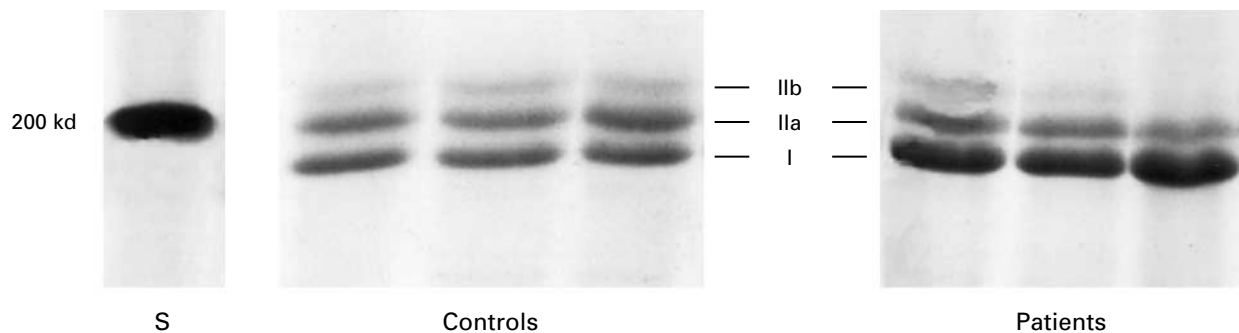
**Quantitative Observations**

As shown in Table 2, the patients with COPD had a higher percentage of type I fibers, whereas controls had a higher percentage of type IIb fibers. Most important, as can be seen from a comparison of the upper and lower portions of Table 2, the percentage of each of the principal types of fibers — I, IIa, and IIb — was markedly similar to the proportion of its corresponding myosin heavy chain in the costal diaphragm.

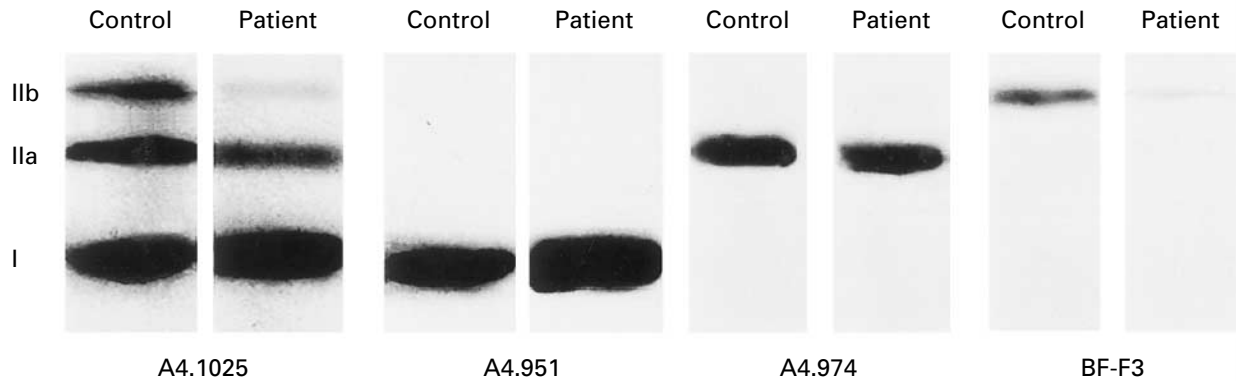
As illustrated in Figure 3, some of the fibers in specimens from both the patient and the control subject were sectioned obliquely. To eliminate these fibers from our comparisons of cross-sectional area, we determined the ratio between the major and minor axes of these elliptical fibers and did not include fibers in which this ratio exceeded 1.25 in our quantitative calculations. Nonetheless, for the three fiber types, the cross-sectional area of the fibers from the patients was 40 to 60 percent less than that of fibers from the controls.

**Composition of Myosin Light Chains**

The purified preparations of myosin from diaphragm-biopsy specimens from controls contained six types of myosin light chains (Fig. 4A, lane 3): 1s<sub>a</sub>, 1s<sub>b</sub>, 1f, 2s, 2f, and 3f (“s” and “f” designate slow



**Figure 1.** Representative Coomassie-Stained SDS-PAGE Gels, Showing the Distribution of the I, IIa, and IIb Isoforms of the Myosin Heavy Chain in the Costal Diaphragms of Three Control Subjects and Three Patients with COPD. A molecular-weight standard (S) is shown at the left.



**Figure 2.** Western Blot Analyses of the I, IIa, and IIb Isoforms of the Myosin Heavy Chain in the Costal Diaphragms of a Representative Control Subject and a Patient with COPD. The monoclonal antibodies used are shown.

and fast isoforms, respectively). Table 3 indicates that the patients had significantly higher percentages of myosin light chains 1s<sub>a</sub>, 1s<sub>b</sub>, and 2s, whereas the controls had higher percentages of 1f and 2f. The two-dimensional gel shown in Figure 4B illustrates these differences in the composition of myosin light chains more clearly than the one-dimensional gel shown in Figure 4A.

**Regulatory Myofibrillar Proteins**

Figure 4A shows the results for a representative patient and control, whereas Table 3 presents a statistical comparison of the percentages of different isoforms of tropomyosin and subunits of troponin in the two groups. As shown in Table 3, control subjects had a higher percentage of fast  $\alpha$ -tropomyosin,

whereas the patients had a higher percentage of slow  $\beta$ -tropomyosin. In addition, the patients had a higher percentage of troponin C<sub>s</sub>, whereas the controls had higher percentages of troponins T<sub>f</sub> and I<sub>f</sub>. The groups did not differ significantly with respect to the percentage of troponin C<sub>f</sub>.

**DISCUSSION**

Our results show that the diaphragms of patients with severe COPD have a higher proportion of type I (slow-twitch) fibers and a lower proportion of type II (fast-twitch) fibers than the normal diaphragm. These data are consistent with the hypothesis that severe COPD transforms fast-twitch fibers to slow-twitch fibers in the diaphragm.

**Is Myosin Heavy Chain IIx Expressed in the Human Diaphragm?**

Much work regarding adaptations of the diaphragm in COPD has been carried out in rats and hamsters, whose diaphragms and limb muscle contain a third fast-twitch myosin-heavy-chain isoform, designated IIx.<sup>16,21,29</sup> The occurrence of this IIx isoform in the human diaphragm has not been evaluated. Using SDS-PAGE gels and immunoblotting, multiple investigators have reported that human limb muscle contains only three myosin-heavy-chain isoforms: I, IIa, and IIb.<sup>30-32</sup> Our results in the diaphragm are consistent with these observations in limb muscle. Therefore, we and other workers have found no evidence of the expression of IIx in human diaphragm or limb muscle.

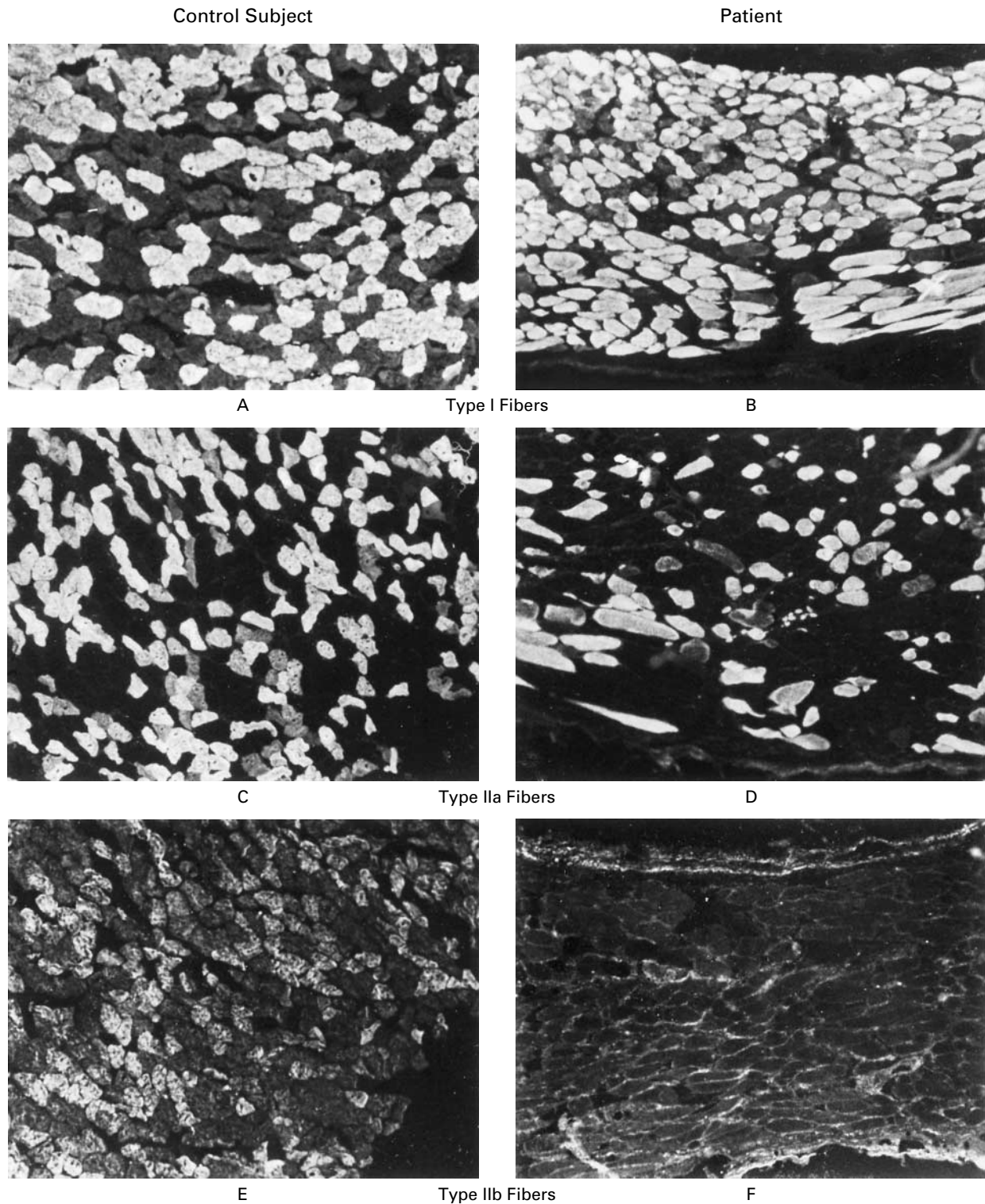
Recently, Smerdu et al.<sup>33</sup> carried out a series of experiments in human limb and trunk skeletal muscle using *in situ* hybridization techniques to detect messenger RNA and immunocytochemical techniques to type muscle fibers according to the expression of myosin heavy chains. They concluded that human muscle contained three principal fiber types (I, IIa,

VARIABLE	I	IIa	IIb
	percent		
Myosin heavy chains†			
Controls (n=10)	45±2	39±2	17±1
Patients (n=6)	64±3	29±3	8±1
P value	<0.001	0.01	<0.001
Type of fiber‡			
Controls (n=4)	46±3	39±2	15±1
Patients (n=4)	61±4	31±3	8±2
P value	0.01	0.09	0.01

\*Plus-minus values are means ±SE. An unpaired t-test was used to compute the statistical significance of the differences between groups. Because of rounding, not all categories total 100 percent.

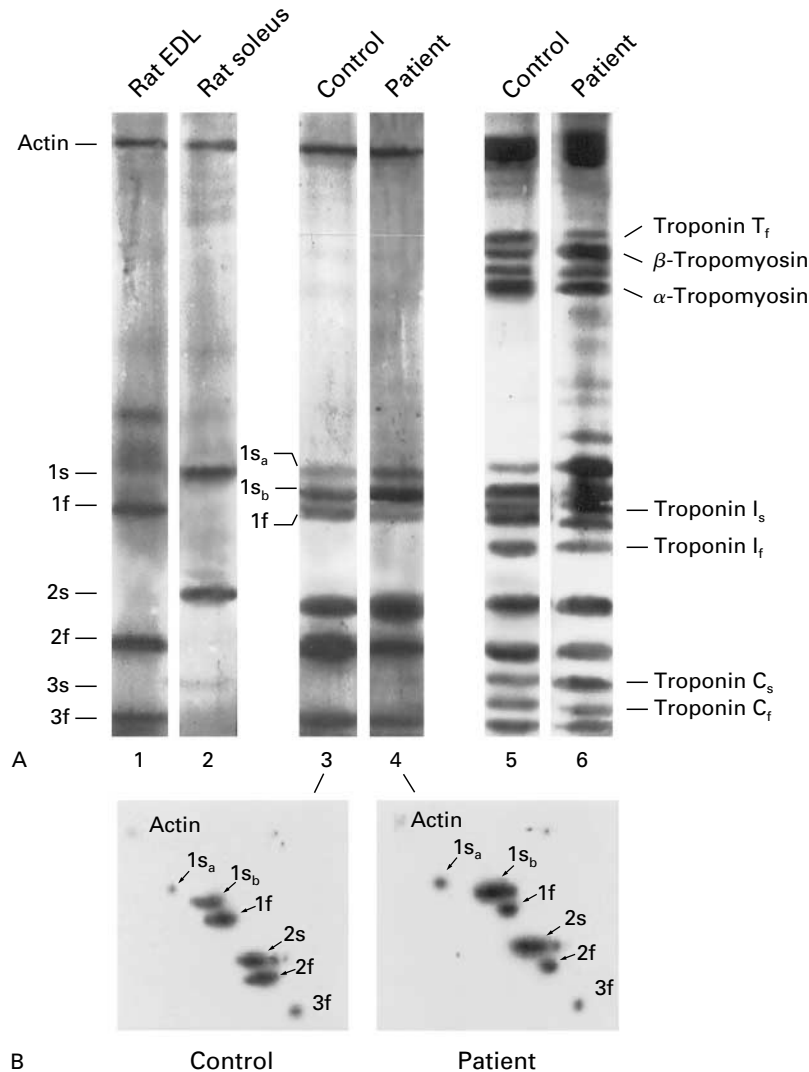
†Values were obtained by SDS-PAGE.

‡Values were obtained by immunohistochemical analysis.



**Figure 3.** Immunofluorescent Staining of Serial Sections of Costal Diaphragm from a Representative Control Subject and a Patient with COPD ( $\times 270$ ).

Cross-sections were preincubated with the following antibodies: NOQ7.54D antibody in Panels A and B, which is specific for myosin heavy chain I; SC71 antibody in Panels C and D, which is specific for myosin heavy chain IIa; and BF-F3 antibody in Panels E and F, which is specific for myosin heavy chain IIb. SDS-PAGE showed the following distribution of myosin heavy chains I, IIa, and IIb: 45, 41, and 14 percent, respectively, in control specimens and 70, 25, and 5 percent, respectively, in patient specimens.



**Figure 4.** Low-Molecular-Weight Region Identified by One-Dimensional SDS-PAGE (Panel A) and Two-Dimensional SDS-PAGE (Panel B).

Panel A shows the results of silver staining. Lanes 1 and 2 show purified myosin from rat extensor digitorum longus (EDL) and soleus, respectively. Lanes 3 and 4 show purified myosin from a representative control and a patient, respectively; and lanes 5 and 6 show purified myofibrils from the same control and patient. The bands were identified on the basis of the migration of proteins with known molecular weights and fast (f) and slow (s) myosin light chains of purified myosin from rat EDL and soleus, commercial preparations of tropomyosin from rabbit back muscle (Sigma, data not shown), and fast troponin isoforms isolated according to the method of Greaser and Gergely<sup>28</sup> from rabbit psoas muscle (data not shown). Panel B shows the results of staining with Coomassie blue.

and IIx), which express a single myosin-heavy-chain transcript, and two populations of hybrid fibers, which express either I and IIa or IIa and IIx myosin-heavy-chain transcripts. However, no specific antibody exists for the IIx myosin heavy chain, and thus, the results of their immunocytochemical fiber typing cannot be accepted unequivocally.<sup>21</sup>

Also, BF-35 is a monoclonal antibody that stains

all fibers except IIx.<sup>21</sup> This antibody stained all fibers in diaphragm-biopsy specimens from both control subjects and patients with COPD. We interpret this observation as ruling out the presence of pure IIx fibers in the human diaphragm. Therefore, we believe that more experimental work is necessary to clarify the status of the expression of IIx in the human diaphragm.

**TABLE 3.** DISTRIBUTION OF MYOSIN LIGHT CHAINS AND REGULATORY PROTEINS IN THE COSTAL DIAPHRAGM.\*

VARIABLE	CONTROLS (N=10)	PATIENTS (N=6)	P VALUE
	mean ±SE		
Myosin light chains (molar ratio)†			
1s <sub>s</sub>	3.7±0.5	5.5±0.6	0.03
1s <sub>f</sub>	14.7±1.6	19.3±1.1	0.05
1f	19.4±1.1	15.0±0.7	0.01
2s	26.3±2.3	34.2±1.3	0.02
2f	25.4±2.0	19.0±0.6	0.03
3f	10.2±0.9	7.3±1.8	0.14
Tropomyosin (%)‡			
α	5.4±0.3	3.4±0.4	0.003
β	5.2±0.3	6.9±0.3	0.01
Troponin (%)‡			
T <sub>f</sub>	4.9±0.3	2.9±0.3	<0.001
I <sub>f</sub>	4.2±0.3	2.7±0.3	0.002
C <sub>s</sub>	4.1±0.2	5.6±0.1	<0.001
C <sub>f</sub>	2.0±0.2	1.7±0.2	0.11

\*Slow and fast isoforms are denoted by s and f, respectively.

†The molar ratios were calculated by dividing the amount of the individual myosin light chains by their molecular weights, which were taken as 24,000, 23,000, 18,000, 19,000, and 15,000 for myosin light chain 1s, 1f, 2s, 2f, and 3f, respectively. Then, the sum of these values was set at 100 percent, and the molar ratio (expressed as a percentage) was calculated.

‡The percentages of tropomyosin and troponin subunits represent the areas of these protein bands relative to the total area (which was set at 100 percent) — that is, the sum of all the bands in the 10-to-45-kd region of our SDS-PAGE; this region contains bands corresponding to actin, myosin light chains, troponins, and tropomyosin.

Finally, with respect to the physiologic consequences of the supposition that all the IIB fibers in our diaphragm samples were really IIX fibers, previous workers have demonstrated that different fiber types classified on the basis of the composition of myosin heavy chains have different maximal velocities (with IIB having the highest velocity, IIX and IIA having intermediate velocity, and I having the lowest velocity) as well as different resistances to fatigue (with I being the most resistant, followed in descending order by IIA, IIX, and IIB).<sup>34</sup> Therefore, even if one suggests that all type IIB fibers in our human diaphragms were type IIX, the decreases in type IIX and the increases in type I fibers noted in the specimens from the patients would be consistent with our concept that severe COPD elicits an increase in slow-twitch characteristics of the human diaphragm.

#### Relation between the Severity of COPD and the Changes in the Fiber Types Present

The data of Sanchez et al.<sup>35,36</sup> indicate that moderate COPD is associated with atrophy of both type I and type II fibers, with no change in the relative proportions of these types of fibers. However, our

patients with COPD had more severe disease, manifested by greater abnormalities in spirometric measurements and appreciably greater hyperinflation. Our results suggest that only the diaphragms of patients with severe COPD show the switch from fast-twitch fibers to slow-twitch fibers.

#### Adaptations in Myosin Light Chains and Regulatory Proteins

Billeter et al.<sup>37</sup> have shown that in human skeletal muscle, fiber types IIA and IIB exclusively express myosin light chains 1f, 2f, and 3f. Similarly, fast forms of troponin and tropomyosin largely occur in type II fibers, whereas the slow isoforms of these proteins predominate in type I fibers.<sup>38,39</sup> Therefore, in view of these previous studies, the decreases in fast isoforms of both myosin light chains (i.e., 1f and 2f) and regulatory proteins (i.e., α-tropomyosin and troponins T<sub>f</sub> and I<sub>f</sub>) that we observed in the diaphragm-biopsy specimens from patients with COPD can be explained by the reduction in type II fibers in these diaphragms.

#### Differential Adaptations of Diaphragm and Limb Muscles

Jakobsson and coworkers<sup>40</sup> demonstrated that the proportion of type I fibers is decreased in the quadriceps muscle of patients with severe COPD. Therefore, the limb muscles and the diaphragm appear to adapt in a qualitatively different manner to severe COPD. Importantly, we recently concluded that severe congestive heart failure elicits similar differential adaptations in diaphragm and limb muscles.<sup>19</sup>

We believe that the difference in the relative activity between the diaphragm and limb muscle accounts for the above-noted differences in adaptations. First, the work of Bellemare and Grassino<sup>11</sup> indicates that the diaphragmatic time-tension index — a measure of diaphragmatic energy expenditure — is greatly increased, even during breathing at rest in patients with severe COPD. Second, Mancini et al.<sup>41</sup> have shown that this index is greater in patients with congestive heart failure than in control subjects during both rest and exercise. Therefore, the diaphragms of both patients with severe COPD and patients with congestive heart failure can be viewed as undergoing constant moderate exercise, and the adaptations that we noted in the diaphragms of our patients resemble those elicited by endurance training in limb muscles.<sup>42,43</sup>

By contrast, patients with severe COPD and congestive heart failure have a sedentary lifestyle, and the muscles of their arms and legs are therefore less active than those of normal subjects. In response to this decreased activity, the limb muscles adapt in ways that resemble those elicited by deconditioning: the proportion of slow-twitch fibers is decreased, and the proportion of fast-twitch fibers is increased.<sup>44</sup>

## Conclusions

Our data show that in patients with severe COPD, the proportion of slow-twitch fibers in the diaphragm increases, whereas the proportion of fast-twitch fibers decreases. There is also an increase in the slow myofibrillar-protein isoforms and a decrease in the fast isoforms. These adaptations may render the diaphragm more resistant to fatigue.

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