

Human Diaphragm Remodeling Associated with Chronic Obstructive Pulmonary Disease

Clinical Implications

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Diaphragm remodeling associated with chronic obstructive pulmonary disease (COPD) consists of a fast-to-slow fiber type transformation as well as adaptations within each fiber type. To try to explain disparate findings in the literature regarding the relationship between fiber type proportions and FEV₁, we obtained costal diaphragm biopsies on 40 subjects whose FEV₁ ranged from 118 to 16% of the predicted normal value. First, we noted that our exponential regression model indicated that changes in FEV₁ can account for 72% of the variation in the proportion of Type I fibers. Second, to assess the impact of COPD on diaphragm force generation, we measured maximal specific force generated by single permeabilized fibers prepared from the diaphragms of two patients with normal pulmonary function tests and two patients with severe COPD. We noted that fibers prepared from the diaphragms of severe COPD patients generated a lower specific force than control fibers ($p < 0.001$) and Type I fibers generated a lower specific force than Type II fibers ($p < 0.001$). Our finding of an exponential relationship between the proportion of Type I fibers and FEV₁ accounts for discrepancies in the literature. Moreover, our single-fiber results suggest that COPD-associated diaphragm remodeling decreases diaphragmatic force generation by adaptations within each fiber type as well as by fiber type transformations.

Keywords: chronic obstructive pulmonary disease; fiber type transformations; single fiber physiology; specific force; within-fiber type adaptations

In recent communications (1, 2), we reported that the diaphragms of patients with severe chronic obstructive pulmonary disease (COPD) exhibited a greater proportion of Type I fibers than did control subjects. However, other investigators (3–6) have reported that the diaphragms of subjects with less severe COPD do not differ from those of control subjects with respect to the proportion of Type I fibers. Therefore, we hypothesized that a curvilinear relationship exists between the proportion of diaphragmatic Type I fibers and the severity of COPD. In the present study, we tested this hypothesis.

We (1) and other investigators (3) have reported that in addition to the fast-to-slow fiber type transformations (2, 7), the diaphragms of patients with severe COPD exhibit adaptations

within each of the fiber types. We hypothesize that both of these processes result in decreases in force generation by the diaphragms of patients with severe COPD. In the present study, we present experiments pertaining to this latter hypothesis.

METHODS

Subject Characteristics

Our study cohort consisted of 24 females and 16 males who were either (1) patients undergoing resection of solitary pulmonary nodules—the results of pulmonary function tests of these subjects indicated normal to moderate obstructive disease (8); or (2) patients with heterogeneous emphysema undergoing lung volume reduction surgery—the pulmonary function measurements of these subjects showed severe COPD (8).

Informed consent for 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 (Philadelphia, PA).

Pulmonary Function Measurements

Before surgery, subjects underwent spirometry ($n = 40$) and measurement of lung volumes by plethysmography ($n = 25$) and values were compared with predicted normal values (9, 10). Prior workers (11, 12) have described artifactually high measurements of lung volumes in patients with COPD and our strategy for avoiding these errors is presented in the online supplement.

Diaphragm Biopsies

Full-thickness biopsies (15–25 mm long by 6–8 mm wide) were obtained from the same region of the right anterior costal diaphragm lateral to the insertion of the phrenic nerve. After allocating tissue bundles for single fibers (*see below*), we prepared the biopsy specimens for quantitative histochemistry (1, 2) as presented in the online supplement.

Determination of Fiber Type Proportion, Cross-sectional Area, and Area Fraction

As previously described (1, 2), we used immunohistochemistry in 28 subjects and myosin ATPase methodology in 12 subjects. In a subset of biopsies ($n = 8$), we compared these methodologies with respect to proportion, cross-sectional area, and area fraction; we noted no systematic differences between the two techniques. Our methodology for making these measurements is presented in the online supplement.

Single-fiber Maximal Isometric Force Analyses

Our technique for preparing single permeabilized fibers from the biopsy specimens (13, 14) as well as our methodology for carrying out measurements of maximal isometric force are presented in the online supplement (13, 14).

Statistics

Univariable analyses. In these analyses, we used our pulmonary function measurements as independent variables and our major dependent variables were the proportion, cross-sectional area (CSA), and area fraction of pure Type I fibers.

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We used a linear model as well as two curvilinear models to characterize our data. The two curvilinear models were (1) exponential growth or decay curves and (2) piecewise linear regression models. These models are fully described in the online supplement and we present data from these models in RESULTS.

Comparison of models with respect to goodness of fit. We used the following two calculations to quantitatively compare our three models with respect to “goodness of fit” to our data: (1) r^2 and (2) mean square error (i.e., residual variance). The formula for these computations is provided as a footnote to Table 3, which summarizes these comparisons. Briefly, the higher the r^2 value, the better the goodness of fit. Conversely, the lower the mean square residual error, the better the goodness of fit.

Multivariable analyses. We performed several multivariable analyses and our methodology is presented in the online supplement.

RESULTS

Vital Statistics

The vital statistics (age, height, weight, and body mass index) for our 40 subjects are shown in Table 1.

Pulmonary Function Measurements

The spirometry data in Table 1 show that the lowest quartile of our subjects—hereafter referred to as lowest spirometric quartile subjects—had FEV₁, FVC, and FEV₁/FVC values less than 22, 58, and 30%, respectively; therefore, all these subjects had severe COPD. In contrast, the highest spirometric quartile of our subjects—hereafter referred to as highest spirometric quartile subjects—had normal airway function manifest by FEV₁, FVC, and FEV₁/FVC values greater than 92, 94, and 76%, respectively.

Table 1 indicates that our lung volume measurements ranged from marked hyperinflation (highest quartile) to little, if any, hyperinflation (lowest quartile). All the patients in our highest quartile with respect to lung volumes were from our lowest spirometric quartile, whereas all the subjects in the lowest quartile with respect to lung volumes were from our highest spirometric quartile.

Diaphragm Biopsy Data

Representative data. The four panels of Figure 1 show immunocytochemically stained serial sections from a subject in the highest

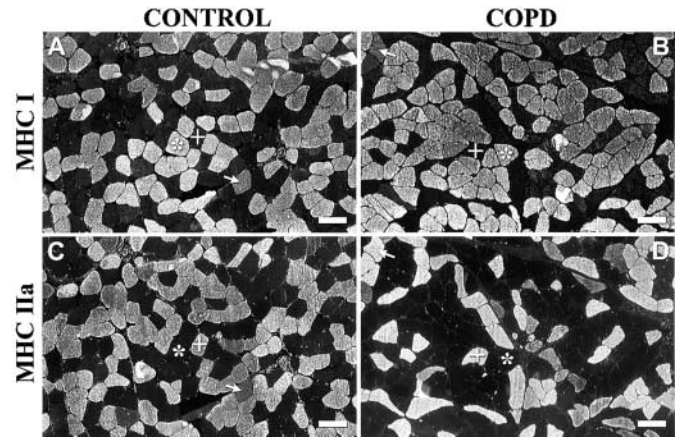


Figure 1. Immunocytochemical studies on cross-sections of diaphragm biopsies. (A and C) Serial sections from a subject in the highest spirometric quartile (i.e., a control subject having normal pulmonary function measurements); (B and D) serial sections from a subject in the lowest spirometric quartile (i.e., all patients in this quartile had severe chronic obstructive pulmonary disease [COPD]). Sections in (A) and (B) were preincubated with NOQ7.5.4D antibody, which is specific for slow (i.e., Type I) myosin heavy chain (MHC), whereas sections in (C) and (D) were preincubated with SC-71 antibody, which is specific for Type IIa MHC. The gray-staining fibers in each of the panels indicate positive reactivity with the particular antibody. This figure shows that the diaphragms of both subjects contain three types of fibers: pure Type I fibers that express only MHC I (asterisks), Type IIa fibers that express only MHC IIa (crosses), and hybrid fibers that express both MHC I and IIa (arrows). See the online supplement for additional details. Calibration bars represent 100 μ m.

spirometric quartile (Figures 1A and 1C) and a subject in the lowest spirometric quartile (Figures 1B and 1D). Inspection of the four panels indicates that virtually all the fibers in each of the subjects express either Type I or IIa myosin heavy chain (MHC). However, a small number of fibers (i.e., hybrid fibers)

TABLE 1. VITAL STATISTICS AND PULMONARY FUNCTION MEASUREMENTS*

Measurement [†]	Median	Percentile		Range	Mean
		25th	75th		
Vital statistics					
Age, yr	59	54	63	25–86	59
Height, cm	166	163	173	152–183	167
Weight, kg	67	62	79	56–102	71
BMI, kg/m ²	24	23	27	19–34	26
Spirometry, % predicted					
FEV ₁	42	22	92	16–118	56
FVC	75	58	94	45–123	77
FEV ₁ /FVC, % [‡]	52	30	76	22–99	55
Lung volume, % predicted					
RV	199	122	237	72–282	181
FRC	157	104	178	74–220	147
TLC	126	111	135	85–148	122
RV/TLC, % [‡]	58	43	65	22–75	55

Definition of abbreviations: BMI = body mass index; RV, residual volume; TLC, total lung capacity.

* In this study, 40 subjects had spirometric measurements and 25 subjects had lung volume measurements. The data of Knudson and coworkers (10) and the data of Kanner and coworkers (9) were used for predicted normal values of spirometry and lung volumes, respectively.

[†] For each of the measurements, we used the following descriptors to characterize our data: (1) median—the value that divides the data set into two halves; (2) 25th percentile—the value that separates the lowest quartile from the remainder of the data set; (3) 75th percentile—the value that separates the highest quartile from the remainder of the data set; (4) range—the set of values between the upper and lower boundaries of the data set, and (5) mean (i.e., average) value for the entire range of data.

[‡] FEV₁/FVC and RV/TLC were calculated as the ratio of their respective values measured in liters times 100.

TABLE 2. DIAPHRAGMATIC FEATURES

Measurement	Median	Percentile		Range	Mean
		25%	75%		
Proportion of pure I fibers, %	58	47	69	33–88	59
CSA of pure I fibers, μm^2	3,400	2,700	4,100	1,900–7,900	3,600
CSA ratio (pure I/all other types*)	1.09	0.94	1.29	0.75–2.28	1.16
Area fraction of pure I fibers, %	60	53	68	33–89	61

Definition of abbreviation: CSA = cross-sectional area.

* CSA ratio was computed as the average CSA of pure I fibers divided by the average CSA of all other types of fiber.

See ¹ footnote to Table 1 for definition of each of our statistical descriptors.

express both Types I and IIa MHC. Hereafter, fibers expressing only Type I MHC are referred to as “pure I” fibers, whereas the combined group of fibers expressing only Type IIa MHC and hybrid fibers are referred to as “all other” fibers.

Figures 1A and 1C indicate that the proportions of pure I fibers and all other fibers are approximately equal. In addition, the relative contributions of pure I fibers and all other fibers to diaphragm cross-sectional area (i.e., area fraction) are also approximately equal. In contrast, Figures 1B and 1D indicate that both the proportion and area fraction of pure I fibers are greater than those of all other fibers in this lowest spirometric quartile biopsy.

Diaphragm biopsy summary data. Table 2 presents a tabular summary of our entire cohort with respect to the following diaphragmatic features: (1) proportions of pure I fibers, (2) CSA of pure I fibers, (3) CSA ratio (the average CSA of pure I fibers divided by the average CSA of all other fibers); and (4) area fraction of pure I fibers. The data in Table 2 show that our diaphragm biopsies exhibited a wide range of values with respect to these features.

Relationship between Proportion of Pure I Fibers and Pulmonary Function Measurements

Figure 2 shows the relationship between percent pure I fibers versus FEV₁ (Figure 2A) and RV (Figure 2B), whereas Figure E3 (see the online supplement) shows the relationship between pure I fibers and the following pulmonary function measurements: FEV₁/FVC, FRC, RV/TLC, and TLC. In all these graphs, the distribution of open symbols (females) and gray symbols (males) suggests that sex does not affect the relationships between the proportion of pure I fibers and our pulmonary function measurements.

Exponential models. The solid black lines in Figures 2 and E3 (see the online supplement) represent exponential regression lines; the *r* and *p* values in each panel are those for the exponen-

tial relationship between percent pure I fibers and the pulmonary function measurement shown in the panel. Inspection of these regression lines indicates that with the exception of the FEV₁ relationship (Figure 2A), these regression lines do not differ appreciably from straight lines. In contrast, Figure 2A shows that as FEV₁ decreases from 100 to 60% of predicted normal, there is little, if any, increase in percent pure I fibers; however, further decreases in FEV₁ are accompanied by appreciable increases in the percentage of pure I fibers.

Piecewise linear regression. Figure 2 and Figure E3 (see the online supplement) present our piecewise linear regression analysis of the relationship between percent pure I fibers and pulmonary function measurements. In each panel of these graphs, we show a regression line that has a slope that does not differ statistically from zero as a dotted line; hereafter, we refer to these lines as zero slope lines. The individual patient points that contributed to these zero slope lines are shown as triangles. The dashed lines (in each panel) show the second regression line that has a slope that is statistically greater than zero; hereafter, we refer to these lines as finite slope lines. The individual points that contributed to this line are shown as circles. Table E1, Part C (see the online supplement) contains the equation for each of the zero slope lines and for each of the finite slope lines. Because the TLC data (see Figure E3D in the online supplement) required virtually all the data points to generate a line with a statistically significant slope, we were not able to use the piecewise technique for this relationship.

Comparison of linear, exponential, and piecewise regression analysis with respect to goodness of fit. Table 3 as well as Table E2 (see the online supplement) indicate that each of our curvilinear regression models (i.e., exponential and piecewise) exhibited a better fit than the linear model.

Relationship Between Fiber Cross-sectional Area and Pulmonary Function Measurements

CSA comparison of pure I versus all other fibers. The mean for pure I fibers was $3,600 \pm 200 \mu\text{m}^2$ and the mean for all other

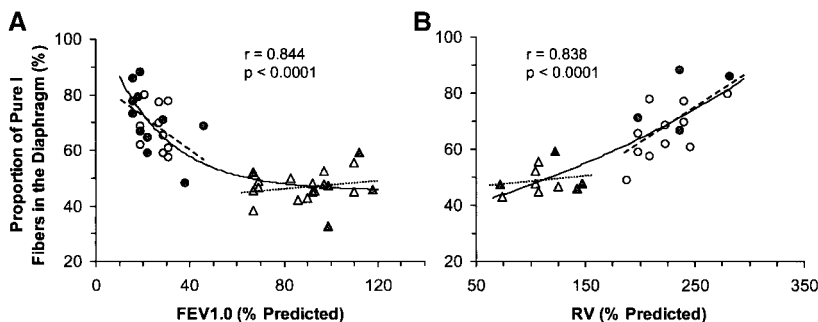


Figure 2. Relationships between the proportion of pure Type I fibers versus FEV₁ (A) and RV (B). Each of these pulmonary function measurements is expressed as a percentage of the predicted normal value (9, 10). In each of the panels, the exponential regression line is shown as a solid line and the correlation coefficient (*r*) and its *p* value are presented. In addition, the finite slope line and zero slope line of the piecewise linear regression model are shown as dashed and dotted lines, respectively. The circles represent data points contributing to the finite slope lines and the triangles represent data points contributing to the zero slope lines. In addition, the open symbols indicate female subjects, whereas the gray symbols denote male subjects.

TABLE 3. COMPARISON OF r^{2*} ASSOCIATED WITH LINEAR, EXPONENTIAL, AND PIECEWISE LINEAR REGRESSION MODELS RELATING PROPORTION AND AREA FRACTION OF PURE TYPE I FIBERS TO PULMONARY FUNCTION MEASUREMENTS

Measurement	Linear		Exponential ^f		Piecewise r^2
	r^2	p Value	r^2	p Value	
Percent pure I fibers versus pulmonary function measurements					
FEV ₁	0.607	< 0.0001	0.712	< 0.0001	0.721
FEV ₁ /FVC	0.631	< 0.0001	0.642	< 0.0001	0.652
RV	0.685	< 0.0001	0.703	< 0.0001	0.733
FRC	0.598	< 0.0001	0.616	< 0.0001	0.615
RV/TLC	0.480	0.0001	0.489	< 0.0001	0.529
TLC	0.347	0.0019	0.338	0.0023	–
Area fraction pure I fibers versus pulmonary function measurements					
FEV ₁	0.372	< 0.0001	0.465	< 0.0001	0.512
FEV ₁ /FVC	0.465	< 0.0001	0.473	< 0.0001	0.515
RV	0.447	0.0003	0.458	0.0002	0.633
FRC	0.320	0.0038	0.332	0.0031	0.551
RV/TLC	0.296	0.0049	0.30	0.0046	0.515
TLC	0.140	0.0657	0.137	0.069	–

For definition of abbreviation, see Table 1.

* r^2 was computed as the ratio of the sum of squares due to regression divided by the total sum of squares.

^f For the FEV₁ relationships, the exponential regression model had the form $y = ae^{(bx)} + c$; all other exponential relationships had the form $y = ae^{(bx)}$ (see METHODS for details).

fibers was $3,300 \pm 200 \mu\text{m}^2$; this difference was significant at the 0.02 level.

Relationship of fiber CSA to pulmonary function measurements. Table 2 shows statistical descriptors for the CSA of pure I fibers; this CSA had statistically significant positive correlations with FEV₁ and FEV₁/FVC, whereas it had statistically significant negative correlations with RV and RV/TLC (see Figure E4 in the online supplement). In contrast, the average CSA of all other fibers showed no statistically significant correlations with any of our pulmonary function measurements (see Figure E5 in the online supplement). Figure E6 and the accompanying text in the online supplement present the relationships between the CSA ratio (i.e., average CSA of pure I fibers to average CSA of all other fibers) and pulmonary function measurements.

Relationship between Area Fraction of Pure Type I Fibers and Pulmonary Function Measurements

Figure 3 shows the relationship between the area fraction of pure I fibers versus FEV₁ (Figure 3A) and RV (Figure 3B), respectively, whereas Figure E7 (see the online supplement) shows the relationships between area fractions of pure I fibers and the following pulmonary function measurements: FEV₁/FVC, FRC, RV/TLC, and TLC.

Exponential models. Figure 3 and Figure E7 have the same format as Figure 2 and Figure E3 (see the online supplement). Inspection of these regression lines indicates that with the exception of the FEV₁ relationship, they do not differ appreciably from straight lines. In contrast, Figure 3A shows that as FEV₁ decreases from 100 to 60% of predicted normal, the regression line indicates that there is little, if any, increase in area fraction of pure I fibers; however, further decreases in FEV₁ are accompanied by appreciable increases in area fraction of pure I fibers. Therefore, these relationships between area fraction of pure I fibers and our pulmonary function measurements are similar to those noted for the relationships between the percent pure I fibers and pulmonary function measurements.

Piecewise linear regression. Figure 3 and Figure E7 (see the online supplement) present our piecewise regression analysis for the area fraction of pure I fibers versus pulmonary function measurements; the symbols and lines in these figures follow the same convention as those used in Figure 2 and Figure E3 (see the online supplement). However, we do not show these data for TLC (see Figure E3D in the online supplement) because the relationship between area fraction of pure Type I fibers and TLC was not statistically significant even when all the data points were used for the finite slope line.

Comparison of linear, exponential, and piecewise linear regression analyses with respect to goodness of fit. Table 3 and Table E2 (see the online supplement) indicate that each of our curvilinear models exhibited a better goodness of fit than the linear model.

Multivariable Analyses

Tests for the possibility that our control variables affected the relationship between predictor and outcome variables. We noted that our control variables (i.e., age, sex, or body mass index) had neither a confounding nor interactive effect on the relationship between predictor variables (i.e., FEV₁ or RV) and major outcome variables (i.e., proportion, CSA, and area fraction of pure Type I fibers).

Relative effects of chronic airflow obstruction and hyperinflation in predicting changes in the proportion and area fraction of pure Type I fibers. Because of the high correlation between our measurements of airflow obstruction and hyperinflation, the multiple regression technique failed to provide us with appropriate data. See the online supplement for discussion of this result.

Single-fiber Measurements

We performed measurements of maximal isometric specific force on single permeabilized fibers prepared from the diaphragms of two subjects from the lowest spirometric quartile and two sub-

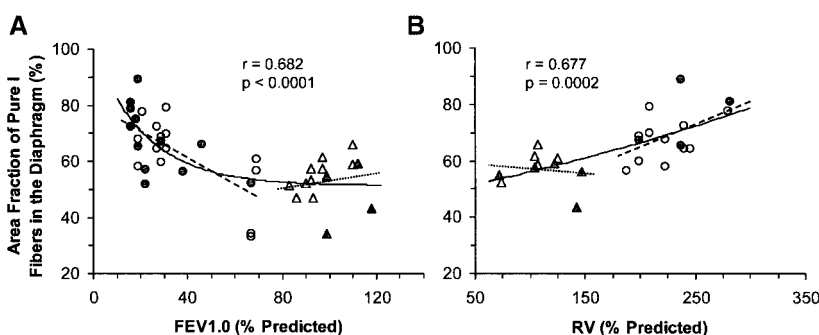


Figure 3. Relationships between the area fraction of pure Type I fibers versus FEV₁ (A) and RV (B). See Figure 2 for definition of symbols and lines.

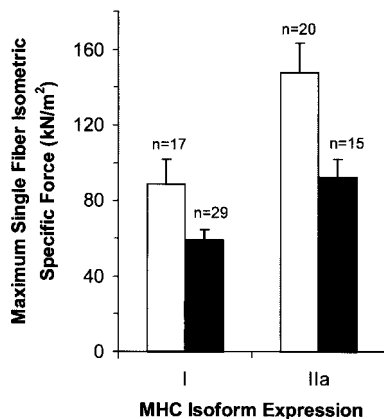


Figure 4. Single-fiber maximal isometric specific force measurements for Type I and IIa fibers prepared from diaphragms of two subjects in the lowest spirometric quartile (i.e., severe COPD) and two subjects from the highest spirometric quartile (i.e., control subjects having normal pulmonary function tests). *Open columns* represent control fibers, whereas *solid columns* represent severe COPD fibers. The *n* value above each *bar* represents the

total number of fibers analyzed and the data are presented as means \pm SEM. A two-way analysis of variance revealed both a statistically significant fiber type effect ($p < 0.001$) and a disease group effect ($p < 0.001$)—see text for more details.

jects from the highest spirometric quartile. Figure 4 shows the number of fibers of each type used in these experiments as well as the results of these experiments. Our two-way analysis of variance (15) indicated no group-by-fiber type interaction. However, the analysis of variance indicated two highly statistically significant group effects. First, fibers from the severe COPD diaphragms generated less specific force than those from control diaphragms ($p < 0.001$). Second, Type I fibers generated less specific force than Type IIa fibers ($p < 0.001$).

DISCUSSION

Summary of Major Findings

Our experimental cohort exhibited a wide range of severity of COPD (ranging from none to severe) and wide ranges of proportions and area fractions of pure I fibers. Our curvilinear regression models indicate that variation in FEV₁ can account for greater than 51% of the variation in both the proportions and area fractions of pure I fibers. Using single permeabilized fibers prepared from the diaphragms of two subjects in our lowest spirometric quartile (i.e., severe COPD) and two subjects in our highest spirometric quartile (i.e., normal spirometry), we noted that severe COPD fibers generated a lower specific force than did control fibers ($p < 0.001$) and Type I fibers generated a lower specific force than did Type IIa fibers ($p < 0.001$).

Critique of Methodology

First, by classifying all of our immunocytochemically determined fibers as pure I or “all other” fibers, we neglected the contribution of hybrid fibers (i.e., those containing both Types I and IIa MHC) to fiber type transformations. The reasons we chose this approach are as follows: (1) the ATPase methodology (used in 12 of our subjects) cannot detect these fibers (16); and (2) our immunocytochemical methodology does not provide us with information about the relative proportions of Type I MHC and the other MHC isoforms in hybrid fibers. Therefore, the major reason for using pure I fibers for our two indices of COPD-associated diaphragm remodeling is that we were able to accurately measure the proportion of pure I fibers in all of our 40 subjects. In addition, the number of hybrid fibers in the diaphragms of our 28 subjects who underwent fiber typing by immunocytochemistry was small—that is, the mean (\pm SEM) proportion of hybrid fibers was $4 \pm 1\%$. Nonetheless, we examined the relationship between all Type I fibers (i.e., the sum of pure I fibers and hybrid fibers) and FEV₁

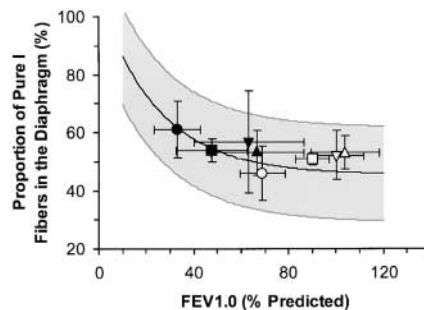


Figure 5. The *black line* and *gray lines* show the mean and the 90% confidence limits, respectively, for the exponential relationship (computed from the data in Figure 2) between the proportion of pure Type I fibers and FEV₁. *Upward- and downward-pointing triangles* depict the two studies by Sanchez and coworkers (5, 6), *squares* indicate the data of Orozco-Levi and coworkers (4), and *circles* indicate data from our initial study in this area (7) (these latter data are not included in RESULTS or in computations of the 90% confidence limits). For each of the studies, *open symbols* indicate control group values, whereas *solid symbols* show data from the COPD group.

in the 28 patients who underwent fiber typing by immunocytochemistry. We noted that the relationship between all Type I fibers and FEV₁ was similar to that noted between pure I fibers and FEV₁. This latter finding indicates that the use of either “pure I fibers” or “all Type I fibers” does not appreciably affect our assessment of the relationships between the proportion and area fraction of pure I fibers versus FEV₁.

Second, Orozco-Levi and coworkers (4) demonstrated that diaphragm fibers from patients with COPD are more prone to injury than are fibers from control diaphragms. Their observation raises the possibility that fibers from COPD diaphragms might generate less force because of either *in vivo* injury or injury during the preparation of our permeabilized single fibers. This is a well-recognized problem with the single fiber preparation (17). On being placed in activating solution, the sarcomeres from fibers that do not contain injured segments will contract isometrically in a uniform manner; therefore, the sarcomere striation pattern—as monitored by laser diffraction—will remain homogeneous. In contrast, when fibers containing damaged segment(s) are placed in activating solution, the sarcomeres in the injured segments will lengthen, whereas the sarcomeres from the noninjured segments will contract. This nonuniform contraction will result in heterogeneity of sarcomere spacing. All the single fiber force measurements reported in this study were obtained from fibers that exhibited homogeneity of sarcomere spacing during contraction. Therefore, we can be certain that damage to myofibers from the severe COPD diaphragms (i.e., either *in vivo* or *in vitro*) cannot account for the decreased specific force measurements noted in our COPD fibers.

Comparison of Our Diaphragm Results with the Literature

Fiber type proportions. Figure 5 presents the 90% confidence limits—computed from our data—relating percent pure I fibers to FEV₁; it shows that the means of all studies in the literature fall within our 90% confidence bands. Figure E8 (see the online supplement) presents the 90% confidence limits (computed from our data) for the relationship between percent Type I fibers and pulmonary function measurements—other than FEV₁—as well as data from studies in the literature regarding these relationships. We interpret all these graphs as being consistent with the hypothesis that a curvilinear relationship exists between the proportions of Type I fibers and our pulmonary function measurements.

Cross-sectional areas. Our CSA observations differ from those of Sanchez and coworkers (5), who noted a decrease in CSA of both Type I and II fibers. However, these workers (6) noted that the CSA of both fiber types exhibited statistically significant positive correlations with the ratio of observed to predicted body weights. These latter relationships raise the possibility that some aspect of undernutrition accounted for the decreases in CSA reported by Sanchez and coworkers (18, 19). Because our subjects exhibited no history of weight loss and no signs of undernutrition, this mechanism cannot account for the decreases in CSA of pure I fibers noted in our patients with severe COPD.

Area fractions. Despite a thorough search of papers containing human diaphragm biopsies and pulmonary function measurements consistent with COPD (1–7, 20, 21), we could not find suitable data to test our hypothesis regarding a curvilinear relationship between area fraction of pure I fibers and pulmonary function measurements.

Relationship between the Proportion of Slow Myosin Heavy Chain Isoform and Pulmonary Function Measurements

Mercadier and coworkers (21) demonstrated a statistically significant linear relationship between the proportion of the slow MHC isoform and some pulmonary function measurements (i.e., FEV₁, FRC, and TLC); however, these authors explicitly state that they did not test any nonlinear model for goodness of fit. Therefore, we compared linear and nonlinear (i.e., exponential) regression models with respect to goodness of fit, using the following data sets: (1) Mercadier and coworkers (21), (2) prior work from our group (2, 7), and (3) the combined data of Mercadier and coworkers and our group. All these analyses indicated that linear models were better than the nonlinear model with respect to goodness of fit (see Table E3 in the online supplement). Figure 6 shows the relationship between the combined data of Mercadier and coworkers (21) and those of our group (2, 7) with respect to these relationships.

Comparison of Different Diaphragmatic Adaptations with Respect to Severity of COPD

Because the relationship between percent slow MHC isoform and FEV₁ is linear (see Figure 6), whereas the relationships between both the proportions and area fractions of pure I fibers versus FEV₁ are curvilinear (see Figures 2 and 3), our data suggest that increases in percent slow MHC isoform will occur at a higher level of FEV₁ than increases in either the proportion or area fraction of pure I fibers. This point is illustrated in Figure E9 (see the online supplement). Mechanistically, these data suggest that a decrease in the concentration of MHC in Type II fibers occurs before the Type II to Type I fiber type transformation associated with increasing severity of COPD.

Relationship between COPD-associated Diaphragm Remodeling and Fatigability

Because of technical difficulties, we do not have physiological data showing that tissues strips from severe COPD diaphragms (i.e., lowest quartile with respect to spirometry) have greater fatigue resistance than strips from control diaphragm (i.e., highest quartile with respect to spirometry—all had normal values). Even in the absence of direct evidence, we believe that severe COPD diaphragms exhibit increased resistance to fatigue at the myofiber level for the following reasons: first, muscles that undergo transformations characterized by an increase in the proportion and area fraction of Type I fibers exhibit an increase in fatigue resistance (22, 23). Second, for any given fiber type, we (1) have previously demonstrated that fibers from severe COPD

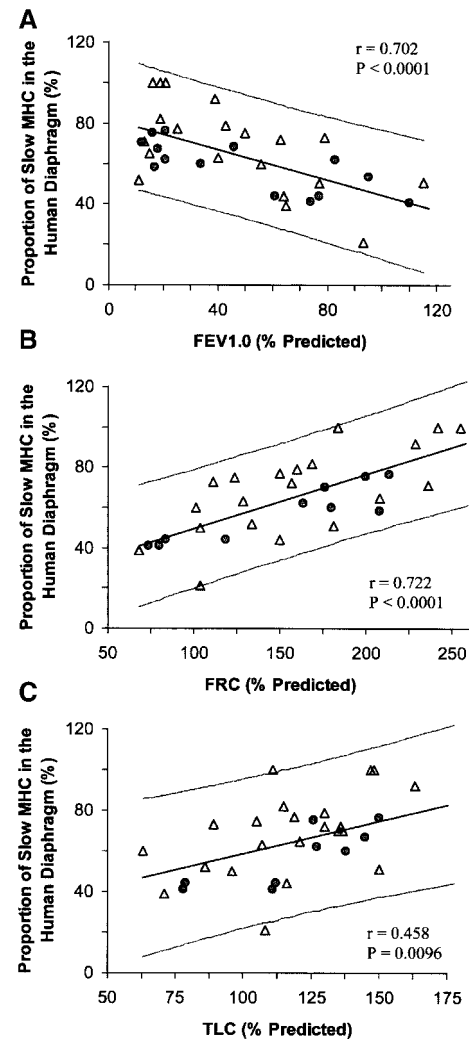


Figure 6. Relationship between the proportion of slow myosin heavy chain isoform from human diaphragms and the following three pulmonary function measurements: (A) FEV₁, (B) FRC, and (C) TLC. In each panel, we show the mean and the 90% confidence limits. In all panels, triangles ($n = 20$) represent data published by Mercadier and coworkers (21), whereas circles represent data from our laboratory (2, 7) (i.e., 14 data points in panel A and 10 data points each in panels B and C). Additional analyses of these data are summarized in Table E3, Part I (see the online supplement).

diaphragms have higher ratios for the maximal rate of succinate oxidation to the maximal rate of MHC ATP utilization (i.e., succinate dehydrogenase:myofibrillar ATPase activity ratio) than fibers from control diaphragms. Because previous workers (24, 25) have demonstrated that the succinate dehydrogenase:myofibrillar ATPase ratio is the best biochemical predictor of myofiber fatigue resistance, our prior observations suggest that fibers from severe COPD diaphragms of a given fiber type are more fatigue resistant than those (of the same fiber type) from control diaphragms.

Maximal Isometric Force Generation

Experimental preparation. The permeabilized single fiber preparation allows one to bypass the sarcolemma, t-tubules, and sarcoplasmic reticulum to directly elicit calcium-induced changes in the regulatory proteins (i.e., troponin subunits, tropomyosins) that permit force generation by the interaction of the MHC head

with actin. At high concentrations of calcium—such as that used in our activating solution (i.e., 10^{-4} M)—the MHC cross-bridges are virtually the only determinant of force generation (26, 27). The determinants of maximal isometric force are summarized in Equation (1):

$$\text{Force} = n \times F_m \times \alpha F_s \quad (1)$$

where n is the number of cycling MHC cross-bridges, F_m is the average force per attached cross-bridge in the force generating state, and αF_s is the fraction of cross-bridges in the force-generating state. To compare myofibers of different cross-sectional area with respect to force generation, force measurements are expressed per unit area (i.e., specific force).

Data in literature before present studies.

Specific force of COPD fibers less than specific force of control fibers. Because of the increases in mitochondrial volume fraction in all fiber types of severe COPD diaphragms (1), fibers from these diaphragms should contain a lower myofibrillar volume fraction than fibers from control diaphragms of the same fiber type (28). This aspect of within-fiber type remodeling should decrease the value of n in severe COPD fibers and thereby account for a decrease in maximal isometric specific force; that is, in Equation (1), the value of n for COPD fibers of any given type should be less than the value of n for control fibers of that type.

Specific force of Type I fibers less than specific force of Type II fibers. The rat diaphragm data of Geiger and coworkers (14) indicate that the force per unit mass of myosin heavy chain per half-sarcomere—the fundamental contractile unit in muscle—is less in Type I fibers than in Type II fibers. Because these authors demonstrated that the fraction of cross-bridges in the force-generating state (αF_s) is the same for Type I and Type II fibers, Equation (1) indicates that decreases in F_m and/or decreases in n account for the lower specific force generation by Type I fibers. We would expect this greater force generation by Type II fibers to be present in both COPD and control diaphragms.

Limitations of our data. Our single fiber observations that COPD fibers generate less specific force than control fibers, and that Type I fibers generate less specific force than Type IIa fibers, are totally in accord with our hypothesized results. However, these experiments were performed only on permeabilized fibers prepared from the diaphragms of two subjects with severe COPD and two control subjects. The highly significant results shown in Figure 4 have the implicit assumption that for each of these comparisons, between-subject differences were appreciably less than between-group differences. Because we cannot obtain an accurate assessment of between-subject variability with only two subjects in each group, we consider our single fiber results to be “hypothesis generating” and not “hypothesis testing.” Nonetheless, we believe that the combination of our single fiber results and the strong theoretical basis for predicting these results mandate repetition of these experiments with an adequate number of subjects (in each group) to characterize between-subject as well as between-group variability.

Clinical implications. Assuming that the specific forces of our 2 subjects with severe COPD and our 2 control subjects are representative of all 10 subjects in the lowest and highest spirometric quartiles, respectively, we calculated the average maximal diaphragm muscle-specific force for each of these 20 subjects as the product of the average fiber type-specific force for the group (computed from the measurements on the 2 subjects from that group) and the area fractions for each of the individual subjects. The details of this calculation are presented in the online supplement. Using these individual subject calculations, Figure 7 indicates that patients with severe COPD would be expected to generate only 60% of the average maximal diaphragmatic spe-

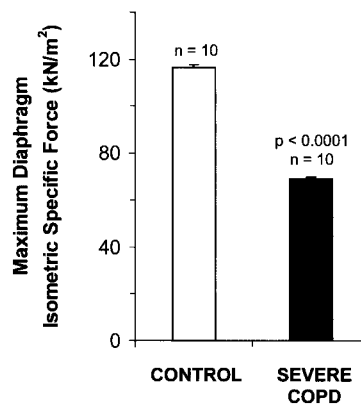


Figure 7. Maximal diaphragmatic muscle isometric specific force (MDSF) calculated for 10 subjects from the lowest spirometric quartile (i.e., severe COPD) and 10 subjects from the highest spirometric quartile (i.e., control subjects). Data are presented as means \pm SEM. The difference between group means was significant at $p < 0.0001$. See the online supplement for details about MDSF calculations.

cific force of the control subjects. Because $P_{di_{max}}$ of subjects with severe COPD is decreased to about 65% of that noted in control subjects (29–33), these calculations raise the possibility that the molecular and cellular remodeling noted in the diaphragms of subjects with severe COPD can fully account for the decreases in $P_{di_{max}}$ noted in these subjects.

Overall Hypothesis to Characterize Diaphragmatic Remodeling Associated with COPD

On the basis of our previous work (1, 2, 7) as well as the present study, we hypothesize that in patients with COPD, the fiber type transformations as well as the within-fiber type remodeling produce the following changes at the organ level (i.e., whole diaphragm) as well as at the individual myofiber level: (1) increases in the capacity for ATP generation; (2) decreases in the rate of ATP utilization; and (3) decreases in specific force. The clinical consequences of this process are an increase in fatigue resistance and decreases in the capacity for force generation.

Summary Statements

First, for patients with COPD, our data show the relationship between pulmonary function measurements and Type II-to-Type I transformations in both fiber type and area fractions of the diaphragm. Second, we hypothesize that diaphragmatic remodeling associated with COPD appears to be characterized by a tradeoff of decreases in force-generating capacity for increases in fatigue resistance. Third, we recognize that further studies are required to fully evaluate some of our hypotheses as well as to characterize the signal transduction pathways involved in the diaphragmatic remodeling associated with COPD.

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