Eur Respir J 1997; 10: 2853–2860
DOI: 10.1183/09031936.97.10122853
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European Respiratory Journal
ISSN 0903 - 1936

Fibre types in skeletal muscles of chronic obstructive pulmonary disease patients related to respiratory function and exercise tolerance

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ABSTRACT: This study aimed to investigate the relationship between skeletal muscle, fibre type composition, functional respiratory impairment and exercise tolerance in patients with moderate to severe chronic obstructive pulmonary disease (COPD).

A group of 22 COPD patients and 10 healthy control subjects were studied. In COPD patients, vital capacity (VC) and forced expiratory volume in one second (FEV_1) were reduced to 79% and 51%, respectively. Diffusion indices (transfer factor of the lung for carbon monoxide ($T_{L,CO}$) and carbon monoxide transfer coefficient (K_{CO})) were also reduced. Arterial oxygen tension (P_{a,O_2}) was normal or slightly altered. A maximal exercise test was performed and anaerobic threshold was calculated. Muscle samples from vastus lateralis were obtained by needle biopsy. Myosin heavy chain (MHC) and light chain (MLC) isoforms were separated by gel electrophoresis and quantified by densitometry. MHC isoforms were considered as molecular markers of fibre types.

The proportion of the fast MHC-2B isoform was increased in COPD patients. $T_{L,CO}$, K_{CO}, VC and FEV_1 were positively correlated with slow MHC isoform content. $T_{L,CO}$ and K_{CO} were also negatively correlated with the content of the fast MHC-2B isoform. No correlation was found between exercise parameters and MHC isoform composition. The co-ordinated expression between MHC and MLC isoforms was altered in COPD patients.

We conclude that reduced oxygen availability, probably in combination with muscle disuse, may determine muscle alterations in chronic obstructive pulmonary disease patients. The altered correlations between myosin heavy chain and light chain isoforms suggest that co-ordinated protein expression is lost in chronic obstructive pulmonary disease muscles.

Eur Respir J 1997; 10: 2853–2860.

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Keywords: Muscle fibre types
myosin isoforms
obstructive lung disease

Received: December 30 1996
Accepted after revision July 21 1997

Patients with chronic obstructive pulmonary disease (COPD) are characterized by poor quality of life and various degrees of limitation in their daily activity, mainly due to breathlessness. They are often self-limiting their level of activity into a downward spiral leading to further inactivity and muscle deconditioning. Exercise tolerance of COPD patients is determined by the level of ventilation that they can reach. Even at a rather low work rate the increase in ventilatory response and blood lactate concentration are higher than in normal subjects.

Rehabilitation of COPD patients involves general exercise reconditioning. This is suggested as the best approach to the rehabilitation of COPD patients, and is usually performed by training large muscles of the lower and, less frequently, upper limbs [1]. The mechanisms underlying the improvement of the performance after general training in COPD subjects are still unclear.

There is evidence that skeletal muscle function and structure are altered in COPD patients. Contractile strength is reduced [2] and energetic and oxidative metabolism are impaired [3–5]. Fibre-type composition is also altered, with a decrease in the proportion of fatigue-resistant slow fibres [3, 6]. Muscle alterations are potentially important in determining both the low tolerance to exercise [5] and the improvement after physical training [7, 8]. Muscle training improves tolerance to exercise without actual improvement of pulmonary mechanics or gas exchange [7]. A shift of the lactate threshold is likely to play a role [7], possibly in relation to an improvement of oxidative potential [8].

It has been shown that in various animal species, including humans, both low oxygen availability [9] and disuse [10, 11] induce a change in fibre type from slow to fast. We can put forward the hypothesis that in COPD patients either the scarce oxygen availability and/or the

inactivity and physical deconditioning cause a fibre transformation with an increase in the proportion of fast fatiguable fibres. These fibres can be responsible for lactate production and fatigue, which arise in patients even at low work rates.

The present study aimed to test this hypothesis. We tried to assess whether fibre-type composition of the skeletal muscle was related to the ventilatory and gas exchange parameters and exercise tolerance in a group of 22 patients with moderate to severe COPD but without respiratory failure. Samples of skeletal muscle were obtained by percutaneous biopsy of vastus lateralis. Quantification of fibre-type composition was based on electrophoretic and densitometric analysis of the myosin heavy chain (MHC) isoforms. A second family of myofibrillar proteins, the regulatory and essential myosin light chains (MLCs), were analysed to test the degree of coordinated expression of muscle proteins.

Materials and methods

Subjects

Twenty two male patients, mean±SD age 65.4±9.1 yrs, were studied. COPD was defined according to the criteria of the American Thoracic Society [12] The enrolment criteria were: 1) airways obstruction demonstrated by a forced expiratory volume in one second (FEV_1) <70% than predicted [13] and lack of reversibility (FEV_1 increase after 200 μg inhaled salbutamol <10% of its baseline value); 2) dyspnoea during exercise (selected patients were unable to climb two or more stairs or to walk slightly uphill); 3) age <75 yrs; and 4) body weight >90% of the ideal weight. The following exclusion criteria were applied: 1) arterial oxygen tension (P_{a,O_2}) <8 kPa, and arterial carbon dioxide tension (P_{a,CO_2}) >6 kPa at rest; 2) bronchitis exacerbation in the month before the enrolment; 3) cardiovascular, metabolic, skeletal and muscular disorders; 4) treatment with systemic steroids during the year before the enrolment.

A group of 10 healthy male subjects (aged 30–40 yrs, body weight 70–74 kg, who were not involved in any regular training or exercise activity) served as control group for muscle fibre composition.

The study was performed after admission to the Division of Pneumology, Medical Center of Tradate. Respiratory function analysis, exercise testing and muscle biopsy were performed on different consecutive days. Subjects were informed about the aims and methods of the study and about the possible risks and gave written consent. The study was approved by the Ethics Committee of the Salvatore Maugeri Foundation.

Respiratory function assessment

Baseline measurement of respiratory function was performed: vital capacity (VC), forced respiratory volume in one second (FEV_1) and residual volume (RV) with the plethysmographic method (Masterlab body, Jaeger, Wurzburg, Germany); transfer factor of the lung for carbon monoxide ($T_{L,CO}$) measured with the single breath method (Masterlab transfer, Jaeger, Wurzburg, Germany) and expressed as the transfer coefficient for carbon monoxide (K_{CO}), *i.e.*, relative to alveolar volume (VA); and maximal inspiratory pressure (MIP) obtained using a mouth pressure meter (Precision Medical Ltd, Pickering, UK). P_{a,O_2} and arterial carbon dioxide tension (P_{a,CO_2}) were measured immediately after sampling from a puncture of the radial artery at rest (Gas analyzer ABL 330; Radiometer, Copenhagen, Denmark).

Exercise testing

Each patient performed an incremental (10 $W \cdot min^{-1}$), symptom-limited exercise test to the maximal tolerated level, on a cycle ergometer (Lode, Groningen, Holland). An electrocardiogram was recorded during the test (Ivy, Biomedical System, Brandford, CT, USA). Maximal oxygen uptake ($V'_{O_2,max}$), maximal minute ventilation ($V'_{E,max}$) and maximal cardiac frequency ($f_{C,max}$) were measured (EOS sprint Jaeger, Wurzburg, Germany). Maximal work rate was calculated from $V'_{O_2,max}$ assuming that 20.14 kJ were produced with 1 L O_2 and that efficiency was 25%. Before the test, a catheter was inserted in the radial artery for arterial blood sampling during the test. Blood samples were obtained simultaneously from the arterial catheter for P_{a,O_2}, P_{a,CO_2}, pH and lactates. Anaerobic threshold (AT) was obtained directly from the lactates curve by using the method proposed by BEAVER *et al.* [14] and was expressed as percentage of $V'_{O_2,max}$. The variations in gas pressures and lactate concentrations ($\Delta P_{a,O_2}$, $\Delta P_{a,CO_2}$, Δlactate) were calculated before and at the maximal level of exercise.

Muscle biopsy

Muscle specimens were obtained using the needle biopsy technique; suction was applied to obtain larger samples. Immediately after withdrawal, samples were frozen in isopentane cooled in liquid nitrogen. Frozen samples were stored at -25 °C and transferred to the Institute of Human Physiology of the University of Pavia for further processing.

Three main fibre types are present in human muscles, type 1, type 2A and type 2B. Their diversity is based on the expression of specific isoforms of myofibrillar proteins, on the profile of the metabolic enzymes in the cytosol and in the mitochondria and on the ability to release and re-uptake activator calcium (for a review see PETTE and STARON [15] and SCHIAFFINO and REGGIANI [16]). Among these features, the expression of specific MHC isoforms represents a distinctive characteristic and can be used as a molecular marker to identify muscle fibre types [17]. The traditional histochemical methods for fibre typing based on adenosine triphosphatase (ATPase) following acid or basic preincubation also utilize the presence of a specific MHC isoform to identify a fibre [15]. A substantial agreement between histochemical and electrophoretic methods has been demonstrated in previous studies [17, 18]. In addition, compared to fibre counting after histochemical staining, the electrophoretical separation of MHC isoforms followed by their densitometric quantification accounts not only for variations in number but also for variation in size of the individual fibre types. In human muscles three MHC isoforms are expressed: MHC-1, MHC-2A and MHC-2B; the latter has recently been identified as the human equivalent of MHC-2X [16].

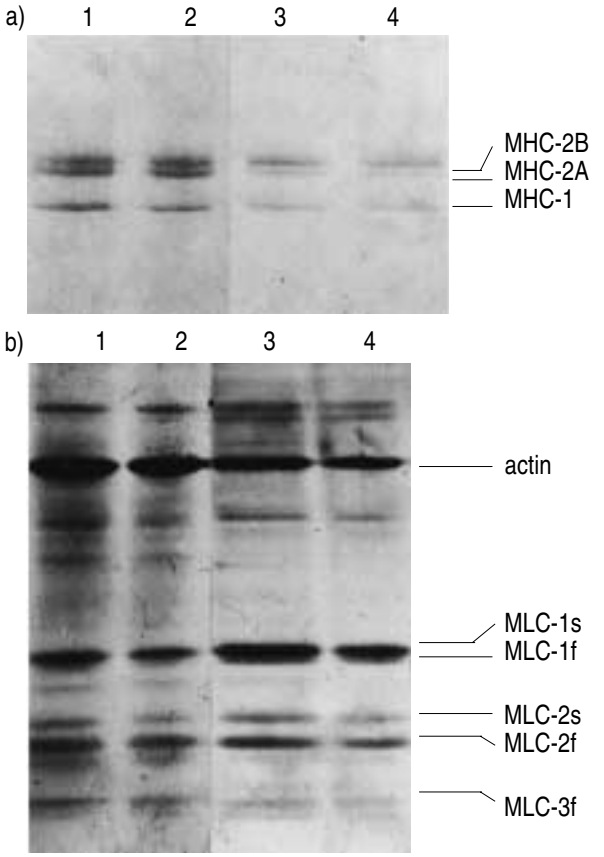

Fig. 1. – Examples of electrophoretical separation of myosin heavy chain (MHC) isoforms (a) and myosin light chain (MLC) isoforms (b) in the muscle biopsy samples from two patients. Lanes 1 and 2 refer to patient 4 and lanes 3 and 4 to patient 9. The difference in MHC distribution is clearly visible.

For these reasons, MHC isoforms were separated by gel electrophoresis and quantified by densitometric analysis. In addition to MHC isoforms, essential and regulatory MLC were also separated and quantified by electrophoresis and densitometry. Examples of electrophoretical separation are shown in figure 1.

MHC and MLC electrophoretic separation

Before electrophoresis, samples were immersed in a solution of the following composition: 62.5 mM Tris-HCI, pH 6.8; 2.3% (w/v) sodium dodecylsulphate (SDS); 10% (w/v) glycerol; and 5% mercaptoethanol at the final protein concentration of 0.1 mg·mL^{-1}. The samples were boiled for 5 min and stored at -25 °C prior to electrophoretic determination of MHC and MLC isoforms. MHC isoforms were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). A small amount of the sample (containing approximately 1.0–1.4 μg of proteins) was loaded on 6% SDS polyacrylamide gels with 4% stacking gels and run overnight (21–22 h) at 100 V. Gels were silver stained. This electrophoretic protocol has demonstrated a clear resolution of MHC. Three bands were separated, corresponding to the isoforms MHC-1, MHC-2A and MHC-2B, from the faster to the slower in migration speed.

MLC isoform determination was performed by SDS-PAGE with the following protocol: a sample (containing approximately 4–10 μg of proteins) was loaded on 10–20% polyacrylamide gradient slab gels with 4% staking gels and run overnight at 100 V. Gels were silver stained. Five MLC bands were separated, corresponding to the fast and slow isoforms of regulatory MLC (MLC-2s and MLC-2f) and to the three isoforms of alkaline MLC (MLC-1s, MLC-1f, MLC-3f).

The relative proportions of MCH and MLC isoforms were determined by means of densitometry followed by planimetric measurement of the areas under the peaks corresponding to different MHC and MLC isoforms.

Statistical analysis

Data were expressed as mean and standard deviations. Student's t-test was used for comparison between means. A correlation matrix was utilized to analyse the matching between functional data and MHC isoform composition and between MHC isoform and MLC isoform distribution. Covariance analysis was used to compare regression lines in control and COPD muscles. Correlations were considered significant at p-values of less than 0.05.

Results

The mean values of respiratory parameters at rest are reported in table 1. COPD patients exhibited a pronounced respiratory impairment. VC (-21%), FEV1 (-49%) and diffusion indices ($T_{L,CO}$ and K_{CO}, -23% and -36% respectively) were all significantly reduced, whereas RV was increased (+71%). P_{a,O_2} and P_{a,CO_2}, however, were only slightly altered, thus indicating that no respiratory failure was present at rest. Table 2 summarizes data obtained during exercise tests. All patients had a symptom limited $V'_{O_2,max}$, *i.e.* early dyspnoea represented the limit to exercise. The low exercise tolerance of patients is indicated by the low value of $V'_{O_2,max}$ and by the low average work rate they could reach. The

Table 1. – Functional respiratory parameters of 22 chronic obstructive pulmonary disease patients at rest

	Mean	SD
VC L	2.8 (79)	0.5 (17)
RV L	4.0 (171)	0.9 (36)
FEV1 L	1.4 (51)	0.4 (12)
$T_{L,CO}$ mmol·min·kPa^{-1}	6.2 (77)	2.2 (27)
K_{CO} mmol·min·kPa^{-1}	1.1 (64)	0.4 (22)
MIP kPa	7.1 (99)	1.7 (22)
P_{a,O_2} kPa	10.8 (101)	1.1 (10)
P_{a,CO_2} kPa	5.4 (102)	0.5 (9)

The values in parenthesis are expressed as percentage of the predicted values in healthy subjects. VC: vital capacity; RV: residual volume; FEV1: forced expiratory volume in one second, determined in basal conditions; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; K_{CO}: carbon monoxide transfer coefficient ($T_{L,CO}$/alveolar volume); MIP: maximal inspiratory pressure; P_{a,O_2}: arterial partial oxygen pressure; P_{a,CO_2}: arterial partial carbon dioxide pressure.

patients did not show any significant decline in P_{a,O_2} during exercise. The alveolar arterial pressure difference for oxygen ($P_{A\text{-}a,O_2}$), however, increased from 4.02±1.56 kPa at rest to 4.86±1.91 kPa during exercise (p=0.02).

Muscle fibre compositions determined using MHC isoform as a molecular marker in COPD and control subjects are summarized in table 3. The proportions of different isoforms are expressed as percentage values. The large majority of muscle samples contained a combination of type 1, 2A and 2B MHC. Seven samples

Table 2. – Parameters determined during the exercise test in 22 chronic obstructive pulmonary disease patients

	Mean	SD
$V'_{O_2,max}$ mL·min^{-1}	1007	270
W_{max} W	85.4	8.4
$V'_{E,max}$ L·min^{-1}	38.9	9.8
$f_{C,max}$ beats·min^{-1}	125	23
$\Delta P_{a,O_2}$ kPa	0.3	1.5
$\Delta P_{a,CO_2}$ kPa	0.4	0.5
Δlactate mM	3.2	1.0
AT % $V'_{O_2,max}$	94.2	5.4

$V'_{O_2,max}$: maximal oxygen consumption; W_{max}: maximal power; $V'_{E,max}$: maximal ventilation; $f_{C,max}$: maximal cardiac frequency; $\Delta P_{a,O_2}$: change in arterial oxygen tension; $\Delta P_{a,CO_2}$: change in arterial carbon dioxide tension; Δlactate: variation of plasma lactate concentration; AT: anaerobic threshold expressed as percentage of $V'_{O_2,max}$.

Table 3. – Distribution of myosin heavy chain (MHC) and myosin light chain (MLC) isoforms in biopsy samples from vastus lateralis of the chronic obstructive pulmonary disease (COPD) patients (n=22) and control healthy subjects (n=10)

	COPD patients %	Control patients %	p-value
MHC isoforms			
MHC-1	36±17	44±16	0.21
MHC-2A	40±13	46±9	0.26
MHC-2B	23±22	7±12	0.04
MLC isoforms			
MLC-2s	13±6	18±10	0.07
MLC-2f	30±6	23±9	0.04
MLC-1s	26±9	42±12	<0.01
MLC-1f	12±10	2±4	<0.01
MLC-3f	21±12	13±5	0.06

Values are presented as mean±SD. A p-value less than 0.05 was considered significant.

Table 4. – Correlations between respiratory parameters at rest and myosin heavy chain (MHC) isoform composition of vastus lateralis: values of the regression coefficient (r)

	VC	FEV1	$T_{L,CO}$	K_{CO}
MHC-1 (%)	0.50*	0.42*	0.52*	0.47*
MHC-2A (%)	-0.20	0.05	0.43	0.42
MHC-2B (%)	-0.27	-0.38	-0.66*	-0.62*

*: significant (p<0.05) correlation. VC: vital capacity; FEV1: forced expiratory volume in one second, determined in basal conditions; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; K_{CO}: carbon monoxide transfer coefficient ($T_{L,CO}$/alveolar volume).

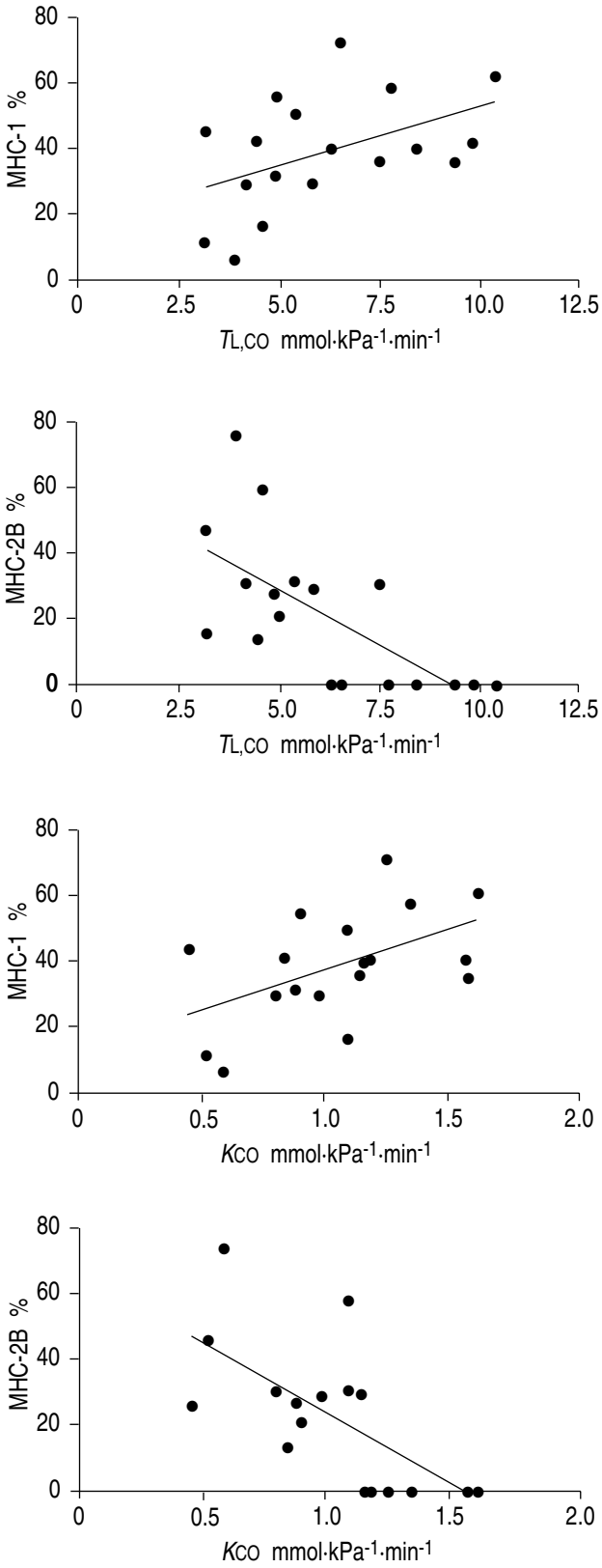

Fig. 2. – Examples of significant correlations between myosin heavy chain (MHC) isoform distribution and transfer factor of the lung for carbon monoxide ($T_{L,CO}$) and carbon monoxide transfer coefficient (K_{CO}). All possible correlations between respiratory parameters at rest and MHC isoform distribution were examined and the results are reported in table 4. Statistical significance was reached by only six correlations, of which four are shown in the figure.

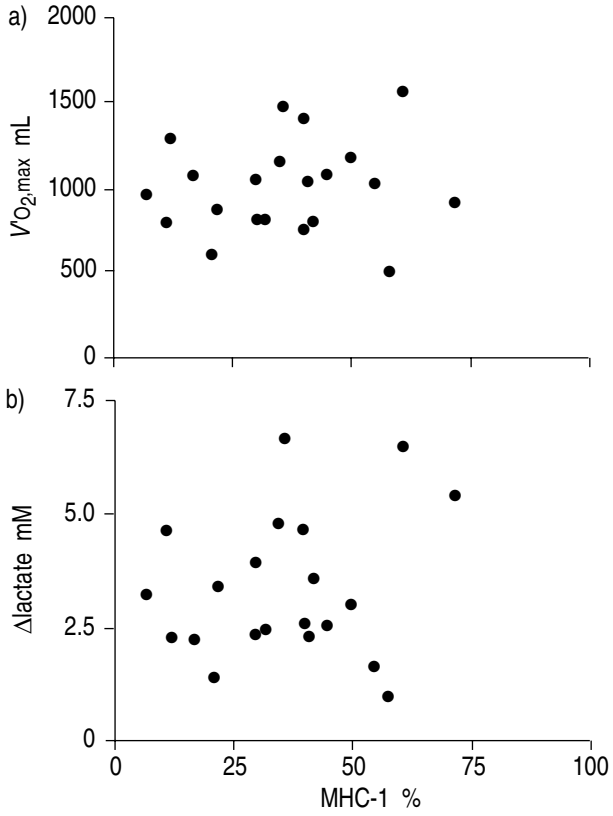

Fig. 3. – Examples of lack of correlation between myosin heavy chain (MHC) isoform distribution and functional parameters: a) maximum oxygen consumption ($V'_{O_2,max}$); and b) variation in plasma lactate (Δlactate) determined during exercise test. The values of the coefficient of determination (r^2) are 0.0098 for the upper diagram and 0.0562 for the lower diagram.

from the 22 COPD patients and six samples from 10 control subjects did not contain the fast isoform 2B. The proportion of MHC-2B was significantly greater in COPD patients than in control subjects. The isoform distribution of regulatory and essential MLC is shown in table 3. All muscle biopsies contained slow and fast isoforms of MLC-1 and MLC-2, with a variable percentage of MLC-3. MLC-1f isoform, however, was not found in two samples of COPD patients and in seven samples of control subjects. Muscle samples from COPD patients were significantly richer in MLC-1f, and MLC-2f, with a trend towards a higher percentage of MLC-3f, and contained significantly lower amounts of MLC-1s.

The analysis of the correlation (table 4) between respiratory parameters at rest and MHC isoform composition showed the following significant correlations: 1) VC and FEV1 were positively correlated with the percentage of MHC-1; and 2) the diffusion capacity indices ($T_{L,CO}$ and K_{CO}) were positively correlated with the content of MHC-1 and negatively correlated with the percentage of MHC-2B (fig. 2). No significant correlation was found with type 2A fibres. No other respiratory parameters at rest correlated with muscle MHC isoform composition.

No correlation was found between muscle composition in type 1, 2A and 2B MHC and exercise parameters including $V'_{O_2,max}$, maximal work rate, anaerobic threshold and variations in P_{a,O_2} and P_{a,CO_2}. Two examples are shown in figure 3, where $V'_{O_2,max}$ and Δlactate are plotted as a function of the proportion of MHC-1, which is a marker of slow, aerobic fibres. As can be seen, no correlation was present.

Table 5 shows the results of the correlations between the percentage distribution of MHC and MLC isoforms. These correlations allowed the degree of co-ordinated expression between different muscle proteins to be tested. All possible combinations were examined in both COPD and control muscles. In control muscles significant correlations were present between MHC-1 and MLC-2s (positive), MLC-2f, MLC-1f (negative), between MHC-2A and MLC-2f, MLC-1f (positive), MLC-1s (negative) and between MHC-2B and MLC-2s (negative), MLC-1f (positive). In muscles of COPD patients the correlations between MHC-1 and MLC-2s and between MHC-1 and MLC-1f disappeared and new significant correlations appeared between MHC-1 and MLC-1s (positive) and MLC-3f (negative). In the same way the correlations between MHC-2A and MLC-2f, MLC-1f and the correlations between MHC-2B and MLC-2s, MLC-1f disappeared, whereas new correlations between MHC-2B and MLC-1s (positive) and MLC-3f (negative) appeared. Examples of correlations between MHC and MLC isoform distributions are given in figure 4.

Table 5. – Correlations between percentage distribution of myosin heavy chain (MHC) isoforms and percentage distribution of myosin light chain (MLC) isoforms: values of the coefficient of determination (r^2), the intercept (a) and of the slope (b)

	MLC-2s %		MLC-2f %		MLC-1s %		MLC-1f %		MLC-3f %	
	Control	COPD	Control	COPD	Control	COPD	Control	COPD	Control	COPD
MHC-1 %										
r^2	0.48*	0.03	0.69*	0.33*	0.21	0.41*	0.50*	0.01	0.05	0.26*
a	-0.97	10.02+	46.75	37.44	25.32	38.63+	10.03	12.47	17.51	5.47+
b	0.43	0.07+	-0.52	-0.23	0.36	-0.34+	-0.18	-0.01+	-0.09	0.35+
MHC-2A %										
r^2	0.04	0.05	0.48*	0.01	0.44*	0.31*	0.76*	0.02	0.26	0.14
a	27.66	16.51+	-9.84	29.52+	82.38	47.24	-0.04	10.65+	-2.02	7.44+
b	-0.21	-0.09	0.73	-0.01+	-0.90	-0.51	0.28	0.06+	0.34	0.34
MHC-2B %										
r^2	0.51*	0.01	0.14	0.21*	0.02	0.63*	0.47*	0.01	0.14	0.40*
a	-0.58	0.01+	21.31	26.07	40.52	18.35+	-9.87	29.65+	14.95	29.45+
b	22.38	12.65+	0.31	0.15	0.14	0.36	0.73	-0.01+	-0.19	-0.35

*: significant ($p<0.05$) correlation; +: $p<0.05$ *versus* control subjects. COPD: chronic obstructive pulmonary disease.

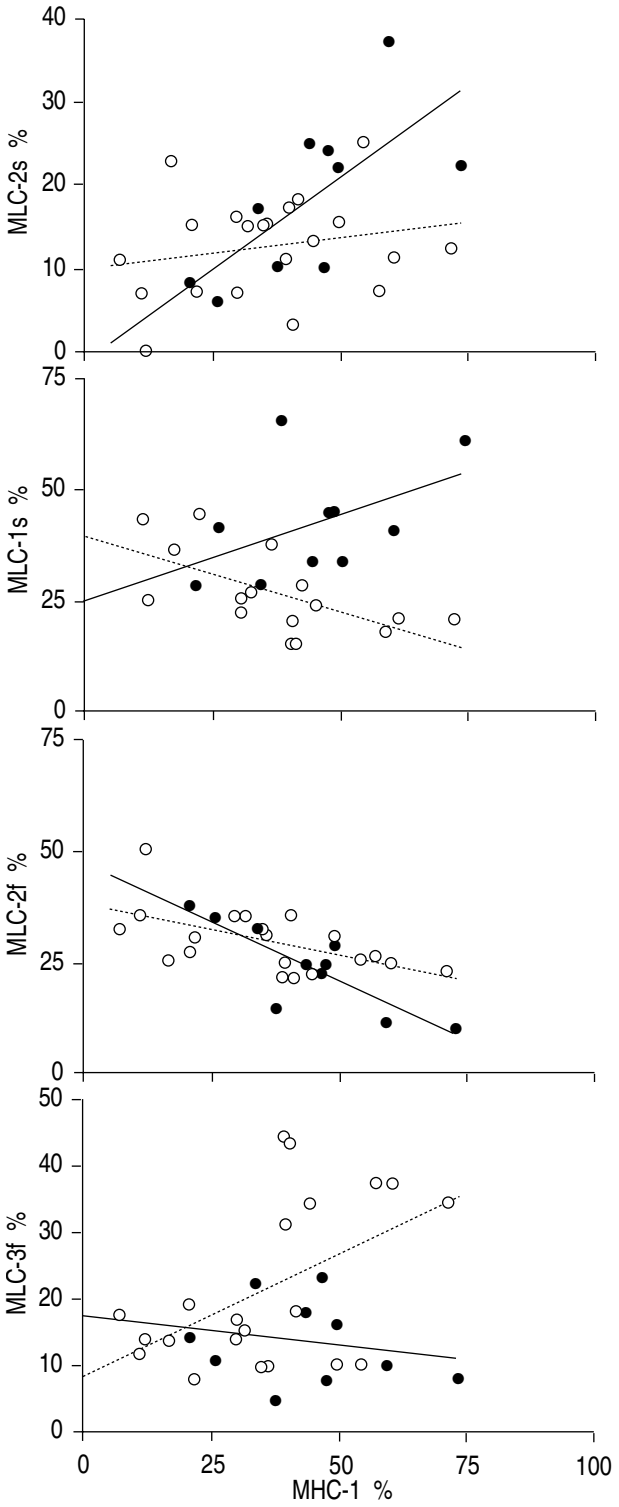

Fig. 4. – Preferential associations between myosin heavy chain (MHC) isoforms and myosin light chain (MLC) isoforms in the samples of vastus lateralis from control subjects (●) and chronic obstructive pulmonary disease (COPD) patients (○). As shown in table 5, all possible correlations between MHC isoforms and MLC isoforms were tested with linear regression analysis both in control and COPD muscles. Four examples are shown in the figure: three examples (MLC2s *versus* MHC1, MLC1s *versus* MHC1, MLC3f *versus* MHC1) show significant differences between the correlations observed in control subjects and those observed in COPD patients, whereas one (MLC2f *versus* MHC1) shows that the same correlation was present in control and COPD muscles. Regression lines are shown for the controls (—) and COPD patients (- - -).

Discussion

The aim of this study was to investigate skeletal muscle fibre composition in a group of COPD patients in whom a rehabilitative programme involving exercise training was indicated. The selected patients had airways obstruction and their exercise capacity was limited by dyspnoea. Pulmonary gas exchange was impaired, but their arterial blood gas values were normal or slightly altered. Care was taken to avoid interference from other factors that can affect muscle composition, such as steroid treatment [19], or respiratory function, such as recent bronchitis exacerbation. The respiratory function of the patients was studied at rest and during exercise. Skeletal muscle samples were obtained by percutaneous biopsy of the vastus lateralis in the COPD patients and also in a group of healthy control subjects. Fibre type distribution was assessed by using MHC isoforms as molecular markers. MLC isoforms were also characterized.

The analysis of the muscle samples revealed that in COPD patients when compared to control subjects: 1) the proportion of fast 2B fibres was increased; and 2) the co-ordinated expression between MHC and MLC isoforms was altered. The slow to fast fibre type transition is in general agreement with previous results obtained with histochemical fibre typing which showed an increase of fast fibres in hypoxic patients [3, 6]. In the present study, however, three fibre types were identified and only the fast glycolitic 2B fibres were found to increase in COPD patients, whereas fast oxidative type 2A fibres shared with slow fibres a tendency to decrease. Three factors can be considered responsible for the differences between COPD patients and control subjects: age, inactivity and low oxygen availability. There is evidence, however, that aging does not substantially change the fibre type composition of human muscles. If there is a change, it consists of an increased proportion of slow type 1 fibres, regardless of whether it is determined with electrophoretical methods [20] or traditional histological methods [21]. On the other hand, as mentioned in the introduction, both disuse [10, 11] and hypoxaemia [6, 9] have been found to induce a transition from slow to fast in animal and human muscles. In human muscles, however, the fibre shift from slow to fast has been observed only when complete disuse occurs, *e.g.* in leg muscles of paraplegic patients [11]. Prolonged periods of reduced activity, for example bed rest, produce significant fibre atrophy but no fibre distribution change [22]. Long-term exposure to hypobaric hypoxia or life at altitude do not induce significant changes in fibre type distribution [23]. It must be observed that the patients participating in this study did not show any decrease in P_{a,O_2} even during exercise. Reduced transfer factor of the lung, however, might be expected to limit oxygen supply to muscle even without producing arterial oxygen desaturation. In addition, overnight saturimetric monitoring revealed that in 17 of the 22 patients there were nocturnal desaturations (saturation less than 90%) with an average duration of 100 min. The two factors together might have combined to produce a chronic condition of low oxygen supply to muscles. The combined action of low oxygen supply and

reduced activity might explain the fibre-type transition. Support for this hypothesis is provided by animal experiments, which show that increase of fast fibres in hypobaric conditions can be antagonized by increased motor activity [24]. Furthermore, the muscle alterations in COPD are consistent with those described in muscles of patients with chronic heart failure, where reduction of the oxidative metabolism and shift of fibre types from slow to fast is generally related with inactivity and reduced oxygen supply [25].

A close match between MHC and MLC isoform expression has been found in several animal species and appears to be a general rule in myosin isoform expression [16]. In human skeletal muscles, slow MHC has been found to associate preferentially with slow isoforms of both alkaline and regulatory MLC [26]. Fast MHC isoforms preferentially associate with fast MLC isoforms [26]. The co-ordinated expression was confirmed in the control subjects examined in this study. The loss of preferential association observed in COPD patients might indicate the presence of fibres in transition phase where some proteins, very likely with faster turnover, are already present with the fast isoform and other proteins are still present with the slow isoforms [27]. In a recent study the disappearance of the preferential association between MHC-2B and MLC-3f has been described in muscles of aged rats [28].

The main results of this study are represented by the correlations between muscle fibre type distributions in COPD patients. A significant positive correlation was found between MHC-1 (*i.e.* type 1 fibre relative content) and functional respiratory parameters (VC, FEV_1) and diffusion parameters. Diffusion parameters were found to be negatively correlated with type 2B relative content. The correlation of diffusion indices with type 2A fibre was positive, as with slow fibres, although the statistical significance level was not reached (table 4). Previous attempts to find correlations between muscle changes and respiratory impairment have yielded controversial results. In a previous study [3], the muscle contents of glycogen, adenosine triphosphate (ATP), creatine phosphate and lactate were found to correlate with arterial blood gases in COPD patients with and without respiratory failure. In particular, the relative proportion of type 1 fibre was correlated positively with P_{a,O_2}. An overall derangement of the main indices of muscle metabolism without any correlation with hypoxemia was found by FIACCADORI *et al.* [29] in COPD patients with acute severe respiratory failure. No correlation between fibre composition and indices of airway obstruction was found by HUGHES *et al.* [30] in COPD patients undergoing thoracotomy for pulmonary neoplasm. It is likely that the choice of patients in a clinically stable state and the exclusion of patients with muscle atrophy or fibre-type shift caused by concurrent disease or steroid treatment are required to observe pure effects of COPD on muscles.

The correlations found in our study extend the results previously obtained by JAKOBSSON *et al.* [3]. Three different fibre types were identified in this study, thus correctly separating fast oxidative 2A fibres from fast glycolitic 2B fibres. Moreover, the fibre type distribution shift was found to occur not only in patients with low P_{a,O_2} [3], but also when gas exchange was impaired (reduced $T_{L,CO}$ and K_{CO}) even without any decrease of P_{a,O_2}. The more lung function, especially gas exchange, was impaired, the less type 1 fibres and the more type 2B fibres were found. As mentioned above, type 2A fibres seems to follow the changes of type 1 more than those of type 2B. Interestingly, fibres of type 1 and 2A are associated with high oxidative metabolic activity, 2B fibres with glycolitic anaerobic metabolism (reviewed by PETTE and STARON [15]). This is in full agreement with the observations that oxidative metabolism, but not glycolytic metabolism, is impaired in skeletal muscles of COPD subjects [5, 4].

To our knowledge, no previous study has correlated parameters determined during exercise with muscle fibre composition. The lack of correlation between maximal exercise capacity, lactate production, respiratory gas behaviour during effort and muscle fibre composition observed in this study is quite striking. On the one hand, it is possible that sampling of vastus lateralis by needle biopsy provides information on local muscle changes in response to reduced oxygen availability, but is not particularly representative of the muscle mass of the whole body or even of the lower limbs. On the other hand, if the information obtained from the biopsy sample is representative, the lack of correlation would argue against the idea that fibre transformation from type 1 to type 2B and the accompanying reduction of muscle oxidative capacity might actually contribute to determining the early rise in plasma lactate, the dyspnoea and the low tolerance to exercise. Indeed, evidence in favour of a causal relationship between decreased activity of muscle oxidative enzymes and increase of plasma lactate has recently been presented by MALTAIS *et al.* [5]. Indirectly, the lack of correlation would also contrast with the observation of positive effect of training on exercise tolerance [7] and the recent finding that endurance training improve muscle oxidative capacity and reduces lactic acidosis [8] in COPD patients. The apparent contradiction, however, might find an explanation if the loss of co-ordinated expression observed between MHC and essential MLC were also true of other proteins and in particular to metabolic enzymes. MHC isoforms would, thus, lose their role as molecular markers of fibre types. The enzymatic changes and the changes in other muscle protein expression would take place independently and the generally existing strict co-ordination would disappear. This might represent an interesting feature of the muscle cell dysfunction associated with COPD.

In conclusion this study provides evidence that muscle structural alterations are present even in patients with moderate chronic obstructive pulmonary disease and without plasma oxygen desaturation. The combined effects of disuse and reduced oxygen supply are likely to be among the causal factors. A fibre type transition along the pathway from 1 to 2A to 2B and disruption of co-ordinated protein expression characterize the muscle alterations.

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